ALIEN WEEDS IN THE TERRESTRIAL ECOSYSTEM OF KERALA

BY

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(2006-21-104)

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SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

Agron. 751 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

I hereby declare that the seminar report entitled **"Alien weeds in the terrestrial ecosystem of Kerala"** is a record of the seminar presented by me during the course (05.05.2007) and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
02.06.2007

Musthafa Kunnathadi
(2006-21-104)

CERTIFICATE

Certified that the seminar report entitled **"Alien weeds in the terrestrial ecosystem of Kerala"** is a record of seminar presented by **Sri. Musthafa Kunnathadi (2006-21-104)** under my guidance and that this report has been prepared by him independently.

Vellanikkara
02.06.2007

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Introduction

India is considered to be one of the megabiodiversity countries in terms of flora and fauna. Biodiversity is in fact the basis of life on earth and a resource to be used and preserved. In the present context of global change biodiversity depletion and biological invasion are of relevant issues. Biological invasion is the spread of non-native or exotic species leading to the loss of native biodiversity.

Organisms are considered alien when they occur artificially in locations beyond their known historical natural ranges. "Alien" can refer to species brought in from other continents, regions, ecosystems and even other habitats. One of the major factors identified during the last decade, directly affecting the resource management schemes such as land use, watersheds and native flora and fauna (biodiversity) are alien species that are aggressive colonists (invaders) outside their natural range that have damaging effect (Sandlund *et al.*, 1996).

A large number of weeds have been introduced to India along with the introduction of some economically important plants as well as planting materials from other countries. Many introduced plants have become naturalized and are replacing the native plant species. One important step to address the problem is to understand better the long term range dynamics of invasive species in different landscapes or habitats and the consequences. This will also help define the needs and actions for management.

Alien species are recognized as the second largest threat to biological diversity, the first being habitat destruction. Economic impact of invasive weeds, both aquatic and terrestrial includes interference with cultivation of crops, loss of biodiversity and ecosystem resilience, loss of potentially productive land, loss of grazing and livestock production, poisoning of human and livestock, choking of navigational and irrigation canals and reduction of water in water bodies. As the invasion by exotic weeds progress the density and diversity of native species have been found reduced which was likely to be caused by the competition and fast growing, aggressive weed species (Mahajan and Azeez, 2001).

Factors effecting colonization/invasion by exotic weeds

1. Invaders in the tropical rainforests of India principally come from regions in Central/South America with a tropical humid climate and highly bleached nutrient soils and this similarity in the climatic and edaphic conditions help many exotic species get established and colonized efficiently (Bennett and Rao, 1978).

2. Efficient reproduction and self dispersal mechanism of the alien species.
Exotic weeds belonging to family Asteraceae such as Chromolaena, Parthenium etc. have higher reproductive capacity which was achieved by production of more number of minute, light and wind dispersed seeds (Saxena and Ramakrishnan, 1982) and this helps in their immediate and fast spread. *Mikania micrantha* produces on an average 45,812 (Abraham, 1999) and *Mimosa invisa* 72,650 (Jayasree, 2005) seeds per plant. Lantana and Chromolaena have capacity of resprouting after serious disturbances such as burning and Lantana also produces abundant seeds that are fed by birds and dispersed through their droppings.

3. Lack of natural enemies in the new locality. In the native locality these species are controlled by the native natural enemies which may not be occurred in the new locality.

4. The exotic weeds invade different habitats successfully as they possess unique adaptive attributes such as fast growth, stress tolerance, high nutrient uptake efficiency etc.

However, human factors are also involved in their regional spread within the country. In a survey in the Western Ghats, Muniappan and Virakthamath (1993) found that Chromolaena is migrating northwards at the border of Karnataka and Maharashtra, apparently its spread is inadvertently aided by the Forest department by the planting of seedlings of forest trees which are raised in Chromolaena seed contaminated soil-filled plastic bags and then moved from infested areas to uninfested areas.

Important alien weeds in Kerala

Scientific name	Common name	Malayalam name	Family
Terrestrial			
Lantana camara	Lantana	കൊങ്ങിണി	Verbenaceae
Mikania micrantha	Mile-a-minute	അമേരിക്കൻ വള്ളി	Asteraceae
Chromolaena odorata	Siam weed	കമ്മ്യൂണിസ്റ്റ് പച്ച	Asteraceae
Parthenium hysterophorus	Congress grass	പാർത്തീനിയം	Asteraceae
Mimosa invisa	Giant sensitive plant	ആനത്തൊട്ടാവാടി	Fabaceae
Mimosa pudica	Touch-me-not	തൊട്ടാവാടി	Fabaceae
Aquatic			
Eichhornia crassipes	Water hyacinth	കുളവാഴ	Pontederiaceae
Salvinia molesta	Kariba weed	ആഫ്രിക്കൻ പായൽ	Salviniaceae
Ipomoea carnea	Gramophone plant	തോട്ടുചീര	Convolvulaceae
Ipomoea aquatica	Water spinach		Convolvulaceae
Alternanthera philoxeroides	Alligator weed	കൊഴുപ്പ	Amaranthaceae
Limnocharis flava	Water cabbage	നാഗപ്പോള	Limnocharitaceae

The important alien weeds commonly observed in the terrestrial ecosystem of Kerala are dealt with in detail below.

1. *Chromolaena odorata* (L.) R.M. King and H. Robinson

1.1 Introduction and spread

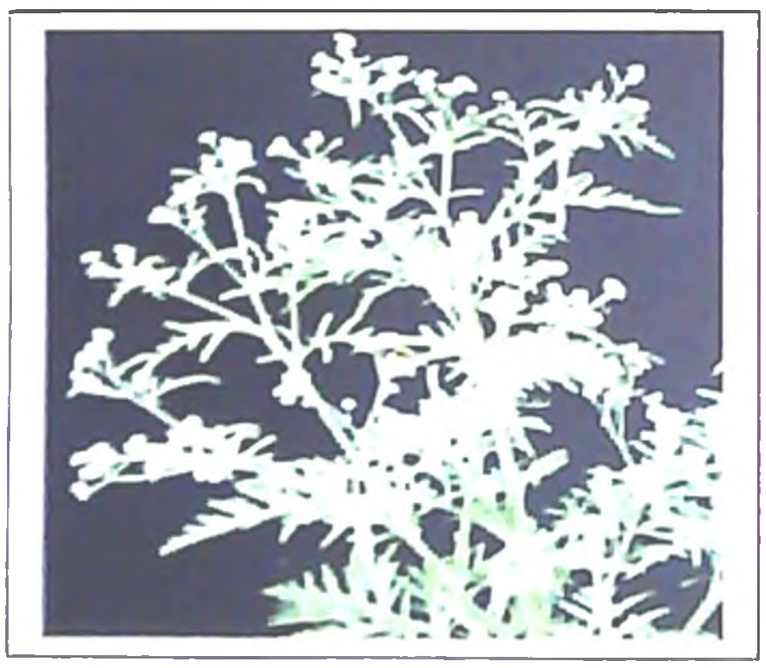
Chromolaena odorata is a native of South and Central America and a weed in the tropics causing severe damage to the crops. It is a successful colonizer in different habitats, growing luxuriantly on tree trunks, straw roofs of huts with minimum amounts of soil and in extremely poor soils. Bright sunlight, high soil moisture and relative humidity and low temperature favour vigorous growth of Chromolaena (Ambika, 1998).

There is considerable disparity of views as to when this neotropical herbaceous perennial shrub was introduced to India. Ambika and Jayachandra (1990) quoted a reference from 1920 as the first reported occurrence. However, Biswas (1934) implied that it was deliberately introduced much earlier from Jamaica, presumably as an ornamental into the Royal Botanic Gardens in Calcutta, and noted that Sir Joseph Hooker (In "Flora of British India 1881") reported its occurrence in Assam in the late 19th century, where it was said to

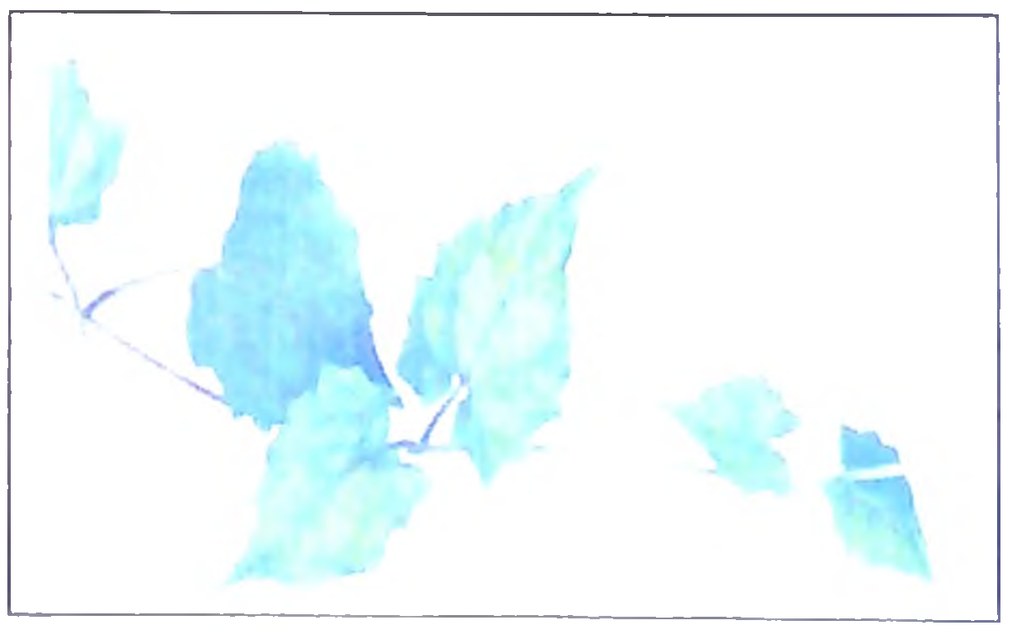
Important terrestrial alien weeds in Kerala

Chromolaena odorata

Lantana camara



Parthenium hysterophorus



Mikania micrantha

Mimosa invisa

Mimosa pudica

rapidly replace the indigenous shrubs. Nevertheless, he also stated that the plant could have arrived in the ballast of cargo boats traveling from the West Indies to Singapore and entered India via Malaysia, Thailand (Siam) and Burma. According to Moni and George (1959) Chromolaena migrated to Assam during the first World War (1914-18) and from there it spread all over the north east region. In 1924-25 it further spread to West Bengal forests and gradually invaded the forests. From West Bengal it spread to Orissa and from the eastern region the weed spread to Kerala in 1942 by the seeds stuck to the beddings and clothes of the labourers returning from the Assam Front.

In Kerala, the siam weed commonly known as "communist pacha" became a problem weed in plantation crops like coconut, rubber, cashew, pepper, cocoa etc. Being a native of the neotropics the weed could establish very well in the humid tropical climatic conditions in the state. It has developed as a serious problem during the last five decades. From Kerala it spread rapidly to all the southern states. In India it is now very well distributed in north east and southern states particularly in Assam, West Bengal, Orissa, Karnataka, Maharashtra, Tamilnadu and Kerala and the distribution is limited to the areas receiving rainfall of 150 cm and above (Singh, 1998). It has now replaced *Lantana camara* in the western parts of the Western Ghats (Muniappan and Virakthamath, 1993).

1.2 Competition with crops

Chromolarena odorata has occupied pastures, marginal lands and open areas and has become a menace in coconut, rubber, oil palm, teak, tea, coffee, cardamom, citrus and other plantations, orchards and forests. It impedes access to the crops and hampers cultural and harvest operations. During dry season, it can be a serious fire risk in the forests. Competition from Chromolaena adversely affects the growth of the crops, especially during the early stages (Singh, 1998).

1.3 Management of *Chromolaena odorata*

1.3.1 Physical/ mechanical

Hand pulling, digging with spade and slashing with sickle are the common practices for controlling this weed. However, these mechanical methods give only short-term control (Muniappan and Marutani, 1991).

1.3. 2 Chemical

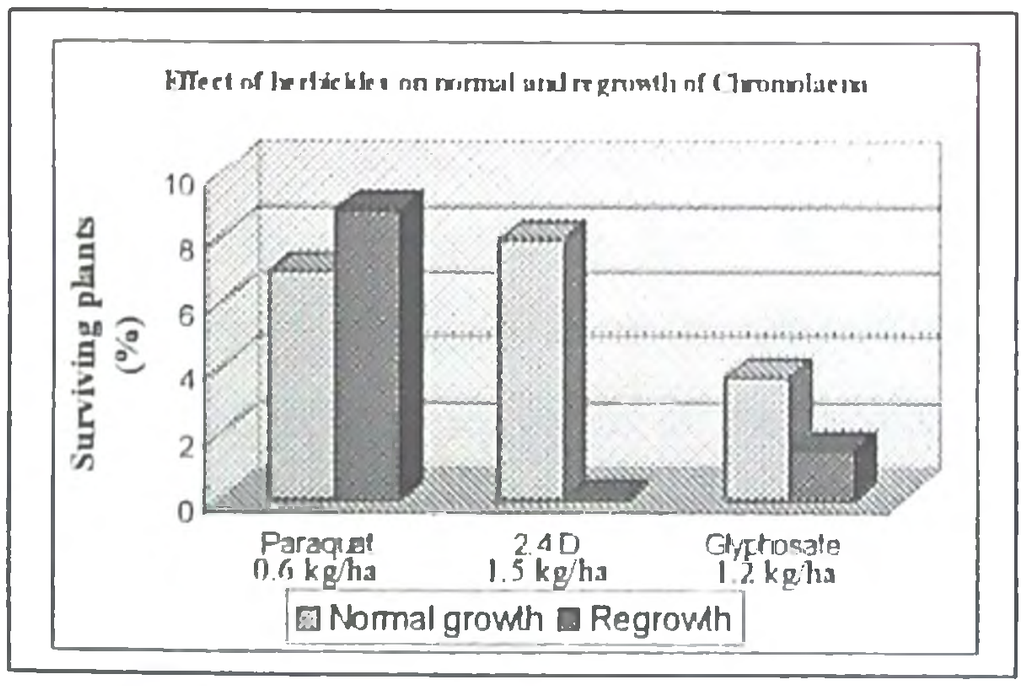
In order to prevent infestation of Chromolaena application of pre-emergence herbicides Atrazine, Metolachlor and Diuron were found useful as reported by Leucas (1989). Abraham *et al.* (1998) reported greater effectiveness of pre-emergence herbicides at higher doses in controlling the weed. They could observe complete control of germination of the weed with Diuron at doses 1.5 kg/ha and above and Atrazine at 2 kg/ha.

Table 1. Effect of pre-emergence herbicides on the germination of *C. odorata*

Herbicide	Dose, kg/ha	No. of Chromolaena seedlings/sq.m
Diuron	1.0	2.0
	1.5	0.0
	2.0	0.0
Atrazine	1.0	1.0
	1.5	1.0
	2.0	0.0
Oxyfluorfen	0.2	32.5
	0.3	10.6
	0.4	9.1
Unsprayed control		35.6
CD (0.05)		2.21

(Abraham et al., 1998)

Abraham *et al.* (1998) reported increased effectiveness with increasing doses of post emergence herbicides in controlling Chromolaena. Application of higher doses of Paraquat (1 kg/ha), 2,4 D (2.5 kg/ha) and Glyphosate (1.6 kg/ha) resulted in the least number and dry matter production of the surviving plants. They also reported better effectiveness of systemic herbicides when sprayed on the new flushes emerging after slashing, than application on normal growth, as illustrated in the following graph. Denny and Naude (1994) also reported higher effectiveness of Imazapyr applied to the cut surface of stumps for killing the plants.



1.3.3 Biological

1.3.3.1 Native insects

Many scientists attempted control of Chromolaena through biological means. Survey of native insect complex of *Chromolaena odorata* was carried out extensively in Karnataka, Tamilnadu and Kerala, but none of the insects was found promising as bio-control agent. Ramani and Haq (1983) recorded an oribatid mite *Aphis fronothrus orboreus* in Kerala. An abundant occurrence of *Aphis citricola* and *Aphis fabae* in Kerala was reported by Joy *et al.* (1979) and Lyla *et al.* (1987). *Myllocerus viridanus* (Fabricus), a common defoliator of teak, caused considerable defoliation of *Chromolaena odorata* in Tamilnadu, Kerala and Karnataka (Ahmad, 1989).

1.3.3.2 Introduced insects

Adults of *Apion brunneonigrum* Beguin-bellecocq, a seed feeding weevil were introduced in 1982 and 1983 to Kerala from Trinidad, but its establishment has not been reported and necessitated its re-evaluation. Another insect *Pareuchaetes pseudoinsulata* Rego Barros was introduced from Trinidad by the Commonwealth Institute of Biological Control, Indian Station Banglore in 1970. In Kerala, the first consignment of about 250 caterpillars was sent in December 1981, from CHES, Chethalli. The second release was done in the premises of the College of Horticulture, Vellanikkara in 1982. Even after repeated consignment of the caterpillars, and their timely release, no sign of establishment was observed. After repeated release of the insect larvae there were clear symptoms of feeding and damage to *Chromolaena odorata* during the year 1985. By July 1985 *Chromolaena odorata* in about 0.5 ha area in the release site was severely defoliated showing the effectiveness of the caterpillar. But bacteria infected these caterpillars, as well as birds of various species were suspected to be predaceous on them. However, the gut contents of the birds showed no remnants of the caterpillars. Later on, the caterpillars spread further and the yellowing of the affected weed was conspicuous at the feeding locations and wild plants like *Ficus sp.*, *Mikania scandens*, *Centrocema pubescens*, *Ipomoea sp.*, *Clerodendron sp.* etc. were found growing in Chromolaena cleared patches. Even though there were further release at different locations in various parts of the state its establishment was failed (Singh, 1998). Climatic factors could be a factor for the low field population of the insect in Vellanikkara and non-establishment of it in many locations in Kerala (Joy *et al.*, 1993).

Reddy and Jacob (1998) reported the feeding behaviour of *Pareuchaetes pseudoinsulata* on the leaves of *Chromolaena odorata*. The minimum amount of leaf matter was consumed by

a larva of 15-17 days age, i.e., 1400-1700 sq. mm leaf area per day, and final star larva was the most efficient feeder. The larvae preferred older leaves to tender ones but did not feed on leaves that turned yellow, and within a leaf, > 70% of larvae started feeding from the lower half, i.e., towards the petiole. The total life cycle of *Pareuchaetes pseudoinsulata* took 52-55 days in Karnataka but Satheesan *et al.* (1987) reported 45 days in Kerala and 39-54 days at Bangalore (Muniappan *et al.*, 1988). The stem galling tephritid *Procecidochares utilis* was also reported to be effective against Chromolaena (Wilson and Widayanto, 1998).

1.4 Uses of Chromolaena

*Chromolaena odor*ata has been found to carry on associative nitrogen fixation with the help of free living nitrogen fixers both in the root surface and in its rhizosphere soil, enabling it to live luxuriantly in the poor soils (Ambika, 1998). As soil cover it reduces soil erosion and the high nutrient contents tells it a good green manure.

Table 2. Nutrient content of *C. odorata* in comparison with other green manures, %

Green manure	N	P_2O_5	K_2O
Pongamia sp	2.79	0.02	0.69
Glyricidia sp	2.80	0.02	1.50
Chromolaena odorata	2.65	0.03	1.90

(Chandrasekhar and Gajanana, 1998)

The leaf of Chromolaena contains oil, which has action of insect repellant. In addition the plant has certain ethno botanical uses also.

2. *Lantana camara* L.

2.1 Introduction and Distribution

This verbenaceous shrub was introduced into India from the neotropics at the beginning of the 19th century as an ornamental plant and noted as a dangerous, invasive weed since the turn of the century (Singh, 1976). This was introduced in India in the National Botanical Garden, Calcutta in 1809 as an ornamental plant because of its beautiful aromatic flowers. It spread soon to wastelands and pastures forming dense thickets.

Rao (1920) studied the distribution of the species in India in 1916. It is a very noxious weed in Coorg, Wynad, West Coast, Travancore, Cochin, Yercaud and Coimbatore, and found up to 1520 m above msl in the Nilgiris. It is well adapted to the tropical and sub tropical climatic conditions and to semi-arid as well as humid regions. The species is seen as a

serious invader and creates problems in forest management. It invades disturbed as well as undisturbed tropical rain forests, occurs along the roadsides, fences, wastelands, pastures and tropical tree plantations. Lantana is a dominant shrub of the dry deciduous forests located at altitudes between 650 m and 1200 m above msl. Disturbances in these forests by selective logging and firewood collection enhanced the colonization and dominance of the species, while it was absent in fallow lands located at higher altitudes (1800-1900 m above msl) (Chandrasekhara, 2001).

Lantana grows best in rich organic soils receiving constant rainfall or where the soil is moist throughout the year. It can tolerate poor soils and almost pure sand, but cannot tolerate waterlogged condition and high soil salinity. It has high efficiency for nutrient extraction and retranslocation of nutrients from senescing leaves enabling it to grow in nutrient poor sites. It also exhibits high foliar N concentration, even on nutrient poor sites. The species combines its nutrient conserving strategies with capacity for higher circulation and low retention of nutrients; rapid decomposition and faster nutrient turn over rate making it fit to expand rapidly (Rawat *et al.*, 1994).

Since World War II, in many areas in the western parts of the Western Ghats, Lanatna has been replaced by invasion of Chromolaena. But in the eastern parts, it is still common, where Chromolaena is rare (Muniappan and Virakthamath, 1993).

2.2 Biology of Lantana

It is a perennial, straggling shrub with prickly stems, spread by seeds but regrows vigorously after cutting. Thus Lantana can multiply through both vegetative and sexual means. Birds eat the seeds, which facilitates the rapid dispersal of the plant.

2.3 Competition with crops

It competes with young trees in forests and plantations thus not allowing them to grow thereby reduce productivity and interfere with harvesting. It may affect economic viability of crops such as coffee, oil palm, coconuts and cotton. The plant contains Lantadene A and as such Lantana has been implicated in the poisoning of a number of animals including cattle, buffalo, sheep and goats. Thus these animals tend to avoid this species and browse native plants, giving a competitive edge to Lantana over palatable native species (Murphy, 2001).

2.4 Management of Lantana

2.4.1 Physical/chemical

The plant can be pulled out, but is a cumbersome process. According to Erasmus *et al.* (1993), chemical control using Imazapyr at 2g/l as the initial treatment followed by Glyphosate at 180 g was an effective method of manual weed control. This can also be controlled by spraying 2,4-D @ 2.0 kg/ha or Glyphosate @ 2.0 – 4.0 kg/ha.

2.4.2 Biological

The seed fly *Ophiomyia lantanae* (Froggatt) was introduced from Hawaii in 1921 and evaluated in South India but it failed to give any visible weed suppression. The lace bug *Teleonemia scrupulosa* Stal imported from Australia in 1941 was effective in combating Lantana. The lace bug is a defoliator and its female starts egg laying in 24 hours of emergence. The eggs are usually inserted on the under side of tender leaves; 48-60 eggs are laid during the lifespan of 15-35 days. The eggs hatch in 4-6 days and 4-5 nymphal instars are completed in 12-22 days (Varma and Sadatullah, 1973).

2.5 Uses of Lantana

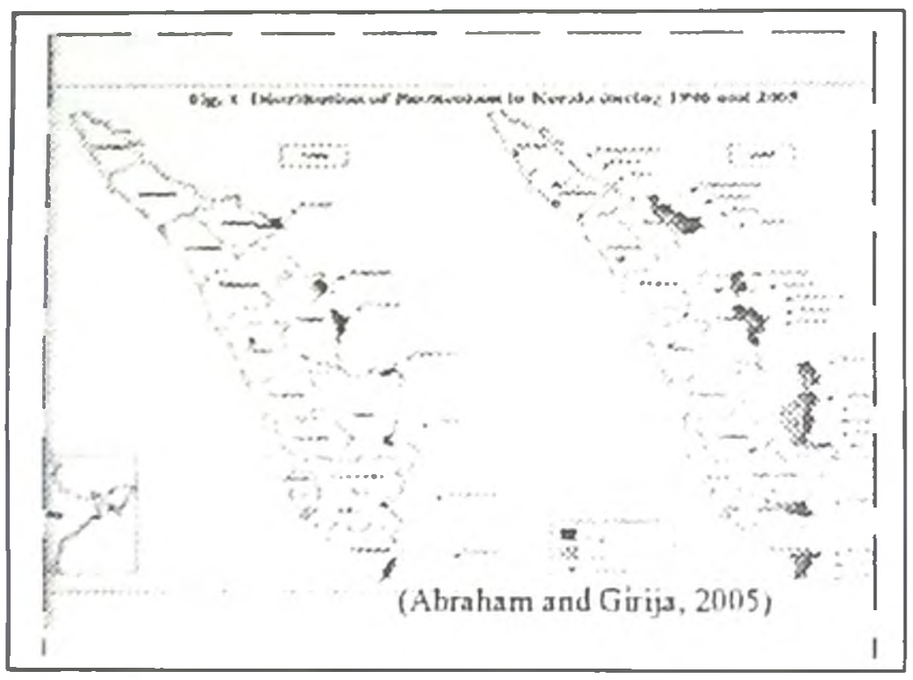
Lantana camara has several uses, mainly as an herbal medicine and in some areas as firewood and mulch. There has been much work conducted, especially in India, on the chemical constituents of lantana; extracts from the leaves exhibit antimicrobial, fungicidal, insecticidal and nematicidal activity.

3. *Parthenium hysterophorus* L.

3.1 Introduction and distribution

Parthenium hysterophorus L. also known as white top or congress grass is an herbaceous erect annual plant belonging to the family Asteraceae (Compositae). It is native to the area Gulf of Mexico including the West Indies, and Central South America. The plant is now widely distributed in India, Africa, China, Vietnam, Pacific Islands, and Australia. It is surmised that Parthenium possibly entered India from the USA through the imported food grains or through imported seed lots (Lonkar *et al.*, 1974). In India it was recorded in 1810 in Arunachal Pradesh and Nagaland and in Pune in 1955. According to Mahadevappa (1996) status of infestation by Parthenium in terms of its seriousness in different states of India is as follows: Uttar Pradesh, Karnataka, Maharashtra, Tamil Nadu, Andhra Pradesh, West Bengal, Madhya Pradesh, Gujarart, Orissa and Bihar. The Government of Karnataka has issued a notification on 23rd Oct. 1975, declaring Parthenium as noxious weed

(Sushilkumar and Saraswat, 2001). Due to the absence of effective natural enemies that keep it under check in its native home, combined with allelopathic effects of the weeds, Parthenium grows in pure stands in almost all the states, where climatic conditions are congenial for its growth, suppressing local vegetation and threatening the natural diversity. It has occupied fallow land along roadsides and railway tracks, pastures and is a serious health hazard to susceptible individuals and cattle (Singh, 2001). Although most of the infestation was restricted to open areas like roadsides and uncultivated wastelands, infestation also noticed in the fields of coconut, mango, banana, vegetables and ginger (Abraham and Girija, 2005).



(Abraham and Girija, 2005)

Infestation of this weed is very severe in the low rainfall regions of the country. However, in the high rainfall regions of the North Eastern states and Kerala in the south. it has not yet become a serious problem. A survey conducted in 1996 in Kerala revealed the presence of Parthenium at Parasala (Trivandrum), Aryankavu (Kollam), Kumily (Idukki) and Anakkatty (Palakkad) on the borders of Kerala with Tamilnadu and Karnataka, which have road connection between the states. However, wide spread infestation was noticed only in Chittur taluk of Palakkad district, a low rainfall area with alkaline soil and climatic condition similar to that of Tamilnadu, and where Parthenium was a major weed along road sides and cultivated areas. Later on a survey conducted in 2005 revealed wide spread infestation of Parthenium during the last decade. The spread was alarming in the districts of Idukki, Palakkad and Wynad, and the spread was come through the cow dung brought in from adjacent areas in Tamilnadu. In the other districts only localized infestation was noticed, especially in town areas and railway stations (Abraham and Girija, 2005).

3.2 Competition with crops

Parthenium dominates pastures and excludes useful forage plants as well as threatens the quality of crop produce. Mahadevappa *et al.* (2001) reported a yield reduction of 40% in agricultural crops and up to 90% in forage due to Parthenium infestation.

3.3 Management

3.3.1 Physical/Mechanical

Ever since the weed became a menace in India and other countries, efforts have been made to manage it by different methods. But so far, no single method has been proved satisfactorily. From the past experience, it is also clear that any single approach is not enough to manage Parthenium. Manual methods, being expensive cannot be employed on a large scale. Mechanical removal is possible to a certain extent and that too only in open fields. As there is regeneration from the cut portions of Parthenium, manual or mechanical methods, if adopted, should uproot the plant well before the onset of flowering.

3.3.2 Chemical

Parthenium can be controlled chemically by spraying pre-emergence herbicide such as Atrazine @ 1 kg/ha or post emergence herbicide Glyphosate @ 1-1.5% or Metribuzin @ 0.3-0.5% (Balyan *et al.*, 1997). Abraham *et al.* (1991) reported that Parthenium can be controlled by spraying NaCl @ 10 - 15 % solution.

3.3.3 Biological

3.3.3.1 Insects

A number of native natural enemies has been recorded on this weed but they do not suppress its population rather utilize it as alternate host (Kumar *et al.*, 1979). Considering the failure of indigenous natural enemies, in 1983, a chrysomelid beetle *Zygogramma bicolorata* was imported from Mexico. Adults and larvae of *Z. bicolorata* feed only on Parthenium leaves and it was declared to be safe to economic plants after testing (Jayanth and Nagarkatti, 1987). The eggs are laid singly or in small groups up to 5, mostly on the under surface of the leaves and hatch in 4-6 days. The early stage larvae feed on the terminal and auxiliary buds and on the leaf blades as they grow. The full-grown larvae enter the soil and pupate. The larval and papal periods under laboratory conditions lasted 14-16 days and 8-10 days, respectively. Females are capable of laying, on an average 800 eggs (Singh, 2001). Field activity of *Z. bicolorata* coincides with receipt of 1.5 mm rainfall. It may therefore, perform better in well-distributed heavy rainfall areas (Patnaik *et al.*, 1988). The beetle has already spread naturally in the entire Karnataka, Tamilnadu, Andhra Pradesh, Maharashtra and Kerala (Singh, 1997).

3.3.3.2 Microorganisms

In India, various pathogenic and nonpathogenic microorganisms are recorded on Parthenium and have been reported by many workers. Kauraw *et al.* (1997) reported the

occurrence of *Fusarium pallido-roseum*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Sclerotium rolfsii and Sclerotinia sclerotiorum* on Parthenium in India.

3.3.4 Competitive replacement

Parthenium can be managed through competitive replacement utilizing *Cassia uniflora* which reduced the Parthenium population to 59.3% of the control (Kandasamy and Sankaran, 1997). Agarkar (1999) recorded the competitiveness of *Xanthium strumarium*, *Tephrosea purpurea*, *Achyranthes aspera*, *Vitex negundo*, *Cassia spp.* and *Zinnia elegans* against Parthenium. Quick growing weeds like *Chromolaena odorata*, *Cassia tora*, *Cassia occidentalis*, *Lantana camara*, *Sida acuta* and *Hyptis suaveolens* that are more adapted to humid conditions, were more competitive than Parthenium which is known to prefer low rainfall areas. Hence the chances of Parthenium replacing the native flora and becoming a major weed in Kerala are limited (Abraham and Girija, 2005).

3.4 Allelopathic effects of Parthenium

The weed was noticed to cause allelopathic suppression of other species in the vicinity of its gregarious stands. Dutta *et al.* (1977) reported the identification by Patil *et al.* (1976) the allelogen as a sesquiterpene "parthenin". Besides parthenin, beta sitosterol, campestrol, stigmasterol, beta-D-glucosides of beta sitosterol, myricyl alcohol, hexacosanol, phenolic acid, an unidentified sterol and a saponin were isolated and identified. The allelogen was found to possess barley and oats at 150 ppm. In guar (*Cyamopsis tetragonoloba*) and egyptian clover (*Trifolium alexandrinum* L.) the germination was either inhibited or delayed at 350 ppm.

3.5 Uses of Parthenium

Parthenium has been well documented for its insecticidal, nematicidal and herbicidal properties besides oxalic acid and biogas production. The weed contained 4.8 per cent of KCl on dry weight basis and it is therefore a potential accumulator from the soil. Ashing of the weed for recovery of MOP appears to be an interesting possibility. In addition, it has multi uses as silage for feeding animals, papermaking, compost preparation etc. Furthermore the sesquiterpene "parthenin" exhibits anticancer & anti-tumor properties (Sushilkumar and Saraswat, 2001).

4. *Mikania micrantha* H.B.K.

4.1 Introduction and distribution

Mikania micrantha H.B.K., a fast growing perennial vine belonging to family Asteraceae is native to Central and South America (Barreto and Evans, 1995) and is commonly known as climbing hemp vine. Because of its quick growth and fast spreading nature, it is also called 'mile-a-minute'. In Asia, *Mikania micrantha* was first introduced to Malaysia, where it was tried as a cover crop in rubber because of its fast growth rate. But its higher competition with the crop than the leguminous cover crop was realized later on (Weng, 1964) and now it has become a major invader and suppressive weed of forestry and plantation crops, particularly in South East Asia (Waterhouse, 1994). In India, Mikania *micrantha* was introduced first to northeast regions during 1940s. It is believed that the American soldiers inducted this plant in this region during World War II for camouflaging purpose. In India, attention was first drawn to this weed by Choudhury (1972) who reported it as a threat to forestry and agriculture in the northeastern region, especially in Assam and later in Bengal. It has since spread to other regions and is a serious threat to both plantation and natural forests in the Western Ghats (Nair, 1988).

In Kerala, it was first reported by Nair (1968) from the Rubber Research Institute, Puthuppally, Kottayam. During the last four decades its spread was very fast and is densely distributed in the districts of Ernakulam, Kottayam, Thrissur, Alleppey, Idukki and Pathanamthitta with only localized infestation in other districts of the State (Abraham, 1999). This was further confirmed by Sankaran and Sreenivasan (2001) who surveyed the distribution of Mikania in the Kerala State and found that of the 163 sites surveyed 111 (68%) showed some level of infestation with Mikania and that the southern zone has maximum proportion of sites infested (82.5%) compared to central (75.5%) and northern zone (45.3%).

(Abraham, 1999)

4.2. Biology of Mikania

Mikania micrantha is a perennial climber with 5-10 cm long cordate leaves and producing subsidiary off-shoots to twine round objects of any kind, including 15-20 m tall trees, and spread from one bush to another in search of light (Choudhury, 1972). The top part of Mikania plant dies out every year but suckers on the ground or main stock may survive for years and the new suckers begin to grow by June-July. Shading has an adverse effect on vegetative growth, dry matter production, leaf area and biomass partitioning (Ipor, 1991). In Kerala flowering takes place from October onwards and fruiting from February to April (Sasikumar and Prakash, 1998). They also reported that the embryo of Mikania seed requires a resting period, as fresh seeds showed dormancy and which could be broken by treatments.

Mikania is a prolific seeder, average seed output per plant being 45812 with an average production of 357 inflorescence, each having 32 flowers. Seeds as well as stem cuttings can propagate the weed, and unburied seeds on the soil surface enhanced the germination where as a slight burying prevented germination (Abraham, 1999). Mikania propagates more profusely by runners than the seeds (Choudhury, 1972).

4.3. Competition with crops

Mikania is a problem weed in forests, plantations and agricultural fields where it smothers the crop reducing the availability of sunlight thereby adversely affecting the growth and yield of the crop and it has been reported as an important weed in coconut, cocoa, pineapple, mango, arecanut, teak and banana (Abraham, 1999), Eucalyptus, Bamboo and Reeds (Nair, 1988) and Acacia (Sankaran and Sreenivasan, 2001). The weed is also seen on road sides, climbing on the fence, hedges, electric stay wires and telephone posts. Its growth is more luxuriant in moist soils and thick perennial growth is noticed on the banks and borders of aquatic areas and canals. Suppression of crop growth due to competition from Mikania on pineapple, banana, rubber, coconut, cocoa, and teak plants as well as delayed flowering and reduced fruit weight in pineapple and banana were reported by Abraham (1999).

Table 3. Effect of competition from Mikania on various crops

Crop	Treatments	No. of days for flowering	Fruit fresh weight, kg
Pineapple	Mikania infestation	330	0.9
	Control	212	1.24
	CD (0.05)	42.8	0.225
Banana	Mikania infestation	229	5.84
	Control	193	8.38
	CD (0.05)	15.8	1.83

(Abraham, 1999)

4.4. Management of Mikania

Mikania could pose serious threat to the crops as well as forest plantations, if proper management is not done through any means. The frequent cultural practices and the thin canopy especially in young plantations, act as catalysts in promoting infestation in plantations (Sankaran and Sreenivasan, 2001).

4.4.1 Physical/Mechanical

Abraham (1999) tried the various methods to properly manage Mikania and found digging the field at monthly interval as an effective physical method of control. As Mikania can form new suckers from the old stock and as the physical method of control is expensive, physical control cannot form an important part of its management.

4.4.2 Chemical

Pre-emergence herbicides Diuron (1.5 kg/ha) and Oxyfluorfen (0.2 kg/ha) were found effective in prevention of germination and establishment of Mikania. Similarly post-emergence herbicides 2,4-D, Glyphosate, Gluphosinate ammonium and Paraquat also could control the weed (Abraham, 1999). Removal of Mikania manually followed by application of Glyphosate (0.5 – 1 kg/ha) on newly emerging shoots controlled the weed effectively and in non-cultivated areas spraying 2,4-D (0.75 kg/ha) was found effective (Gogoi, 2001).

4.4.3 Biological

4.4.3.1 Insects

Abraham (1999) in her study to find out strategies to control Mikania could identify eighteen insect pests and four fungal pathogens that were found attacking Mikania. The major pests included aphids, tea mosquito bug, thrips and lepidopteran pest *Spilosoma obliqua*. As all these were polyphagous in nature, their utility as biocontrol agent is limited.

Among the introduced insects *Liotrhips mikaniae*, though effective, was not fully successful (Ellison, 2001).

Table 4. Natural enemies of Mikania identified in Kerala

Insetc pests	**Family**	**Order**
Teranychus neocaledonicus	Tetranychidae	Acari
Eurommaticera vittata	Cerambicidae	Coleoptera
Apophylia viridis	Chrysomelidae	Coleoptera
Derectina collina	Chrysomelidae	Coleoptera
Diapromorpha turcica	Cerambicidae	Coleoptera
Hipsa armigera	Chrysomelidae	Coleoptera
Lacoptera quadrimaculata	Chrysomelidae	Coleoptera
Luperomorpha bombayensis	Chrysomelidae	Coleoptera
Myllocerus blandus	Curculionidae	Coleoptera
Aphis citricola	Aphididae	Hemeptera
Krishna strigicollis	Cercopidae	Hemeptera
Helopeltis theivora	Miridae	Heteroptera
Spodoptera litura	Noctuidae	Lepidoptera

(Abraham, 1999)

4.4.3.2 Microorganisms

The fungal pathogens infecting the weed were identified as *Colletotrichum glocosporioides*, *Alternaria alternata*, *Curvularia lunata var. aria* and *Corynespora cassicola*. The toxic metabolites isolated from Colletotrichum *glocosporioides* and *Alternaria alternata* produced necrotic symptoms on mikania leading to complete drying of leaf by four days. In addition to the above fungal pathogens Sankaran and Sreenivasan (2001) reported the pathogenicity of *Fusarium solani*, *Myrothecium roridum*, *Ascochyta sp.*, *Pestalotiopsis sp.*, and *Phoma sp.* on *Mikania micrantha*. *Puccinia spegazzinii*, the rust fungus has been found a very successful bioagent which is able to infect all vegetative parts of the plant causing necrosis, cankering and often leading to plant death (Ellison, 2001).

4.5 Uses of Mikania

4.5.1 As green manure

The possibility of using mikania leaves as green manure was studied by many workers. Alam *et al.* (1994) reported >1.8 per cent N, 0.22 per cent P, 0.22-0.35 per cent Ca and

>0.12 per cent Mg and a higher content of K in Mikania leaves. Similarly Abraham (1999) estimated the nutrient contents as 2.35 per cent N, 0.39 per cent P, 3.58 per cent K, 0.82 per cent Ca, 0.42 per cent Mg and traces of minor elements. Owing to its large biomass yield (2.25 kg fresh weight /plant) and fast growth rate (on an average 6.29 cm/day) combined with high nutrient content, its potential as a green manure cannot be neglected and its possibility in paddy cultivation has been reported by Saha (1986) and Abraham (1999).

4.5.2 As fodder

Sankaran and Sreenivasan (2001) reported the use of Mikania as fodder in certain parts of Kerala during summer season when availability of grass becomes limited. Estimating the chemical composition and fibre fraction of Mikania, Abraham (1999) studied its fodder value. It contained an average of 14.63 per cent crude protein, 2.36 per cent fat, 23.2 per cent crude fibre, 48.75 per cent nitrogen free extract, 8.92 per cent ash, 0.87 per cent Ca and 0.42 per cent P. The fibre fractions contained 42.42 per cent neutral detergent fibre, 33.22 per cent acid detergent fibre, 0.096 per cent cellulose, 9.2 per cent hemicellulose and 10.84 per cent lignin. The fodder value was compared with that of the traditional fodder crop, guinea grass.

Table 5. Comparison of fodder values of *M. micrantha* and guinea grass

Fodder quality attributes	*Mikania micrantha*	Guinea grass
Crude protein%	14.63	8.4
Crude fibre %	23.21	32.97
Total ash %	8.92	10.4
Calcium %	0.87	0.53
Dry matter (t/ha)	4.02-6.04	7.58 -11.51

(Abraham, 1999)

4.6 Allelopathic effects of Mikania

Mikania, when used as mulch or incorporation brought about an inhibitory effect on the growth of rubber but not on rice the growth of which was enhanced by the application, and the Mikania extract showed a clear allelopathic effect on germination and radicle length of cowpea (Abraham, 1999). Mikania contained growth-inhibiting substances like phenolic and flavanoid constituents, which depressed the growth and yield of rubber (Weng, 1964).

5. *Mimosa invisa* Martius ex. Colla

5.1 Introduction and distribution

Mimosa invisa Martius ex. Colla, commonly known as giant sensitive plant, is a native of Tropical America, especially Brazil (Waterhouse, 1994) and the first report on its introduction to India was to some coffee plantations in South India as a cover crop (Coffee Board, 1955). It is emerging as a new threat to natural forests, forest plantations and agricultural systems throughout India, especially in the North Eastern states (Rajkhowa *et al.*, 2003) and Kerala (Sankaran, 2001). The weed has even become a threat to the single horned rhinoceros in the world famous Kaziranga National Park in Assam, now declared a world heritage (Vattakkavan *et al.*, 2004).

In Kerala, *Mimosa invisa* locally called Anathottavady or Padayincha or vishamullu, was first reported in 1964 from Perunna near Changanachery in Kottayam district (Nair, 1964). Since then it started gradual spread and has become naturalized under Kerala conditions over the last four decades. Sankaran (2001) reported widespread incidence on the central and southern parts of Kerala. However, a survey conducted by Jayasree (2005) showed the spread of *Mimosa invisa* in all the fourteen districts of Kerala with very severe infestation in Kottayam, Pathanamthitta, Ernakulam, Thrissur and Palakkad districts. As per the survey the infestation was sparse to medium in higher altitude areas as in Idukki and Wynad districts as well as in the northern districts.

5.2 Biology

Mimosa invisa, belonging to the family Fabaceae and the subfamily Mimosoideae, is a fast growing, abundantly thorny, biennial or perennial shrub with angular branching stems that become woody with age (Waterhouse, 1994). However, Sreenivasan and Sanakran (2000) reported the weed as an annual generally, which will grow as a biennial when water is available throughout the year. Based on the presence or absence of spines on the stem and other plant parts there are two types of *Mimosa invisa*. They are *M. invisa* var. *invisa*, called spiny or thorny mimosa, which is armed with sharp downward pointed prickles with broad

bases arranged in 3-4 rows, and *M. invisa* var. *inermis*, called smooth mimosa, which is unarmed or spineless and instead, the stem is covered with soft pubescent hairs.

The main mode of propagation is by seeds and it can sprout vigorously from the cut base, soon after onset of monsoon (Sanakran, 2001). Further, Jayasree (2005) reported that the plant cannot be propagated by stem cuttings while the root clumps after digging could survive the summer months if soil moisture is available. Rajkhowa *et al.* (2003) reported the seed production ability of *M. invisa* var. *inermis* as ranged from 8,000 to 12,000 per sq. m, while Jayasree (2005) estimated it to range from 46200 to 127620 with an average of 72650 seeds per plant. The seeds are hardy and capable of remaining in soil for a long time (Muniyappan and Virakthamath, 1993). However, Jayasree (2005) reported a reduction in germination percentage or viability with time of storage. She also reported seed dormancy in *M. invisa* which could be broken by hot water treatment at 60^0 C for five minutes, at 80^0 C for two minutes, scarification with conc. sulphuric acid for up to 1.5 minutes or by flaming for 30 seconds, resulting in germination of the seed above 85 per cent.

5.3 Competition with crops

The weed was found to infest mainly the open spaces like roadsides, sides of railway tracks, and along the banks of irrigation and drainage channels. In severely infested areas the plant was found climbing on trees, stay wires, advertisement and location boards etc. Upon growing the plant smothers all the native vegetation including crops like banana, tapioca, jack tree, mango tree, coconut etc. and it was even found infesting the upland paddy fields.

5.4 Control Methods

5.4.1 Physical/ Mechanical

Although sickle weeding at monthly interval was found effective (Jayasree, 2005) manual weeding is difficult and labour intensive (Sanakran, 2001). In nursery beds and small compact areas where high value crops are raised, solarization for 40 days could effectively prevent the establishment of Mimosa seedlings (Jayasree, 2005).

5.4.2 Chemical

Trials to study the effect of herbicides on control of *M. invisa* showed that pre-emergence herbicides – Atrazine, Diuron, Fluchloralin, Metolachlor and Pendimethalin gave almost 100 per cent control, and Glyphosate was the most effective post emergence herbicide at 0.6 kg/ha (Jayasree, 2005). They also reported the ineffectiveness of 2,4-D in controlling the

weed even at high dose of 5 kg/ha. This was found to be due to the recovery of the growing tips from the epinasty caused by 2,4-D within a few days. However, Muniappan and Virakthamath (1993) reported that mechanical and chemical control methods were expensive and did not provide a long lasting effect.

5.4.3 Biological

5.4.3.1 Insects

Garcia (1982) listed 67 insects including *Psygida walkeri*, a lepidopteran moth as well as nymphs and adults of the coreid bug, *Scamurius sp.* attacking *M. invisa*. A Hemipteran psyllid defoliator seen in Brazil *Heteropsylla spinulosa* is an effective bioagent for the control of *M. invisa*, and is used in Papua New Guinea (Ablin, 1992). Jayasree (2005) could identify six species of insects belonging to five different families of the order Lepidoptera as pests of *M. invisa*. These were mainly flower bud or leaf feeders as shown in the following table.

Table 6. Nature and extent of damage caused by insect pests

Insect pests	Family	Nature of damage	Extent of damage
A. Flower bud feeders			
Euproctis scintillans Wlk	Lymantriidae	Larvae feed on flowers and flower buds	Moderate
Rapala sp.	Lycaenidae	Larvae feed on flowers and flower buds	Negligible
B. Defoliators			
Porthesia sp	Lymantriidae	Larvae feed on leaves in batches leaving behind the midrib	Moderate
Ericeia optature Wlk.	Noctuiidae	Larvae feed on the leaves and pupate in the soil	Negligible
Adoxophyes moderanata	Tortricidae	Larvae feed on the leaves	Negligible
Unidentified species	Pyraloidea	Larvae feed on the leaves	Negligible

(Jayasree, 2005)

5.4.3.2 Microorganisms

Among the pathogens *Corynespora casiicola*, a stem spot fungus which caused shedding of the leaflets and stem dieback, is found to kill *M. invisa* in Queensland, Papua New Guinea and Western Samoa (Ablin, 1992). *Fusarium pallidoroseum* isolated from *M. invisa* in the Philippines proved excellent control of *M. invisa* seedlings when applied as spray of crude

culture filtrate (Mabbayad and Watson, 2000). Jayasree (2005) noticed the small circular spots on the stem caused by *Alternaria sp.*

5.5 Uses of *Mimosa invisa*

5.5.1 For reclamation of degraded soils

Liu *et al.* (1999) studied the effect of intercropping Chinese fir with *M. invisa* as a method to control soil erosion, which was decreased by 17.76 to 52.25 per cent compared with controlled burned land. The growing of *M. invisa* also improved the soil physical properties and nutrient conditions and enhanced the growth of Chinese fir trees.

5.5.2 As green manure/ cover crop

The effectiveness of growing *M. invisa* as a green manure crop has been reported by many workers. Mohankumar (1996) reported that *M. invisa* grown as a green manure crop in coffee proved as an effective method to improve the soil fertility status and as a cover crop it considerably suppressed weed growth and prevented soil erosion. Thomas *et al.* (2001) found that growing *M. invisa* in the coconut basin could add 20.5 kg wet biomass and 134.8 g N per basin at 140 days growth. A higher N fixation efficiency of *M. invisa* was also observed by them, as evidenced by nodule biomass and acetylene reduction activity of nodulated roots. The very high drymatter accumulation of up to 3.63 kg/plant and the ability to decompose sixty per cent of the litter within the first fortnight of incorporation in soil along with high content of N (3.85%) and exceptionally high content of Zn (90-100 ppm) combined with N fixing capacity upholds the plant as a green manure crop (Jayasree, 2005).

The plant can be used as a substrate for vermicomposting as well. The earthworm sp. *Eisenia foetida* could effectively compost *M. invisa* and banana pseudostem (1:1) within sixty days, thus enriching the content of primary and secondary nutrients in the substrate (Jayasree, 2005).

5.5.3 Applications of inhibitory effects of Mimosine

Lalande (1996) reported that the plant amino acid mimosine, found in *Mimosa sp.* and *Leucaena sp.* reversibly blocks the mammalian cell cycle, which can be used in treatment of cancer or for synchronizing various cell lines. Largo *et al.* (1997) reported that the chloroform extract of air dried leaves of *M. invisa* showed anti-tumor and anticancer potential. Jayasree (2005) could observe the highest mimosine content of 10.4 per cent in the immature leaves of *M. invisa* at active vegetative stage.

5.6 Harmful effects

5.6.1 Biodiversity of native flora

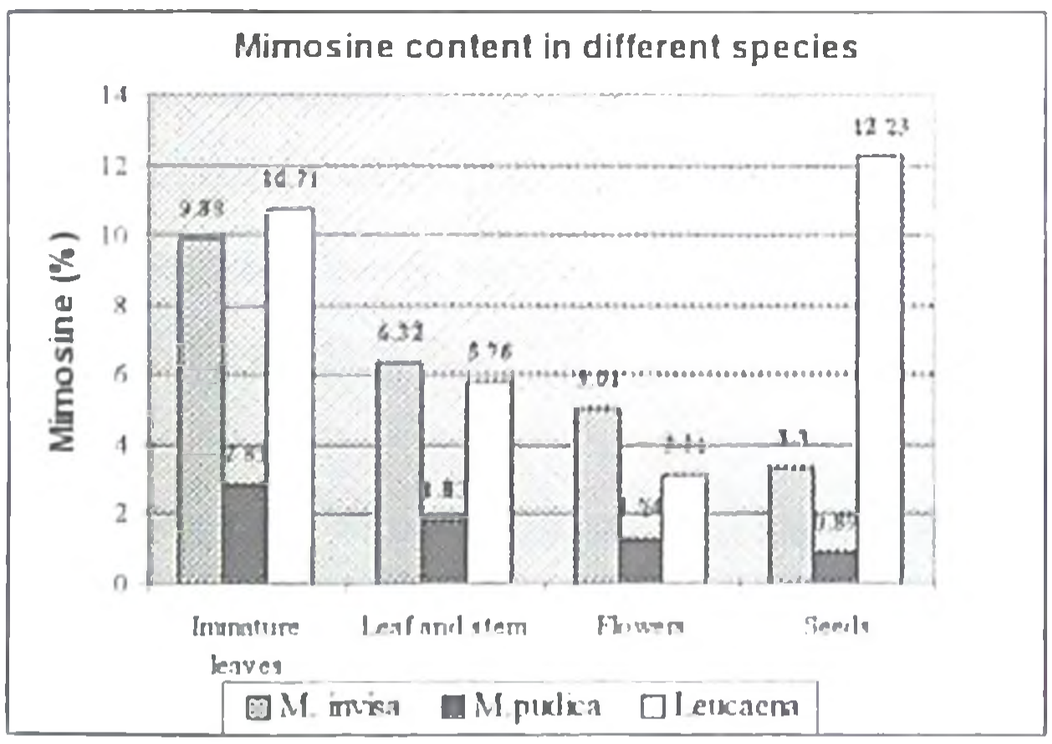
M. invisa has a smothering effect on the native flora of the infested area and a progressive influence on reducing the biodiversity of the area over the years of infestation. Jayasree (2005) observed that the smothering efficiency of *M. invisa* increased from 14.08 to 38.55 per cent within a period of three years of infestation. The heliophytic nature of *M. invisa* helped it to have an uppermost layer of canopy in any type of vegetation it is competing (Vitousek *et al.*, 1996) and this smothering effect was more severe on the grasses, which are heliophytic, but are not tall enough to compete with *M. invisa* (Jayasree, 2005). Sankaran (2001) reported that *M. invisa* is found to over grow and exceed *Mikania micrantha* in forest plantations.

5.6.2 Allelopathic effects

Application of *M. invisa* plant as mulch or water extract had noticeable allelopathic effects on the germination of rice and cowpea and the reason was attributed to be the presence of miomosine, an allelochemical (Jayasree, 2005).

5.6.3 Mimosine toxicity

The toxic amino acid, mimosine, present in the plant is an anti-nutritional factor contributing to clinical cases of toxicity in livestock. Jayasree *et al.* (2006) reported a higher content of mimosine in *M. invisa* (9.22%) compared to that in *Mimosa pudica* (2.85%). Many workers in different animals reported the cases of mimosine toxicity due to feeding on *M. invisa* plant. Rajan *et al.* (1986) from Kerala reported the nature of toxin, mimosine on animal tissues, during their studies in calves. The study revealed that the toxic principle in *M. invisa* caused vascular endothelial damage, nephrosis, necrosis of heart and liver and anaemia. Alex *et al.* (1991) reported a clinical case of mimosine poisoning due to ingestion of *M. invisa* in two years old heifer in Kerala. Intake of mimosine through *M. invisa* caused nephrotoxic and hepatotoxic symptoms in rabbits and the affected animals developed alopecia (hair fall) and moderate diarrhoea (Jayasree, 2005). Ensiling *M. invisa* infested pasture/fodder grass is an effective method to reduce the



mimosine content of the mixture before feeding to livestock as ensiling the *M. invisa* admixed with fodder grass in different proportions ranging from 10 to 90% for 60 days lowered the mimosine content by 32 to 46 per cent (Jayasree *et al.*, 2006).

6. *Mimosa pudica*

Mimosa pudica, native of South America is a prickly herb of the family Mimosoideae with sensitive leaves and hence the name sensitive plant. It is also known by the name Touch-me-not. The plant is deep rooted and thorny. It is commonly seen in wastelands, bands of arable lands, river banks and disturbed forests. The plant was not observed in eucalypt plantation, evergreen forest, shola and wattle plantation (Mahajan and Azeez, 2001). *Mimosa pudica* was not considered as a serious weed but recently it has become problem in plantations such as cashew, coconut, coffee, teak etc. as well as in orchards. The thorny nature of the weed makes it a troublemaker while carrying out cultural operations as well as crop harvest as is noticed during the collection of fallen nuts in cashew. Application of Paraquat gives temporary drying while Glyphosate gives a complete control, and 2, 4-D has been found less effective as it causes only slight yellowing.

Conclusion

The only way to deal with the problem of biological invasion is designing effective Natural Resource Management strategies to enhance biodiversity in the landscape, to keep bio-invasion under check. There is an urgent need to develop an Integrated Weed Management programme involving various stakeholders, as there is excellent opportunity to protect the non-invaded ecosystems.

To prevent entry of new alien species the quarantine should be strengthened. Early detection and eradication of small invasions and prevention of new invasive species would be the most cost effective approach to manage alien weeds. The invasion of *Merremia vitifolia*, an alien species belonging to Convolvulaceae family that has just started spreading in Thrissur district and nearby areas, has to be prevented immediately.

In addition, although alien species are considered as unwanted plants or nuisance, studies have to be undertaken in detail to understand the medicinal, industrial and commercial potential so as to exploit them for the benefit of human kind.

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DISCUSSION

1. Can two alien weeds coexist? What is the impact of one alien weed over the other?

   ☑ Alien weeds can exist together. But what we have observed in nature is the domination of the most competitive species over the other resulting in the suppression of the dominated species. *Merremia vitifolia* and *Mikania micrantha* can be seen existing together but *Merrimia* is more heliophytic and hence takes the dominance. Muniappan and Virakthamath (1993) have reported the replacement of *Lantana camara* by the invasion of *Chromolaena odorata* in the western parts of the Western Ghats.

2. Why Parthenium is called "congress grass"?

   ☑ The flower of Parthenium plant is white in colour and so it is known as "white top" or *safed topi* in Hindi. Traditionally Congressmen wear white cap. And hence Parthenium came to be known as "congress grass", though it is not a grass rather a dicot plant.

3. What is solarization and how does it bring about control of *Mimosa invisa*?

   ☑ Solarization is the technique of covering the soil with transparent polyethylene to trap the radiations reflected back from soil resulting in raising the soil temperature, which would be lethal to the soil inhabiting pathogens, nematodes and weed seeds. Solarization can be practised as a method to prevent germination of seeds of *Mimosa invisa* in nursery beds as well as small compact areas where high value crops are planned thereby reducing the weed problem. On a larger scale it may be neither feasible nor economic.

4. By which is invasion more, alien weeds or native weeds?

   ☑ Native weeds undergo destruction by their natural enemies and hence their invasion is restricted. While in the case of alien weeds, as their natural enemies are not occurred in the introduced locality the invasion by them is very fast.

5. As the "Parthenin" content in Parthenium plant is of much use in medical as well as other fields, is there any chance for Parthenium being cultivated?

- ☑ Parthenin, the major sesquiterpene present in the Parthenium plant is reported to exhibit anticancer and anti-tumour properties, as well as many other applications. As such it would be far fetched to assume 'cultivation of Parthenium' as suggested by Yaduraju *et al.* (2005) in the 2nd International Conference on Parthenium Management.

6. Can all the pathogens mentioned be considered as bioagent against theses weeds?
   - ☑ Although these pathogens have infected the weeds and caused disease symptoms none of them has shown the potential as a bioagent for the control of any of the weeds mentioned.

7. Why Lantana is not seen in cultivated fields?
   - ☑ Lantana usually grow and invade in non cultivated or undisturbed soils and not commonly seen in cultivated fields. Generally perennial weeds prefer undisturbed areas while annual weeds are more in cultivated fields. Upon cultivation the vegetative propagules of the perennial weeds are lost and hence they cannot establish there.

8. Is there any activity being conducted at KAU or government level for prevention and management of Parthenium?
   - ☑ Under the AICRP on Weed Control a week long Parthenium campaign is being conducted annually from 2004 onwards so as to make awareness among the public about the importance of Parthenium management.

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KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara, Thrissur

Agron. 751 - Seminar

Alien weeds in the terrestrial ecosystem of Kerala

Student: Musthafa Kunnathadi (2006-21-104)
Time: 9.15 am, 05.05.2007
Venue: Audio Visual Laboratory
Dept. of Pomology & Floriculture

Abstract

"Alien" can refer to species brought in from other continents, regions, ecosystems and even other habitats. A large number of weeds have entered India through the introduction of economically important plants as well as due to increased trade and travel (Murphy, 2001). Most of these are native to the Neotropics. The similarity between the humid tropics of India and the Neotropics, absence of natural enemies as well as high reproduction and dispersal rates cause their successful invasion and colonization in our country, and this bioinvasion has reduced the density and diversity of native species (Mahajan and Azeez, 2001).

Terrestrial alien invasive weeds found in Kerala include *Lantana camara, Chromolaena odorata, Parthenium hysterophorus, Mikania micrantha, Mimosa invisa and Mimosa pudica*. They have been found to infest plantation crops, forests, crop fields and waste lands and reduce the crop productivity and the biodiversity. Detailed studies on *Mimosa invisa* by Jayasree (2005) and *Mikania micrantha* by Abraham (1999) have revealed their heavy infestation in the southern districts of Kerala. Recently Abraham and Girija (2005) reported an alarming spread of *Parthenium hysterophorus* along the borders of the state with Tamilnadu and Karnataka. Although several methods have been attempted from time to time to control the alien weeds, no single method has been found effective on a sustainable basis.

Several of the alien weeds are known to be allelopathic. Although the fast growing and invasive nature of many alien weeds holds promise for their exploitation as green manure, fodder etc., their negative qualities far outweigh their benefits. Proper management is needed to contain the spread of these weeds to the non-infested areas, and the quarantine at entry points is to be strengthened to prevent entry of new weeds from other countries.

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HERBICIDES OF NATURAL ORIGIN

BY

Musthafa Kunnathadi

(2006-21-104)

Department of Agronomy

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

Agron. 752 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

I hereby declare that the seminar report entitled **"Herbicides of Natural Origin"** is a record of the seminar presented by me during the course (06.07.2007) and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
08.08.2007

Musthafa Kunnathadi
(2006-21-104)

CERTIFICATE

Certified that the seminar report entitled **"Herbicides of Natural Origin"** is a record of seminar presented by **Sri. Musthafa Kunnathadi (2006-21-104)** under my guidance and that this report has been prepared by him independently.

Vellanikkara
08.08.2007

Dr. C. T. Abraham
Major Advisor

CONTENTS

1. Introduction

Among the chemical pesticides, insecticides are used to the extent of 52 per cent in India followed by fungicides (30%), herbicides (16%) and other chemicals (2%). In most of the western countries, share of herbicides is the highest. The world average for herbicide use is about 45 per cent followed by insecticides (36%), fungicides (17%) and other chemicals (2%) (Wahab, 2003). Global herbicides sale in 2002 had reached US $ 28 billion. Indiscriminate use of herbicides has resulted in serious ecological and environmental problems as toxicological effects on the environment and living organisms including human beings, development of herbicide resistant weed biotypes, shift in weed flora, residues in crop produces, and persistence in soil and water bodies. In order to overcome these problems we have to reduce the reliance on synthetic herbicides and shift to integrated weed management, a component of sustainable agriculture and also we have to search for natural chemicals that are effective, eco-friendly, possessing novel mode of action and capable of replacing the existing herbicides (Narwal *et al.*, 2004).

Sustainable agriculture aims at long-term maintenance of natural resources and agricultural productivity with minimal adverse impact on the environment. It emphasizes optimal crop production with minimal external inputs, reducing dependence on commercial inputs (fertilizers and pesticides) and substituting them with internal resources and relying on sustainable practices, which may maintain the productivity over long periods (Narwal, 1994). Exploitation can be made of the herbicidal potential of microorganisms as well as of the compounds or secondary products from plants and microorganisms for an eco-friendly and environmentally safe weed management. This seminar report discusses the various herbicides of that kind.

2. Herbicides of natural origin

The microorganisms, microbial toxins, allelochemicals and synthetic derivatives based on the chemistry of these natural compounds, all of which show capability to destroy the weed species or to suppress their germination, growth etc. are included under the herbicides **of natural origin** from nature.

2.1 Microorganisms as herbicides

Microorganisms are being utilized for the control of weeds, and are called bioherbicides. The microorganisms that can control the weed species belong to fungi, bacteria and viruses. Among these, fungi have been found to be highly efficient and the most successful cases of weed control have been obtained from the fungi. Thus the bioherbicides are commonly known as Mycoherbicides. The concept of mycoherbicide was first introduced by Daniel *et al.* (1973), who demonstrated that an endemic pathogen might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particularly susceptible stage for weed growth. The use of the pathogen in a "product form" and an "application technique similar to the chemical tactic" are salient features distinguishing the mycoherbicide from classical agents.

2.1.1 Important bioherbicides developed are:

2.1.1.1 DeVine® : This is the first registered mycoherbicide (Kenney, 1986), and the registration was granted in 1981. The mycoherbicidal product consists of a liquid concentrate of chlamydospores of a pathotype of *Phytophthora palmivora* native to Florida. It is capable of killing seedlings and adult stranglervine (*Morrenia odorata*) , a weed of South American origin that is troublesome in Florida's citrus groves. De Vine is applied as a postemergence, directed spray. More than 90 per cent control is obtained, and control lasts two years after initial application (Charudattan, 1991). DeVine® was developed by Abott Laboratories, North Chicago. The material has a shelf life of only about six weeks and must be handled like fresh milk and the marketing area is small enough to make the refrigerated distribution possible (Ridings *et al.*, 1975).

2.1.1.2 Collego® : *Colletotrichum glocosporioides* f. sp. *aeschynomene*, an anthracnose-inciting pathogen on northern jointvetch (*Aeschynomene virginica*), was the first fungus to be evaluated as a mycoherbicide. The commercial product, a wettable powder formulation of dried spores produced by liquid fermentation, was registered in 1982 (by the Upjohn Company). Collego® is applied postemergence, and is capable of killing seedlings as well as mature northern joint vetch, a hard-seeded, leguminous weed in Arkansas and Lousiana rice and soybean fields. It has provided consistently high levels of weed control, typically above 85 per cent.

2.1.1.3 CASST: *Alternaria cassiae*, a foliar blight-inducing pathogen, was discovered in Mississippi and shown by Walker *et al.* (1985) to be a safe and efficacious mycoherbicide for sicklepod (*Cassia obtusifolia*) in soybean and peanut. CASST was developed as an experimental formulation by the Mycogen Corporation (San Diego).

2.1.1.4 Dr. Biosedge: The rust fungus *Puccinia canaliculata* has potential for controlling yellow nutsedge (*Cyperus esculentus*) , which could reduce the plant population by 46 per cent and tuber formation by 66 per cent and completely inhibited flowering. The fungus dehydrates the plant and kills it (Pathak *et al.*, 1987).

2.1.1.5 BioMal®: This mycoherbicide contains the spores of *Colletotrichum gloeosporioides* f.sp. *malvae* and has been found effective against round-leaved mallow (*Malva pusilla*). An experimental formulation of BioMal provided over 90 per cent control of this weed in the field. The wettable powder formulation of this hydrophilic fungus disperses easily in water and is applied as a spray (Boyette *et al.* 1991).

2.1.1.6 ABG-5003: The fungal pathogen *Cercospora rodmanii*, discovered in 1973 in Florida, induced leaf spots, leaf necrosis and root rot on water hyacinth (*Eichhornia crassipes*). ABG-5003 was an experimental formulation in the form of wettable powder containing mycelial fragments and spores. Because of the technical difficulties in assuring efficacy of the microbial herbicide, say restrictive environmental requirements and economic uncertainties of the market place the formulation was not registered (Charudattan, 1991).

2.1.1.7 Velgo: This contains *Colletotrichum coccodes* for the control of velvetleaf (*Abutilon theophrasti*) in corn and soybean (Wymore and Watson, 1989). As the fungus requires restrictive environmental conditions and as it has alternate host causing black dot disease in potato, its herbicidal potential could not be exploited fully.

2.1.1.8 LUBOA II: *Colletotrichum gloeosporioides* f. sp. *cuscutae*, under the trade name LUBOA II, is used against dodders (*Cuscuta* spp.) in China.

Several other candidates showing herbicidal property have undergone extensive testing for the commercial development, for example, *Bipolaris sorghicola* for johnsongrass

(*Sorghum halepens*) (Winder and Van Dyke, 1989). *Fusarium lateritium* has been identified as the first pathogen to show biocontrol over more than one weed species, i.e., against velvet leaf (*Abutilon theophrasti)* and prickly sida (*Sida spinosa*) (Boyette and Walker, 1985). The culture of *Gliocladium virens* can be used as a pre-emergence, soil applied broad spectrum mycoherbicide, which on soil incorporation produces a toxin – Viridiol. The production of Viridiol begins on 3rd day and peaked at 5-6 days after incorporation, and causes severe necrosis of germinating seed radicles with subsequent death of seedling (Jones *et al.*, 1988).

2.1.2 Limitations of Mycoherbicides

- Restricted commercial potential of mycoherbicides. This is because of the technological problems in mass culturing, formulations, shelf life, delivery systems etc., and low economic viability as compared to the chemical herbicides.
- Dew period and temperature regime for disease development – Although the optimum duration of free moisture and the temperature-moisture relationships necessary for infection and disease development can be easily identified, it is often difficult to create these conditions on a field scale.
- Incompatibility with chemicals – In situations where mycoherbicides are to be used in combination with chemical pesticides, viability and efficacy of the biological agents may be adversely affected by the chemicals. A careful sequencing of fungicide and mycoherbicide applications has to be done through researches.

- Multiple applications may be necessary to get effective control - Limited host range is a constraint in the popularity of mycoherbicides. Probably the simplest method to overcome this limitation is to apply a mixture of pathogens, i.e. consortia of pathogenic organisms to mixed weed populations. For example, the rice weeds northern jointvetch and winged water primerose, *Jussiaea decurrens*, can be simultaneously controlled with a single application of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and *Colletotrichum gloeosporioides* f. sp. *jussiaea*, and a mixture of these two pathogens along with *C. malvarum* effectively controlled northern jointvetch, winged water primerose and prickly sida (TeBeest and Templeton, 1985).

2.2 Herbicides from nature?

Nature is full of bioactive materials and compounds with unexploited properties. Many of the hundreds of thousands of secondary products generated by plants, microbes, and animals are the results of co-evolution of the producing organism with pests. Thus, the compounds have biological activity. Sometimes, the function of the compound in nature is as a phytotoxin, as with phytotoxins produced by plant pathogens or allelochemicals produced by allelopathic plants. In short, biological activity is more certain with secondary compounds from nature than with randomly synthesized compounds.

Because of worldwide growing concern about the environmental effects of selected pesticides, the need for the use of easily degradable pesticides with good selectivity is greater than ever. Recent advances in microbial and plant biochemistry have stimulated scientific interest into the possible role of secondary plant products and microbial toxins as natural pesticides. Furthermore, advances in plant cell culture, fermentation technology, molecular genetics and genetic engineering make it now possible to exploit biotechnologically plants and microorganisms as potential sources of naturally occurring chemical compounds that could be developed as herbicides.

The advantages of using natural compounds as herbicides or as the chemical basis for the development of new herbicides are:

- The wide array of phytotoxic compounds produced by plants provides many complex chemical structures that are unlikely to be discovered in the traditional synthetic strategies used by pesticide companies.
- Degradation of natural compounds in the environment proceeds faster than that of synthetic compounds and thus reduces the environmental pollution, ground water contamination etc.
- The natural compounds pose little health hazards and therefore, are environmentally safe.
- Purified natural compounds have longer shelf-life, wide range of storage conditions, broader environmental range for application, less storage space required and greater ease of application.

Extensive research during the last few decades has demonstrated that several plant secondary metabolites (allelochemicals) as well as fungal and other microbial toxins

possess good herbicidal activity. At present, two major areas of research appear attractive because of their potential commercial applications. They include (A) isolation and characterization of microbial toxins or secondary plant metabolites that could be used effectively as herbicides, and (B) evaluation of plant secondary metabolites and microbial toxins with novel chemistries which could be used as leads for the chemical synthesis of new herbicides.

2.2.1 Microbial toxins and secondary plant products

In this approach biologically derived chemicals are used as herbicides and are based on fermenting bacteria and fungi, testing fermentation broths for activity, and isolating active compounds from these broths. Plant cell cultures could be used in a similar fashion. The procedures are tedious and long-term operations. Japan is considered to be the most advanced country in the development of microbial pesticides and pharmaceuticals. Pesticides of microbial origin, commonly referred to as "agricultural antibiotics", are highly specific for target organisms and supposed to be inherently biodegradable because they are synthesized biologically. In addition, purified natural compounds appear to have many practical advantages over bioherbicides as weed control agents such as longer shelf life, wider range of storage conditions, broader environmental window for application, lower storage space requirements, and greater ease of application (Hatzios, 1987).

2.2.1.1 Phytotoxins from Microorganisms

2.2.1.1.1 Products of plant pathogens

Plant pathogens produce a myriad of phytotoxins that are apparently useful in weakening the plant in the infection process. Most of these compounds are not host selective, even though the producing pathogen might infect only one or a limited number of host species. However, some of them are reported to be highly active against only one species or even certain genotypes of that species. Unless, the host is a huge weed problem, there is little interest in herbicides that are extremely selective. AAL-toxin has been claimed to be a host-selective phytotoxin, affecting only certain tomato varieties yet it is one of the most generally phytotoxic natural products evaluated for herbicidal activity (Abbas *et al.*, 1995). Other somewhat host-selective phytotoxins for weeds that have been proposed but not adequately tested include pyrenophorol for wild oat (*Avena fatua* L.) control and maculosin for spotted knapweed (Duke *et al.*, 2002).

2.2.1.1.2 Other Microbial Compounds

2.2.1.1.2.1 Toxins from Fungi/actinomycetes

Some of the more potent natural phytotoxins have come from nonpathogenic microbes. The actinomycete-produced compound, actinonin (also known as butanediamide), is a potent inhibitor of peptide deformylase (Chen *et al.*, 2000), an important enzyme of bacteria and chloroplasts (Dirk *et al.*, 2001). Peptide deformylase is necessary for the post transitional processing of some chloroplast genome-encoded proteins. This is a site of action unique to plants and bacteria that has potential for the development of a new herbicide class. Although it is not extremely active on whole plants, it is highly active (I_{50} < 100 nM) on one out of two chloroplast peptide deformylases (Dirk *et al.*, 2001).

Table 1. Microbial Phytotoxins with promising herbicidal activity

Name of the toxin	Name of the organism
Anisomycin	*Streptomyces* sp.
Bialaphos	*Streptomyces hygroscopicus* *Streptomyces viridochromogenes*
Herbicidins	*Streptomyces saganonensis*
Cytochalasins	*Phomopsis* sp.
Cercosporin	*Cercospora* sp. *Pseudocercosporella capsella*
Phosalacine	*Kitasatosporia phosalacinea*
Tentoxin	*Alternaria alternata*
Mevinolin	*Aspergillus terreus*
Moniliformin	*Fusarium moniliforme*
Patulin	*Penicillium* sp.
Phaseolinone	*Xylaria* sp. *Macrophomina phaseolina*
Stemphyloxin I	*Stemphylium botryosum*
Toyocamycin	*Strephtomyces toyocanensis*
Viridiol	*Gliocladium virens*
Ziniol	*Alternaria carthami*
Thizobiotoxine	*Rhizobium japonicum*
Tabtoxin	*Pseudomonas tabaci*
Cyanobacterin	*Scytonema hofmanni*

i) Anisomycin: It is the first commercial herbicide produced from *Streptomyces* sp. 638 and is strongly phytotoxic to *Echinochloa crusgalli* and *Digitaria* spp.

ii) Bialaphos and phosphinothricin: Both are *Streptomyces* spp. products, and are successful herbicides. Bialaphos is a microbial product isolated from the fermentation broth of *Streptomyces hygroscopicus* and *S. viridochromogenes* and exhibits strong herbicidal activity against a wide spectrum of grass and broad leaf weeds following application to their foliage. Bialaphos exhibited very little phytotoxicity when applied in soil, and this is believed to be due to its remarkable biodegradability by soil microorganisms and its half life is only 18-20 days (Hatzios, 1987).

Bialaphos is currently marketed as a commercially developed herbicide in Japan under the trade name Herbiaceae®. Utilization of blocked mutants and of biosynthetic intermediates was instrumental in the elucidation of the biosynthetic pathway used by *Streptomyces hygroscopicus* to synthesize Bialaphos (Hatzios, 1987).

Mode of action: Bialaphos produced by *Streptomyces hygroscopicus* is a tripeptide consisting of two L- alanine residues and an analogue of glutamic acid called phosphinothricin (PPT). It is a proherbicide, requiring metabolic conversion to phosphinothricin in the target plant for herbicidal activity. In sensitive plants, bialaphos is metabolized to phosphinothricin {L-2 amino-4-[hydroxyl)-(methyl) phosphinoyl]-butyric acid}, which inhibits the enzyme glutamine synthetase involved in the assimilation of ammonia and glutamine synthesis in plants, and hence functions as a potent herbicide. Treatment of plants with phosphinothricin causes ammonia to accumulate at levels exceeding those known to uncouple photophosphorylation and as a result CO_2 assimilation is greatly reduced (Hatzios, 1987)

iii) Hydantocidin: Is a *S. hygroscopicus* product with good herbicidal activity (Duke *et al.*, 2002). It is a proherbicide that must be phosphorylated by the target plant to be an inhibitor of adenylosuccinate synthetase. Hydantocidin is a nonselective herbicide of at least the same potency as the commercial products glyphosate and bialaphos. With regard to its mode of action hydantocidin is converted within the plant to hydantocidin-5'-phosphate, the actual herbicide which efficiently inhibits adenylosuccinate synthase. Many

analogues have been patented, but none has been marketed. Its nucleic acid synthesis site of action may be a cause for toxicological concern.

iv) Pyridazocidin: Is a weak phytotoxin from *Streptomyces* sp., but it is the only natural phytotoxin known to act by the same mechanism as paraquat, by accepting electrons from Photosystem I and transferring them to molecular oxygen to generate a superoxide radical (Duke *et al.*, 2002).

v) Herboxidiene: Isolated from a culture of *Streptomyces chromofuscus* A7847 by Monsanto researchers, who reported the remarkable herbicidal potency of this metabolite. At 70 g/ha it fully controlled several weed species without affecting wheat and soybean. The herboxidiene induces apoptosis in the G2 phase of the cell cycle.

Table2. Toxins from other species of *Streptomyces* spp.

Toxin	Organism	Action/ crop
AT-265	*S. albus*	Potent post emergent
Homoalanosine	*S. galilaeus*	Broad spectrum in paddy
Vulgamycin	*Streptomyces* sp.	In cotton, barley or maize

vi) Phosalcine: Is another microbial compound containing phosphinothricin and its herbicidal behaviour is similar to that of bialaphos.

vii) Tentoxin: Is a cyclic tetrapeptide produced by *Alternaria alternata* and causes marked chlorosis in many grass and broadleaved weed species. Several crops such as corn (*Zea mays* L.) and soybean are tolerant to this toxin. It kills virtually all major weeds in soybean and is the only toxin known to kill johnson grass in maize (Duke *et al.* 2002). However, in spite of its clear-cut crop selectivity and excellent activity, tentoxin has not been developed commercially as a herbicide owing to difficulties in its synthesis (Narwal, 1994).

viii) Moniliformin: This is a potent phytotoxin produced by *Fusarium moniliforme*, which inhibits the growth and causes chlorosis and necrosis in several weed species. The analogue of moniliformin, CGA 49445 shows better activity.

'Sud 96', an isolate from *Fusarium solani* inhibited germination of striga seeds. This inhibition was brought about by an inhibition of ACC oxidation and ethylene action which are otherwise needed for the germination of striga. Chromatographic separation of 'Sud

96' indicated several compounds with inhibitory activity. In addition to this, metabolites - Solaniol, Javanicin and Fusaric acid from *Fusarium* spp. showed herbicidal activity on striga (Ahmed *et al.*, 2001).

Table 3. Effect of *Fusarium* metabolites on striga germination

Concentration, µg ml^{-1}	Germination, %		
	Javanicin	**Fusaric acid**	**Solaniol**
100	45^{d}	42de	10^{f}
50	56^{cd}	69^{b}	33^{e}
25	45^{d}	64^{bc}	52^{cd}
10	76^{ab}	73^{ab}	78^{ab}
1	75^{ab}	71^{ab}	81^{a}
0.1	77^{ab}	75^{ab}	79^{a}

(Ahmed *et al.*, 2001)

Although all the three metabolites inhibited the germinationof striga seeds. Sloaniol was found to be the most effective one.

ix) Cornexistin and hydrocornexistin: From *Paecilomyces variotii*, are very phytotoxic compounds and have been patented as herbicides (Fields *et al.*, 1996). Cornexistin has a unique molecular target site, aspartate amino transferase. The two compounds have different selectivity on crops and weeds (Duke *et al.*, 2002).

x) Phomalactone: Causes rapid electrolyte leakage of plant plasma membranes, leading to plant death, and resormycin and nigrosporins are phytotoxins with activity against both grasses and broadleaf weeds.

xi) Phosphonothrixin: Isolated from a culture of *Saccharothrix* sp., induces chlorosis in a nonselective way when applied to plant leaves, but the mode of action is not yet known. Several mono- and dicotyledonous weeds are controlled at an application rate of 500 g/ha.

2.2.1.1.2.2 Bacterial Toxins

i) Monic Acid Derivatives: In 1993 researchers from *Zeneca* reported on the herbicidal activity of monic acid derivatives. Pseudomonic acid A, a bactericidal metabolite of *Pseudomonas fluorescens* was hydrolysed to monic acid which subsequently was reesterified to the derivative. In the greenhouse and in the field it was proved to he a very

potent postemergence herbicide against broadleaf weeds at application rates of 50-250 g/ha. There is no information on the mode of action, but the parent compound is reported to act as an inhibitor of isoleucyl-tRNA synthase.

ii) Rhizobiotoxine: This is a product from certain strains of *Rhizobium japonicum* and causes chlorosis and other phytotoxicity symptoms in many weed species and has no effect on soybean, the host of the producing bacterium.

2.2.1.1.2.3 Toxins from Lichens

Lichens, a symbiotic relationship between algae and fungi, produce several different secondary compounds. There is little information concerning the phytotoxic activity of specific lichen products. Most information concerns the antimicrobial or human health benefits of lichen secondary products. The depsides barbatic acid and lecanoric acid, and the tridepside gyrophoric acid act like PS II inhibiting herbicides (e.g., atrazine, diuron) by interrupting photosynthetic electron transport in isolated chloroplasts (Duke *et al.*, 2002). The ability of usnic acid to inhibit carotenoid biosynthesis through the enzyme HPPD (Romagni *et al.*, 2000) may contribute to an allelopathic effect. Synthetic inhibitors of this enzyme, eg., sulcotrione and mesotrione, are currently being used as herbicides.

In another study, Lasceve and Gaugain (1990) documented the effects of usnic acid on sunflower and corn plants. There was 40% decline in transpiration as well as dwarfism and root malformation. The concentration of usnic acid was 500 µM, and this might have caused secondary toxicity effects.

2.2.1.2 Phytotoxins from Plants

Green plants produce hundreds of compounds that are not involved in primary metabolism of the plants, and hence called the secondary products. The specific functions of these secondary products are largely unknown. Many of them are thought to be involved in interactions of plants with other organisms. The compounds involved in interspecific chemical interactions with higher plants are often phytotoxic or herbicidal to other species or even to the species producing them. Such compounds, called allelochemicals can be used for effective wed management. Exploitation of allelopathy for weed management can be made use of either through the crude extracts or the phytotoxins/allelochemicals.

2.2.1.2.1 Crude Extracts:

A more fruitful approach has been to use waste products from plants that are processed for food or oil. Many crop residues including straw from wheat, barley, rye and sorghum contain allelopathic compounds (Duke *et al.*, 2002). A green house experiment in Taiwan showed that phytotoxins are present in the soil during the first month of rice straw decomposition (Chou and Lin, 1976) leading to affect the weed growth. Other experiments have shown that rice straw inhibits germination of oat, wheat, lettuce, lentil, field bind weed (*Convolvulus arvensis*), winter wild oat (*Avena ludoviciana*) and littleseed canarygrass (*Phalaris minor*) (Lee *et al.*, 1991). Efficacy of rice hull extract in inhibiting the germination of barnyardgrass (*Echinochloa crusgalli*) was reported by Chung *et al.* (1997) and the personal communication by M. Sugiyama that use of rice bran @ 200g m^{-2} for weed control and fertilization in transplanted rice by the Japanese farmers resulted in weed reduction and high quality grain, was reported by Kuk *et al.* (2001). They also evaluated the herbicidal activity of rice bye-products on various weeds and crops and found that medium-grain fatty rice bran was the most efficient one in reducing the weed emergence and shoot weight of many broad leaf weeds including palmer amaranth, ivyleaf morningglory, sicklepod, hemp sesbania and prickly sida, and that grasses were not as susceptible to rice bran as broad leaf weeds. Inhibition of germination of *Striga hermonthica* by certain herb extracts including the undiluted extracts from *Curcuma longa* was reported by Ma *et al.* (2004).

i) Corn gluten meal: A by-product of the wet milling process, is being used for pre-emergence weed management and fertilization (Bingamen and Christians, 1995; Gough and Carlstrom, 1999) especially in the proper management of turf. Chemicals in the protein fraction inhibit root growth of germinating weeds. Even though the peptides in corn gluten meal are short-lived, leaving no residual effects, a high use rates makes its application expensive and thus limited in use.

Corn gluten hydrolysate, produced by the action of a bacterial proteinase, is more active than the gluten meal as herbicide (Liu and Christians, 1994a). The hydrolysate is active on both grasses and broadleaf weeds (Liu and Christians, 1997). The hydrolysate contains five phytotoxic dipeptides, with alanylalanine being the most active (Liu and Christians, 1994b). Numerous physiological effects of alanylalanine have been reported but the molecular target site is unknown. Later, a more active pentapeptide (leu-ser-pro-ala-gln)

was isolated from corn gluten hydrolysate (Liu and Christians, 1996), but very little information is available on it.

ii) Crambe seed meal: Another seed meal waste that could be used for weed management is that of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries), a crop grown for oil and erucic acid. A major phytotoxin in this material is 1-cyano-2-hydroxy-3-butene. Seed meals from phytotoxic, glucosinolate-containing plants have also been shown to be potentially useful for weed management as a soil amendment (Vaughn and Berhow, 1998).

2.2.1.2.2 Allelochemicals

Plants produce a very large number of interesting phytotoxins with potential use as herbicides. For example, leptospermone is an allelochemical from which the triketone class of herbicides was developed. This is perhaps the most successful development of a commercial herbicide from a phytochemical. Most of the allelochemicals involved in plant-plant interactions are not as phytotoxic as commercial herbicides. Therefore, they are toxic only at very high concentrations and sometimes become antagonistic in combination and inactivated quickly in the soil. (Duke *et al.*, 2002).

Table 4. Allelochemicals with promising herbicidal activity

Name of the chemical	Source
Caffeine	Coffee plants
Trimethyxanthine	
Dhurrin	Sorghum plants
Sorgoleone	
Gallic acid	Spurge plants
Juglone	Black walnut trees
Phloridzin	Apple roots
Psoralen	Psoralea plants

i) Monoterpene cineoles: Are natural products commonly found in the essential oils from aromatic plants such as *Laurus nobilis* L., *Salvia* spp., *Eucalyptus* spp., and *Artemisia* spp. Many volatile monoterpenes are phytotoxic. Of these compounds, 1,8-cineole has been identified as one of the most potent allelochemicals released by *Artemisia* spp. 1,4-cineole,

present in much lower concentrations has been reported to be a structural analogue of the herbicide Cinmethylin, and a potent inhibitor of aspargine synthetase (Romagni *et al.*, 2000). **Artemisinin** is a sesquiterpenoid lactone peroxide constituent in the flower and leaf of annual wormwood (*Artemisia annua*) that is used as an antimalarial drug. It inhibited the germination of lettuce and annual wormwood and also inhibited the root and shoot growth in lettuce, *Amaranthus retroflexus*, *Ipomoea lacunosa*, annual wormwood and common purselane (*Portulaca oleraceae*) at 33µM concentration. It has been identified as a selective phytotoxin and is equally effective to Cinmethylin, a synthetic chemical based on the chemistry of 1, 4 cineole, in reducing the growth of lettuce (Duke *et al.*, 1987)

ii) Rocaglamide: This is obtained from the bark of *Aglaia congylos* (Meliaceae) and exhibits quite potent herbicidal activity. The metabolite, rocaglamide responsible for the herbicidal activity has also been described as insecticidal. The chemical showed postemergence and good preemergence activity at 0.5-1 kg ha^{-1} against a range of mono- and dicotyledonous weeds.

iii) Quassinoids: Are phytotoxic compounds produced by several plant species of the Simaroubiaceae family (Heisey, 1990). **Chapparrinone** and its derivatives are active on plants at 1 to 5 µM concentrations. Chapparrinone-type quassinoids have broad weed spectrum activity when applied either as preemergence or post emergence herbicide and can provide 100% control of green foxtail (*Setaria viridis*) and sicklepod (*Senna obtustfolia*) at rates of 0.125 kg ha^{-1}. Quassinoids cause strong inhibition of the latter stages of mitosis but do not affect prophase, suggesting that these compounds do not prevent induction of cell cycle. The primary site of action appears to be the inhibition of reduced nicotinamide adenine dinucleotide oxidase, and the presence of an oxymethylene bridge between C8 and C11 of the quassinoid backbone was required for the herbicidal activity (Morre *et al.*, 1998). **Ailanthone,** from *Ailanthus altissima* (Simaroubaceae), is yet another strongly herbicidal plant metabolite in the quassinoid group, which shows pronounced postemergence activity against several weeds when applied at about 1 kg/ha.

iv) Podophyllotoxin

There are many plant-derived compounds that inhibit mitosis of plants (Vaughan and Vaughn, 1988). Podophyllotoxin, an aryl tetralin lignan extracted from the leaves of mayapple (*Podophyllum peltatum* L.) was found phytotoxic, more against the

monocotyledonous Italian ryegrass (*Lolium multiflorum*) than against the dicotyledonous lettuce (*Latuca sativa*) species tested. The inhibition of root growth observed in the bioassays suggested that podophyllotoxin affected either cell division or cell elongation. Its inhibitory effect on the different mitotic phases, and especially on prophase, indicates that this compound affects mitosis. Although the precise molecular mechanism of action of this compound remains to be identified in plants, a primary effect is the alteration of the formation of multiple spindle poles, leading to an asymmetrical convergence of the chromatids.

v) Sorgoleone: Is an allelochemical exuded in oily droplets from the root hairs of sorghum (Netzly and Butler, 1986). The concentration of sorgoleone in soils growing sorghum can reach 10^{-4} to 10^{-5} M (Netzly *et al.*, 1988) and lead to suppression of weed growth (Forney and Foy, 1985). This potent phytotoxin represses the growth of large crabgrass seedlings (*Digitaria sanguinalis*) with a GR_{50} of 10 µM for shoot and root growth (Nimbal *et al.*, 1996). Inhibition of shoot and root growth of velvet leaf (*Abutilon theophrasti*) and barnyardgrass has also been observed at concentrations from 10 to 200 µM. Sorgoleone inhibits photosynthetic electron transport in thylakoids by competing for the plastoquinone binding site on photosystem II. This compound has the same level of inhibitory activity of photosynthetic electron transport as diuron and DCMU with an I_{50} of 100 and 120 nM, respectively (Nimbal *et al.*, 1996).

Table 5. Rhizosphere products of sorghum-sudangrass hybrid on seedling growth

Weed species	Root length, mm		
	Control	Treated	Inhibition %
Alfalfa	24.0	7.9	67
Johnson grass	29.6	17.8	40
Common lambsquarters	16.1	10.1	37
Annual rye grass	34.8	25.9	25
Large crab grass	18.0	13.5	25
Curly dock	23.6	22.3	6

(Forney and Foy, 1985)

Allelopathy from sorghum has been found to be due to the phenolic acids as well as HCN released upon degradation of dhurrin from the roots. The SSH rhizosphere products showed selectivity, i.e., alfalfa was most sensitive and curly dock (*Rumex crispus*) the least (Forney and Foy, 1985).

vi) Resin glycosides: Multicoloured morningglory (*Ipomoea tricolor*) is used in traditional agriculture in Mexico for weed management. It contains resin glycosides, of which tricolorin A is the principal constituent (Pereda-Miranda *et al.*, 1993). This compound is highly phytotoxic and a potent inhibitor of plasma membrane adenosine triphosphatase.

2.2.2 Synthetic derivatives of naturally occurring compounds as herbicides

The problems of high phytotoxicity, limited crop selectivity, and instability under field conditions associated with several naturally occurring compounds hinder their commercial development as herbicides. These problems can be overcome by biorational synthesis of more selective and stable analogues of these chemicals. Thus, microbial toxins and allelochemicals provide us with novel chemistries that could be manipulated in order to produce commercial herbicides.

Table 6. Commercially developed herbicides based on natural chemistry

Natural product	Source	Herbicide	Manufacturer/ country
Microbial products			
Phosphinothricin	*Streptomyces viridochromogenes*	Gulfosinate	Hoechst/ Germany
Anisomycin	*Streptomyces* sp.	Methoxyphenone	Nihon / Japan
Irpexil	*Irpex pachydon*	Benzadox	Gulf / U.S.A.
Fusaric acid	*Fusarium* sp.	Picloram	Dow / U.S.A.
Moniliformin	*Fusarium moniliforme*	3,4-dibutoxy-moniliformin	CIBA-GEIGY/ Switzerland
Plant products			
Cineole	Widespread in plants	Cinmethylin	Shell / U.S.A.
Benzoxazinones (Hydroxamic acids)	Gramineae plants	Benzazin	BASF / Germany
Quinolinic acid	*Nicotiana tabacum*	Quinclorac	BASF / Germany

(Hatzios, 1987)

i) Glufosinate (Basta®, Liberty®)

Phosphinothricin, the active ingredient of the microbial herbicide bialaphos (Herbiaceae), is synthetically produced as glufosinate. It is the only herbicide that inhibits glutamine synthetase. It is non-selective, and many crops have been engineered to be resistant to it by inserting a transgene that encodes a detoxification gene from the producing *Streptomyces* sp. Phosphinothricin (glufosinate) is the biggest success story for a natural product-based herbicide. It is relatively inexpensive, toxicologically and environmentally safe, and efficacious on wide spectrum of grass and broad leaf weeds following foliar application. Thus, with herbicide-resistant crops, it has many of the same advantages of glyphosate in glyphosate-resistant crops. The ammonium salt of glufosinate, a synthetic racemic mixture based on the chemistry of **phosphinothricin**, has been introduced by Hoechst Aktiegesellschaft Co. (West Germany) as a nonselective herbicide and marketed under the trade name Basta® (Hatzios, 1987).

ii) Methoxyphenone: Methoxyphenone is marketed in Japan as a selective herbicide for the control of barnyardgrass (*Echinochloa crusgalli*) in rice and is easily degraded in soil (Duke *et al.*, 2002). It is the synthetic analogue of **Anisomycin**, and is manufactured by Nihon, Japan.

iii) Benzadox: Irpexil is produced by the basidiomycete *Irpex pachydon*, which is structurally similar to the herbicide benzadox. Benzadox is the synthetic analogue of **Irpexil**, both appear to act as pro-herbicides. Following application to plants they are activated by being converted to amino-oxyacetic acid, a potent inhibitor of pyridoxyl phosphate-requiring enzymes (Duke *et al.*, 2002).

iv) Picloram: Fusaric acid is a marasmin produced by many species of *Fusarium* fungi, and has been detected in infected tomato plants and wilted cotton. Picloram, a chlorinated analogue of **fusaric acid**, has been marketed as an herbicide, which caused desiccation and wilting in red maple plant (*Acer rubrum* L.) resembling the symptoms caused by fusaric acid (Peterson *et al.*, 1974).

v) Analogues of lichen products

Several analogues of lichen-derived anthraquinones have strong herbicidal activity. Certain analogues of **emodin**, an anthraquinone found in nonlichen as well as

lichenicolous fungi, are highly specific for grasses, causing malformation and bleaching in early seedlings. Other analogues of **rhodocladonic acid** cause root malformations in both dicot and monocot seedlings. Several of these completely inhibit germination.

Most of the lichen compounds are chemically simple, making them relatively easy to synthesize in the laboratory. Doing so would provide large amounts of material without harming the ecosystems. In addition, many of these compounds can be used as lead structures based on their particular mechanism of action and can then be optimized in the laboratory to fit specific applications.

These examples demonstrate that the structures of naturally occurring phytotoxins can serve as leads for the synthesis of new successful herbicides. Undoubtedly, the use of biorational design for the discovery of new herbicide chemistries will become increasingly important in the future, following the isolation and characterization of additional microbial toxins and allelochemicals from higher plants.

2.2.3 Natural compounds from other sources

Secondary products with many kinds of biological activity are produced by all types of marine organisms and animals. For example, certain ants apparently produce herbicidal compounds to kill the vegetation surrounding their habitat (Renner and Ricklefs, 1998). Relatively little effort has been made to examine such compounds for their potential as herbicides.

3. Progress of works in India

Most of the works in the foregoing areas have been undertaken in countries other than India. The works that were carried out in India gave stress on classical biocontrol rather than on the exploitation of the herbicidal potential of microbial toxins and secondary metabolites from plants. Some of the research works done in our country are the following.

Abraham (1999) identified the fungal pathogens such as *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Curvularia lunata* and *Corynespora cassicola* infecting mile-a-minute (*Mikania micrantha*), an alien invasive weed in many parts of the country. She also studied the effects of exotoxic and endotoxic metabolites of *Colletotrichum*

gloeosporioides and *Alternaria alternata* on *Mikania micrantha* and reported that both the toxic metabolites produced necrotic spots on the leaf, and the diameter of the spots increased with time, leading to complete drying of the leaf after four days of inoculation. Necrotic lesions produced by toxic metabolites of *Colletotrichum gloeosporioides* were also reported on *Plumbago indica* by Varma (1991).

Puccinia spegazzini, the rust fungus developed by the scientists of CABI, U.K., has shown potential as a biocontrol agent for the control of *Mikania micrantha* in the Western Ghats. The fungus infects all vegetative parts, causing necrosis, cankering and often leading to death of the plant (Ellison, 2001).

Even though successful cases of classical biocontrol using insects have been reported for the control of water hyacinth (*Eichhornia crassipes*), the plant still remains as a troublesome aquatic weed in many parts of the country. A spore concentration of 10^{11} ml^{-1} as well as the cell free metabolite of *Fusarium pallidoroseum* was found destroying the water hyacinth plant in Kerala. The plant showed symptoms of blighting of leaves within 7-10 days of inoculation (Naseema and Balakrishnan, 2001). Based on these findings the Kerala Agricultural University, Vellanikkara, Thrissur has recommended to spray 40% WP formulation of *Fusarium pallidoroseum* (5%) on water hyacinth plants pre-treated with 5% cashew nut shell liquid (CNSL), so that the plant exhibits typical blighting (KAU, 2007)

Studies have shown that oils from *Eucalyptus globulus* and *Eucalyptus citriodora* have a deleterious effect on *Parthenium hysterophorus*. The germination of the weed was inhibited and the chlorophyll content and cellular respiration of mature plants exposed to Eucalyptus oil were reduced significantly. These were accompanied by an increased water loss resulting in complete wilting of the plants after 15 days of exposure to the volatile oils. The oil from *Eucalyptus citriodora* was more effective in causing injury to the weed compared to that from *Eucalyptus globulus* (Kohli *et al*., 1998).

4. Limitations of natural compounds

- Many natural products are too expensive to be seriously considered for use as agrochemicals because of their structural complexity. The cost of compound isolation and structure elucidation is very high.

- Some highly phytotoxic natural products show high mammalian toxicity also. This aspect of such natural phytotoxins has terminated interest in them for the development for weed management.
- Passing laws to retain ownership of compounds discovered from organisms within a country with a feeling that their biological resources have been exploited by institutions from the developed world for the discovery of pharmaceuticals, has discouraged the discovery efforts that use the biological diversity of certain places (Duke *et al.*, 2002)

5. Future prospects

- Modern instrumentation such as Liquid Chromatography, Mass Spectrophotometry, Nuclear Magnetic Resonance Spectrometry, etc., and automated determination of compounds within a complex extract has greatly reduced the time and effort needed to identify isolated compounds.
- Short half-life of most natural compounds in the field makes them environmentally safe.
- Natural compounds or preparations may require less regulatory scrutiny for registration than synthetic compounds, thus reducing the cost of commercializing the product.
- New synthetic analogues of natural products having greater selectivity, stability and efficacy to control weeds may be developed at lower cost of commercial production.

6. Conclusion

As mentioned earlier, indiscriminate use of pesticides, including herbicides has posed many problems in the world. One of the measures to cure such wounds is to reduce the continuous use of synthetic chemicals, instead look for alternatives such as exploiting the weed management potential of microorganisms/natural compounds, and utilizing the novel chemistries of the natural compounds for synthesis of cost effective, eco-friendly herbicides. In addition we have to strengthen the research for identifying organisms/natural compounds that have herbicidal potential in the Indian scenario, for which we can utilize the modern instrumentation and improved methods.

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DISCUSSION

1. What will be the effect of bioherbicides on crop plants?
   - ☑ The bioherbicides so far developed and applied in the field are highly host specific, or they are having their own host range. Hence the f. sp. fungus will not infect on plants other than its host species.
2. Is there any report on resistance developed by weeds against mycoherbicdes?
   - ☑ No such report has been noticed.
3. Can we go for application of bioherbicides and pesticides at a time?
   - ☑ Application of bioherbicides is not recommended usually with other pesticides especially fungicides as they will affect the growth and establishment of the fungal microorganism within the mycoherbicide. And there are reports that mycoherbicides exhibits compatibility with certain chemicals.
4. What is meant by bioherbicide?
   - ☑ Bioherbicides are cultures of pathogenic microorganisms (the bio part) mixed with some carrier materials (the herbicide part). That means the microorganisms will be applied to kill the weeds as normal chemical herbicides are used.
5. What is the dose of bioherbicdes?
   - ☑ The mycoherbicides are usually recommended based on the count of spores, and not based on the quantity as of the normal chemical herbicides.
6. What about the mode of action of natural herbicides?
   - ☑ Most of the natural compounds having herbicidal activity have the same target sites as that of the chemical herbicides. In addition many natural compounds act on other targets which had not been acted upon by the chemicals. Phosphinothricin has an inhibitory effect on the glutamine synthetase enzyme.
7. How *Eupatorium* was vanished from our area?
   - ☑ Invasive and smothering nature of *Mikania micrantha* has suppressed the growth and existence of *Eupatorium*.
8. Do you have any idea on the use of saw dust for weed control?
   - ☑ The saw dust of teak plant is being used as a means of wed control.
9. Name the fruit plant that does not permit weeds to grow under the canopy.
   - ☑ Garcinia (Kudampuli)

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KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara, Thrissur

Agron. 752 - Seminar

Herbicides of Natural Origin

Student: Musthafa Kunnathadi (2006-21-104)
Time: 10.15 am, 06.07.2007
Venue: Conference Hall attached to the Library

Abstract

About 45 % of the pesticides used in the world is occupied by herbicides followed by insecticides (36%), fungicides (17%) and other chemicals (2%) (Wahab, 2003). Herbicides will continue to be a key component in most integrated weed management systems. A growing concern about the environmental and public health consequences of the chemical pesticides has brought a greater need than ever for the use of easily degradable and environmentally safe pesticides with good selectivity (Kuk *et al.*, 2001). Many plant pathogens, the cultures of which have the potential to kill specific weeds, have been identified and commercialized as bioherbicides. Successful examples are Collego®, DeVine® etc. (Boyette *et al.*, 1991).

Recent advances in microbial and plant biochemistry have stimulated scientific interest into the possible role of secondary plant products and microbial toxins as natural pesticides. Many of these compounds are phytotoxic and have the potential as herbicides or as templates for new herbicide classes (Duke *et al.*, 2002). The two approaches for the development of herbicides from nature include (i) isolation and characterization of microbial toxins or secondary plant metabolites that could be used effectively as herbicides, e.g., 'Bialophos' (Herbiaceae®) from *Streptomyces* spp., and (ii) evaluation of plant secondary metabolites and microbial toxins with novel chemistries, which could be used as leads for the chemical synthesis of new herbicides, e.g., the ammonium salt of glufosinate (Basta®), a synthetic analogue of Phosphinothricin (Hatzios, 1987). Natural products from plants, lichens, marine organisms and animals etc. have also shown promise for use as herbicides.

The natural products that are commercialized as herbicides limit in number due to the high cost involved in their development because of their structural complexity. But improved instrumentation has considerably reduced the cost of isolation and identification of natural compounds from what it was a decade ago. This has caused renewed interest in natural products in herbicide discovery programmes.

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Boyette, C.D., Quimby, P.C., Connick, W.J., Daigle, D.J. and Fulgham, F.E. 1991. Progress in the production, formulation and application of mycoherbicides. In: TeBeest, D.O. (ed.) Microbial Control of Weeds. Chapman and Hall, New York. pp. 209-222.

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Importance of extranuclear genes in crop plants

BY

Vidhu Francis Palathingal

(2006-21-105)

Department of Plant Breeding and Genetics

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

PbGen. 751- Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala

2007

DECLARATION

I hereby declare that the seminar report entitled '**Importance of extranuclear genes in crop plants** ' is a record of the seminar presented by me during the course on 08.06.2007and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
8.06.2007

Vidhu Francis Palathingal
(2006-21-105)

CERTIFICATE

Certified that the seminar report entitled '**Importance of extranuclear genes in crop plants**' is a record of seminar presented by **Smt. Vidhu Francis Palathingal (2006-21-105)** under my guidance and that this report has been prepared by her independently

Vellanikkara
8.06.2007

Dr. V.V.Radhakrishnan
Major Advisor

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1.Introduction

Inheritance of most of the characters in crop plants show different characteristic features. First, the contributions by both male and female parents are equal so that the results from reciprocal crosses are identical. Secondly, the segregation pattern produces the characteristic 3:1 ratio in the F_2 generation of a monohybrid cross and a typical 9:3:3:1 ratio in dihydrid crosses. These features of inheritance were first demonstrated by Mendel, consequently, such an inheritance pattern is referred to as Mendelian inheritance. It is universally accepted that genes showing Mendelian inheritance are located in the chromosomes of eukaryotic nuclei. Therefore, Mendelian inheritance pattern is regarded as a sufficient evidence for a gene to be located in the chromosomes, such genes are termed as nuclear genes or, more commonly, simply as genes. But there are some characters in several crop plants do not show Mendelian inheritance, or they show a nonmendelian inheritance pattern.

2.Nonmendelian inheritance

Nonmendelian inheritance is also referred to as extranuclear inheritance, extrachromosomal inheritance and maternal inheritance. In this type of inheritance, generally the character of only one of two parents (usually the female parent) is transmitted to progeny. As a result, reciprocal crosses exhibit consistent differences for such characters and there is a lack of segregation in F_2 and the subsequent generations. But in many cases, traits following nonmendelian inheritance show somatic segregation.

2.1.Characteristics of nonmendelian inheritance

1. Reciprocal differences

Reciprocal crosses show marked differences for these characters. In most cases, characters from only one parent, generally female parent, are

transmitted, this phenomenon also known as maternal inheritance. As a consequence, F_1s and subsequent generations from reciprocal crosses show marked and consistent differences for such traits.

2. Lack of segregation

In general, F_2, F_3 and the subsequent generations do not show segregation for a extranuclear inherited trait. This is because the F_1 individuals generally receive plasma genes from one parent only.

3. Somatic segregation

Traits exhibiting extranuclear inheritance show somatic segregation, that is the genes show somatic segregation during mitosis, a feature of rare occurrence in case of nuclear genes. This indicates a lack of association between the segregating trait and the mitotic spindle apparatus.

4. Association with organellar DNA

Extranuclear inheritance arises due to genes located in cell organelles (chloroplast and mitochondria). Many such genes have been mapped in organellar DNA. The demonstration of an association of a gene with these known markers is a definite evidence for the trait produced by former is extranuclearly inherited.

5. Transferred through backcross

Extranuclear traits follows maternal inheritance pattern. As a result, these traits are transferred to progeny by repeated backcross (Singh, 2003).

2.2.Evidence for extranuclear inheritance

Evidence for extranuclear inheritance was first presented by Correns in *Mirabilis jalapa* in 1908. He observed that leaves of *Mirabilis jalapa*, four o'clock plant, may be green, white or variegated and some branches may have only green, only white or only variegated leaves. Correns made reciprocal crosses in all combinations among the flowers produced on three types of branches. When flowers from a green branch were used as female, all the progeny were green irrespective of phenotype of branch used as male parent. Similarly, progeny from the crosses involving flowers from white branches as the female parent were all white irrespective of the male phenotype. The phenotype of progeny was same as

that of female parent. The results was explained by assuming that gene producing variegation in *M. jalapa* is located in plastids (Strickberger, 1976).

3.Extranuclear genes

Genes governing the traits showing extranuclear inheritance are located outside the nucleus, that is, in the cytoplasm. Hence they are referred to as plasmagenes, cytoplasmic genes, extranuclear genes or extrachromosomal genes. The sum total of genes present in cytoplasm of a cell is known as plasmon. All the genes present in a chloroplast constitute a plastome, while chondriom denotes all genes present in mitochondria. All available evidence indicates that plasmagenes are located in DNA present in mitochondria (mtDNA) and in chloroplasts (cpDNA). Together the mtDNAs and cpDNAs are termed as organellar DNA.

3.1.Evolution of organelles and organellar DNA

The evolution of organelles and orgenellar DNA is explained by endosymbiont hypothesis. Organelles showed homology with primitive prokaryotic cells. It was identified that a symbiotic association existed between eukaryotic cells and the primitive aerobic bacteria. Later these bacteria was engulfed by the eukaryotic cell and became an endosymbiont. In the course of time, some of bacterial genes were transferred to eukaryotic nucleus, but certain other genes maintained their existence as organelle genome (Jain, 2000)

3.2.Nuclear organelle interaction

In organelles, there is dual type genetic control, that is by the nuclear genes and by organelle genes. Since the expression of plasmagenes is associated with either mitochondrial or plastid function, it is not surprising that their expression is greatly modified by nuclear genes. Nuclear DNA produce RNA and protein which is imported to organelle where it produce genetic control of organellar DNA

An unusual case of interaction between nuclear and cytoplasmic genomes is reported in maize. A type of variegation, called iojap, is produced by a

recessive nuclear gene ij, plants homozygons (ijij) for this gene develop the typical iojap variegation. But once this variegation is produced by the nuclear gene ij, it shows a typical cytoplasmic inheritance. Clearly, the nuclear genotype ijij has a mutagenic effect on plasted genome. Once this mutation is induced in some cpDNA molecules, the variegation is inherited cytoplasmically. The cross between normal (IjIj) plants as female and iojap (ijij) plants as male produces all green plants in F_1 with the nuclear genotype Ijij. In F_2 generation of this cross, ¼ progeny are ijij and develop iojap variegation, the remaining ¾ of progeny are normal green. When the iojap F_2 plants are mated with normal green plants, a marked reciprocal difference is observed in progeny. When iojap plants are used as males and green plants as females, all the progeny are normal green. But in the reciprocal cross, green, white and iojap progeny are recovered, the ratio between the three types of progeny is quite variable.

3.3.Chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA)

Chloroplasts and mitochondria have genomes that show nonmendelian inheritance. Typically they are maternally inherited. Organelle genomes undergo somatic segregation in plants. They are transcribed and translated in same organelle compartment in which they reside. Since organelle DNA has a different replication system from that of nucleus, error rate during replication may be different. Organelle genomes are usually circular molecules and DNA is of unique sequence. There are few exceptions where mitochondrial DNA is a linear molecule, generally in lower eukaryotes. Usually there are several copies of genome in individual organelle. Since there are multiple organelles per cell, there are many organelle genomes per cell. Although organelle genome itself is unique, it constitutes a repetitive sequence relative to any nonrepititive nuclear sequence.

Chloroplast genomes are relatively large, usually approximately 140 kb in higher plants and <200 kb in lower eukaryotes. There are multiple copies of genome per organelle, typically 20-40 in a higher plant and multiple copies of organelle per cell, typically 20-40. Chloroplast genomes vary in size, but are large enough to code for 50-100 proteins as well as rRNAs and tRNAs. With respect to

mitochondria, chloroplasts has more genes. Role of chloroplast is to undertake photosynthesis. Many of its genes code for proteins of complexes located in thylakoid membranes.

Total amount of mitochondrial DNA relative to nuclear DNA is small <1%. Plants show an extremely wide range of variation in mitochondrial DNA size, with a minimum of ~100 kb. Size of genomes makes it difficult to isolate intact, but restriction mapping in several plants suggests that mitochondrial genome is usually a single sequence, organized as a circle. Within this circle, there are short homologous sequences. Recombination between these elements generate smaller, subgenomic circular molecules that coexist with the complete, "master genome", explaining the apparent complexity of plant mitochondrial DNAs. Total number of protein coding genes is rather small, but does not correlate with the size of genome. Plants with much larger mitochondrial genomes codes for more protein. Two major rRNAs are always coded by mitochondrial genome. The number of tRNAs coded by mitochondrial genome varies from none to full complement (Lewin, 2004)

4.Genome sequencing

Chloroplast and mitochondrial genome sequencing have been attempted in different crops. Chloroplast and mitochondrial genetic maps of several crops including *Arabidopsis*, rice, tobacco, maize, liverwort etc. have already been characterized. Proteomics projects to identify organellar DNA coded proteins have been conducted. The big challenge in any proteomics project aimed at identifying the members of a proteome is to find proteins of high and low abundance and also to distinguish between individual members of protein families. Organelle proteins of model plant *Arabidopsis thaliana* have already been characterized.

4.1 *Arabidopsis thaliana* chloroplast proteins

The thylakoid proteome seems particularly challenging because it is so dominated by the photosynthetic apparatus and its hydrophobic chromophores

Therefore an extensive analysis of thylakoid membrane proteome was performed by sequential extractions with salt, detergent and organic solvents, followed by multi-dimensional protein separation steps (reverse - phase HPLC and one- and two-dimensional electrophoresis gels), different enzymatic and nonenzymatic proteins cleavage techniques, mass spectrometry and bioinformatics. Thylakoid proteins, that is 198 identified proteins, functionally classified into 1. non chloroplast, 2 translation (ribosomal subunits, RNA binding proteins, etc.), 3. chaperons, protein insertion and assembly, proteases and protein isomerases, 4. primary and secondary metabolism, 5. thylakoid photosynthetic apparatus, 6. alternative electron flow, 7. oxidative stress (peroxyredoxins, thioredoxins, ascorbate peroxidase etc.), 8. new chloroplast proteins without obvious function, 9. other luminal proteins without obvious function. Thylakoid photosynthetic apparatus has four multisubunit protein complexes - photosystem I (PS I), photosystem II (PS II), ATP synthase and cytochrome b6f complexes, each with multiple cofactors. These four complexes are composed of at least 70 different proteins that perform the photosynthetic reactions (Friso *et al*., 2004).

4.2. *Arabidopsis thaliana* mitochondrial proteins

Mitochondria perform a variety of biochemical functions within the eukaryotic cell. Their primary roles are the oxidation of organic acids via the tricarboxylic acid cycle and the synthesis of ATP coupled to the transfer of electrons from reduced, NAD^+ to oxygen via the electron transport chain. However, in plants, mitochondria perform many important secondary functions, such as the synthesis of nucleotides, amino acids, lipids and vitamins. A novel insight into *Arabidopsis* mitochondrial function was revealed from a large experimental proteome derived by liquid chromatography-tandem mass spectrometry. Within the set of 416 identified proteins, a significant number of low-abundance proteins involved in DNA synthesis, transcriptional regulation, protein complex assembly, and cellular signalling were discovered. Nearly 20% of experimentally identified proteins are of unknown function, suggesting wealth of undiscovered mitochondrial functions in plants.

Table 1. Functional breakdown of the Arabidopsis mitochondrial protein set

Function	Total
Energy	98 (24%)
Metabolism	81 (19%)
Protein fate	53 (13%)
DNA synthesis and processing	9 (2%)
Transcription	8 (2%)
RNA processing	14 (3%)
Protein synthesis	15 (4%)
Defense, stress, detoxification	16 (4%)
Communication / Signalling	19 (5%)
Transport	19 (5%)
Miscellaneous	4 (2%)
Unknown	71 (17%)
Total	416

(Heazlewood *et al*., 2004)

5.Role of chloroplasts and mitodrondrial genes in crop plants

5.1. Maintenance of carbon and nitrogen balance

The improvement of nitrogen use efficiency, particularly in cereals, is a major goal of crop improvement. Such improved crops would make better use of nitrogen fertilizer supplied, they would also produce higher yields with better protein content. This might be achieved, at least in part, by a better understanding of nitrogen metabolism and its regulation, and by identifying likely target genes for manipulation by either direct gene transfer or marker - assisted breeding.

Key enzymes involved in maintaining carbon and nitrogen metabolism are Glutamine synthetase (GS) coded by chloroplast genes and glutamate dehydrogenase (GDH) coded by mitochondrial genes. Glutamine synthetase was first purified and characterized from plants in 1956. One particular important characteristic is its high affinity for ammonia and thus its ability to incorporate

ammonia efficiently into organic combination. Carbon compounds important in stimulating glutamine synthetase include sucrose and 2-oxoglutarate (Figure 1).

Glutamine synthetase convert glutamate to glutamine, that is, NH_3 is fixed as glutamine. Glutamine along with other compounds produced like asparagines, arginine etc. act as nitrogen transport compounds.

GDH functions in the deaminating direction in tissues converting amino acids into transport compounds with a low C/N ratio. The operation of a GDH shunt is that it convert glutamate to NH_3 and 2-oxoglutarate. 2-oxoglutarate goes to carbon pool and acts as a carbon transport compound with low C/N ratio. That is, GDH acts a shunt to the glutamate synthase cycle to release carbon from amino compounds in the form of keto-acids and to enable the synthesis of compounds with low C/N ratios.

Strategies to improve the nitrogen use efficiency of crop plants are being explored. A number of transgenic plants with different GS transgenes have been made. Most of these involve relatively unsophisticated control of the transgene expression because of use of 35S promoter. Given the complexity of the system, many different approaches may need to be tried to obtain robust results (Miflin and Habash, 2002)

5.2. Nitrogen assimilation in tropical nodulated legumes

Synthesis of purine ring is a central metabolic function of all cells. The products, AMP and GMP, provide purine bases for DNA and RNA, as well as for a number of essential coenzymes and signaling molecules. ATP serves as the energy source for many chemical reactions. Despite the essential functions for purines, salvage pathways, which retrieve the purine ring after nucleic acid or coenzyme breakdown, recycle nucleotides to meet day-to-day needs. The purine pathway also functions in pathways that are different and distinct from these "housekeeping roles".

In nodules of tropical legumes, such as soyabean and cowpea, purine biosynthetic pathway plays a dominant role in primary nitrogen metabolism

Within cells infected with rhizobia, bacterial nitrogenase activity leads to secretion of fixed N principally as NH_3 or NH_4^+ (Figure 2).

Two types of cell in the central infected tissue zone of nodules are required for the synthesis of ureides from fixed nitrogen. The enlarged infected cells (IC) contain as many as 50,000 rhizobia, differentiated into bacteroids and enclosed in groups within membrane vesicles of plant origin. These bacteria express nitrogenase and export ammonia to the host cell cytosol where it is assimilated as the amide groups of glycine. Glycine, together with other aminoacids is used by both plastids (P) and mitochondria (M) to form purines. The purines are oxidized within the infected cell cytosol to urate that is transferred to uninfected cells (UC) of the central zone and further oxidized within enlarged microbodies (Mb) to allantoin and allantoic acid (Ureides). Ureides are exported from the nodule in xylem to provide the majority of the nitrogen for the plants nutrition.

The localization of purine biosynthesis pathway in plants is different to that of all other organisms in that it is organelle based. The fact that purine synthesis is also involved in generating ureides for N storage in some species suggests that perhaps a more extensive investigation of pathways for nitrogen assimilation in a wider range of plants will reveal this complex mechanism to be more common than is presently appreciated (Smith and Atkins, 2002).

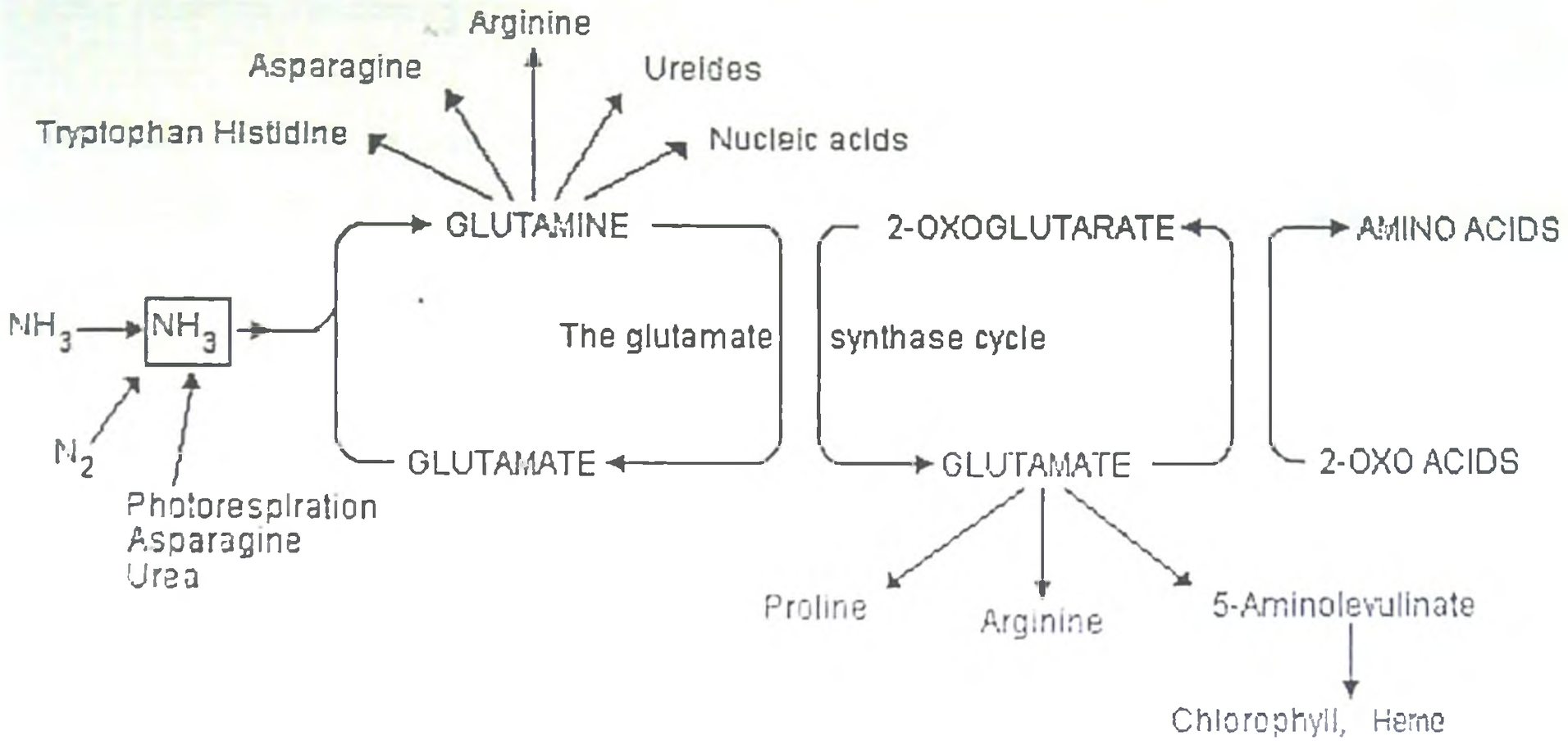

Figure 1.Glutamate synthase cycle

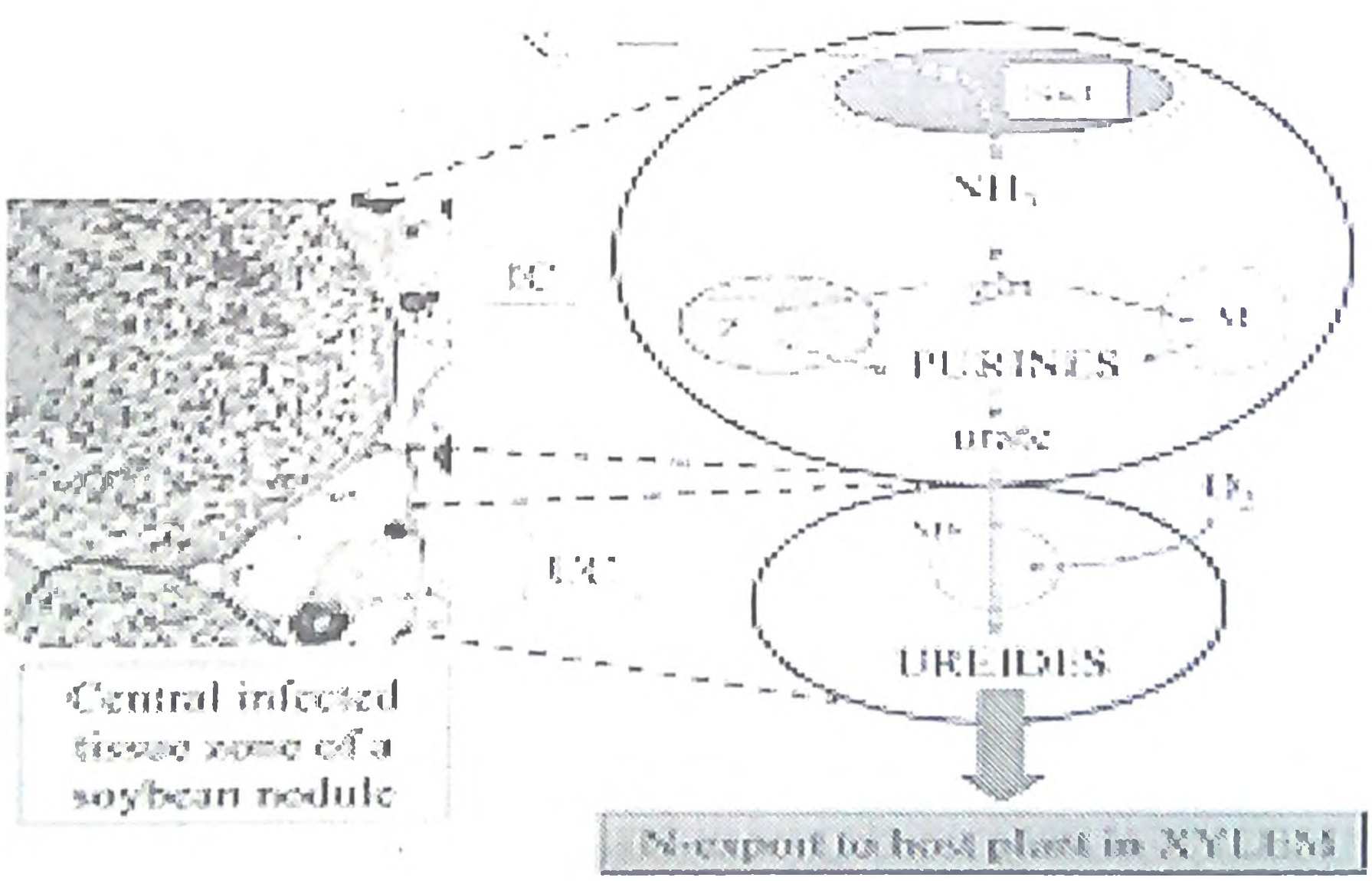

Figure 2. Nitrogen assimilation pathway

6.Role of mitochondrial genes

6.1. Cytoplasmic male sterility

In spite of their agronomic importance in hybrid seed production, mutations that encode cytoplasmic male sterility (CMS) provides a means to probe the role of mitochondrion in reproductive development. In natural plant populations, the most widespread manifestation of disturbed, mitochondrial - nuclear interaction is altered floral development, particularly the loss of the male gametophyte. In many species, CMS halts pollen development at a very early developmental stage, potentially saving considerable output of resources. In other species, the disruption occurs late in development, after considerable energy has been expended. The phenomena of CMS and fertility restoration have been exploited by plant breeders to synthesize hybrid lines of a number of crop species.

Cytoplasmic male sterility are of different types - Autoplasmic and alloplasmic. Autoplasmic is CMS which arise within a species by spontaneous mutational changes in mitochondrial genome. Alloplasmic CMS arises from intergenic, interspecific or intraspecific crosses. Here incompatibility is due to poor co-operation between nuclear genome of one species and organellar genome of other species (Hayward *et al* , 1993)

The usual procedure to produce a CMS line is to cross the fertile inbred line onto selected, completely pollen-sterile plants of approximately the same maturity. By repeated backcrossing with the same fertile inbred on to sterile plants that were selected as the nearest in type and time of flowering to the inbred used as the pollinator, it was possible to convert a fertile line into a similar sterile line after from three to five backcrossings. This is easily accomplished since all that is necessary is to put a complete chromosome set from one type into the cytoplasm of the sterile type. This system of male sterility produce male sterile progeny, which cannot be used commercially for hybrid seed production in case of grain crops, but can be used only in case of vegetatively propagated ornamental crops. So in case of grain crops, a modification of this CMS system called CGMS

system is used for hybrid seed production. In this system, fertility is restored in male sterile line by a nuclear restorer gene.

6.1.1.Hybrid seed production-

In CGMS system or three line system of hybrid seed production, there are 3 lines - A line (male sterile line), B line (maintainer line), R line (restorer line). B line is crossed with A line to produce male sterile line and thus B line maintains male sterility in male sterile line (A line). Restorer line or R line when crossed with A line restores fertility in male sterile line by the nuclear restorer gene. Cross between A line and R line, thus produces fertile F_1 hybrid (Figure 3)

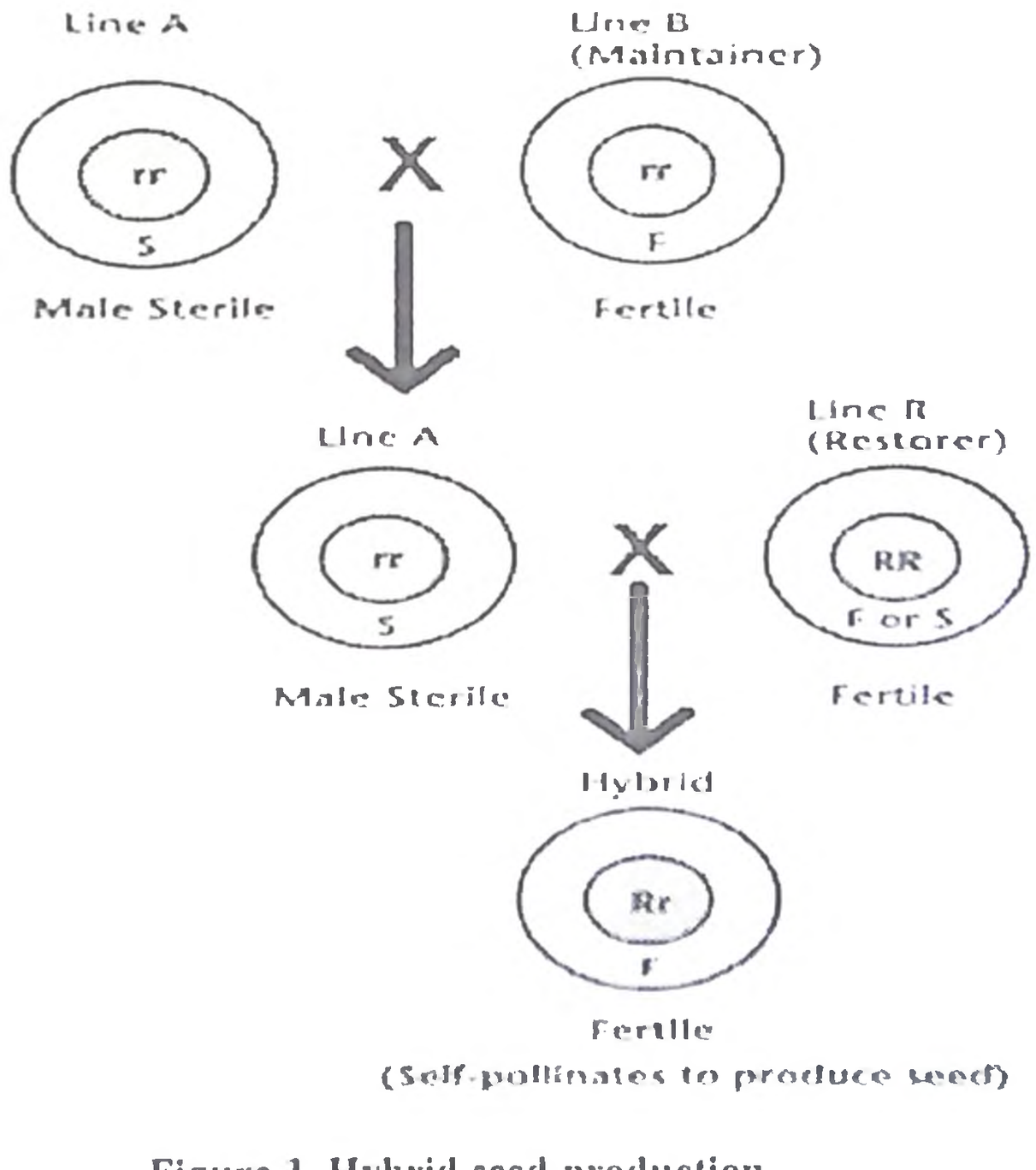

Figure 3. Hybrid seed production

6.1.2.Fertility restoration by restorer loci

All restorers are known to affect either a) the transcript profile or b) the protein accumulation of the CMS - associated locus, and some have been observed to affect both RNA and protein products.

a). Restorer genes affect transcript processing, that is, either increase or decrease the abundance of transcript. Analysis of transcripts of Brassica nap and pol CMS associated region has revealed that restorer gene Rfp results in enhanced processing of a dicistronic transcript so that two monocistronic transcripts increase in abundance. Processing of transcripts of the maize CMS-T urf13 gene has been shown to be correlated with Rf_1 locus (restorer locus). This processing in maize produced decrease in abundance of transcript from CMS locus.

b) Restorer genes affect protein stability - An alteration in transcript profile could be the indirect result of defective translation. In *Brassica napus* CMS restored lines, ORF138 mRNA was found to sediment with polysomes extracted from anthers in polysome analysis, indicating that translation could occur, but no ORF138 (protein from CMS locus causing male sterility) accumulated in floral buds of the restored cybrid line. These results suggest that Rfo (restorer locus) acts post-translationally to affect protein stability.

6.1.3.Mechanism of action of CMS - associated genes or mitochondrial genes

a) Increased levels of a toxic protein in tapetal tissue or sporogenous tissue

Expression of wild-type mitochondrial proteins and mitochondrial transcripts varies between different tissues in the plant, so aberrant proteins could possibly change in concentration during anther development. The enhanced levels of a mitochondrial proteins are an indirect result of an increase in mitochondrial number, which was observed in case of maize.

b). Aberrant protein expressed only in reproductive tissue

In certain species of crops, CMS is due to the aberrant protein expressed only in reproductive tissues. In case of other tissues, this aberrant protein is degraded. In common bean, the CMS - associated gene product is apparently degraded by a protease in mitochondria of vegetative tissue.

c). Impairment of mitochondrial biosynthetic products

Mitochondria play a special role in the development of reproductive tissues like tapetum, male meiotic cells and developing microspores. At this stage in development, demand for energy or particular mitochondrial biosynthetic products may be especially high, so that impairment of mitochondrial function becomes devastating.

d). Antisense down regulation of enzymes

Down regulation of enzymes in various reproductive tissues causes male sterility. Antisense down regulation of alternative oxidase or pyruvate dehydrogenase in the tobacco tapetum resulted in microspore death.

e). Deletion in gene encoding enzymes

Deletion in gene encoding enzymes also cause male sterility. In *Nicotiana sylvestris*, deletion in a gene that encodes a mitochondrial NADH dehydrogenase subunit causes male sterility (Subramanianand Sadasivam, 2001).

6.1.3.1.Effect of CMS - encoding genes on floral morphology

CMS lines of many species exhibit perfectly normal floral morphology, the only obvious difference between flowers of fertile and CMS lines is the absence or presence of pollen or fully developed anthers. In other species, however, male sterility is accompanied by apparent homeotic alterations in floral tissue identity. These changes often have been noted by plant breeders who have performed interspecific crosses. For example, in case of maize, fertile lines exhibit exserted anthers, unlike CMS-T line. For Brassica, flowers containing Ogura cytoplasm exhibit altered stamen morphology. For petunia, CMS and fertile flowers are indistinguishable except for degenerating anthers and a lack of pollen in the sterile line. For sunflower, flowers in CMS lack the pollen evident on wild-type flower. For carrot, stamens in CMS have been converted to petal - or bract - like structures. For tobacco, CMS flowers have no exserted stamens, which are fused with carpels (Hanson and Bentolila, 2004). Cytoplasmic male sterile lines in crops are of different types (Kalloo *et al.*, 2006)(Table 2).

Table 2. Cytoplasmic male sterile lines in crops

Crop	CMS type	Utility
Rice	WA type	Widely used
	BT type	Used
	Gam type	Used
	O-Shan-Tao-Bai	used
Maize	CMS C	Reversion high
	CMS S	Commercially used
	CMS T	Commonly used
Sorghum	Milo	Commercially used
Bajra	Tifton	Commercially used

6.2. Role of mitochondria in optimizing photosynthesis

Photosynthesis is a process of reduction and respiration is a process of oxidation. Both the processes provide ATP for cellular needs. The nature of these two metabolic pathways implies that they complement each other. The sites of photosynthesis and respiration are chloroplasts and mitochondria. Although chloroplasts and mitochondria are traditionally considered to be autonomous organelles, recent studies have established that these two organelles are not only interdependent in their functions but also are mutually beneficial in their interaction. The optimization of photosynthetic carbon assimilation requires the co-ordination of different components : generation and use of assimilatory power (ATP and NADPH), induction of photosynthesis and maintenance of metabolite levels. The function of chloroplast is optimized by the complementary nature of mitochondrial metabolism in multiple ways: facilitation of export of excess reduced equivalents from chloroplasts, shortening of photosynthetic induction, activation of enzymes, maintenance of photorespiratory activity and supply of ATP for sucrose biosynthesis as well as for oxidation of excess chloroplastic reductants. Mitochondrial respiration can prevent over reduction of photosynthetic electron transport chain by oxidizing the excess reductants generated in

chloroplast. Chloroplast reductants are exported to cytosol through malate/ oxalo-acetic acid (OAA) shuttle (Figure 4).

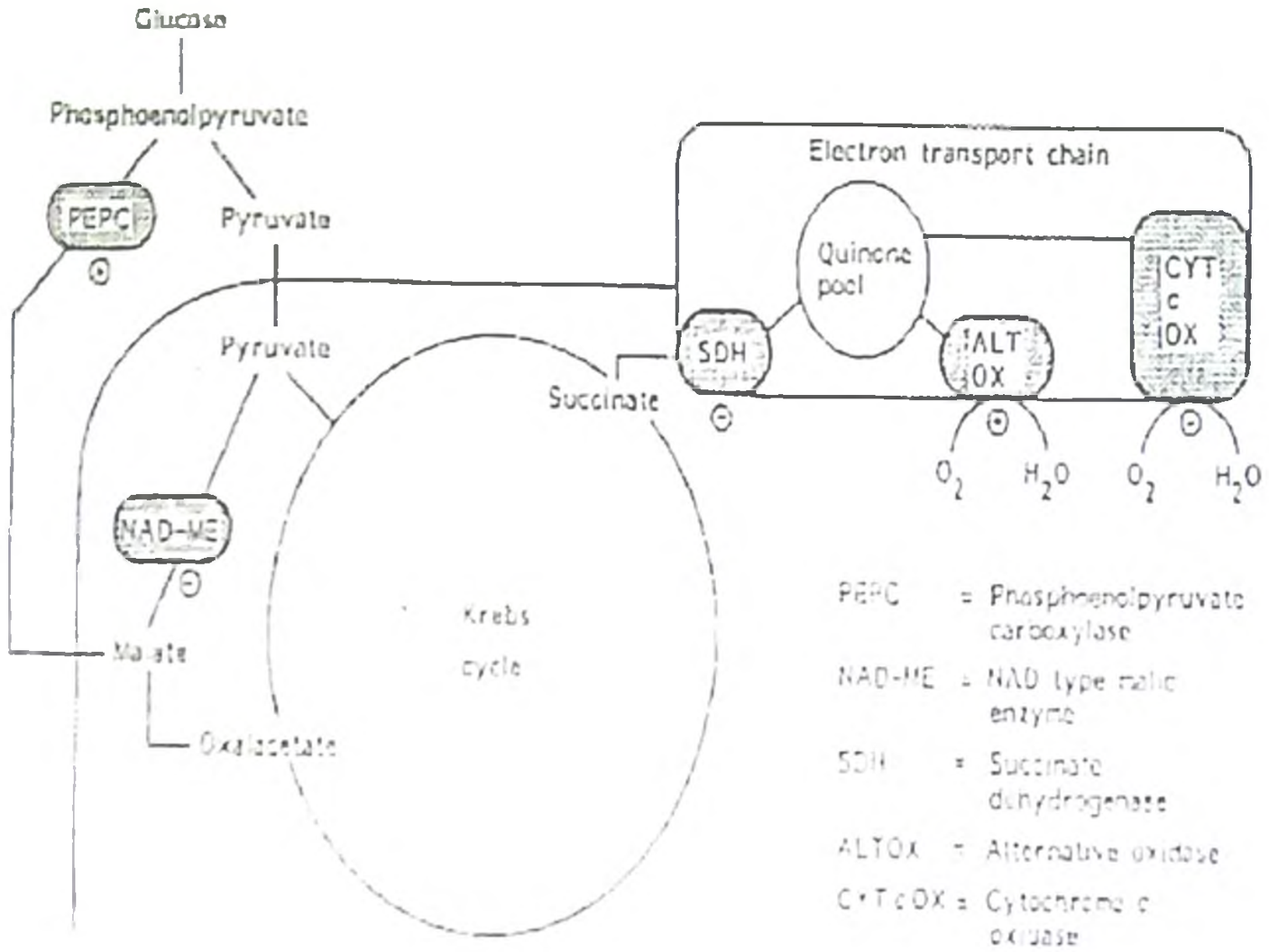

Figure 4. Malate/ oxalo-acetic acid (OAA) shuttle

Deviation from normal respiratory pathway occurs at malate-oxaloacetate point or shuttle Malate/OAA shuttle refers to the inter conversion of these compounds according to necessity of cell

1) Malate possibly enters mitochondria through mito chondrial OAA translocator, where it will be oxidised to OAA by mitochondrial malate dehydrogenase. The NADH thus released in mitochondria could then be used for production of ATP or dissipated through alternative pathway of electron transport This occurs when there is a need for excess energy within the cell

2) If excess energy is not needed by cell, OAA to malate conversion occurs. Malate thus produced pass through reverse process of glycolysis and converted to storage form of glucose, that is, starch Inhibition of alternative pathway by SHAM (salicyl hydroxamic acid) led to a drastic increase in the cellular malate/

OAA levels, indicating an important role for the alternative pathway in dissipating excess photosynthetic reductants through malate/OAA shuttle (Padmavathi *et al.*, 2001).

6.3. Mitochondrial heterosis and complementation

Shull coined the term heterosis and referred it to the superiority of heterozygotes with respect to some measurable attributes in comparison with the corresponding homozygotes. The nuclear DNA molecules are undoubtedly the major genetic determinants and are objects of intraorganism competition and natural selection, but it has not been generally appreciated that abundant and genetically distinct DNA in cytoplasmic organelles fulfill similar conditions of intra cellular competitive interactions. The co-operative intra organism genetic interaction between cellular organelles and between organelles and their nucleus may be responsible for the integration of genetic information available on different genomes, energy supply and substance flow in cellular functions

Mitochondrial heterosis is the increased levels of respiratory function and higher enzyme activities in hybrids observed in artificially mixed mitochondria of certain inbred lines Mechanism of heterosis involve complementation of polypeptide subunits of both mitochondria Resulting products due to this interaction ensures enhanced structural, catalytic and regulatory functions leading to heterosis and adaptive advantage Thus mitochondrial complementation is enhanced oxidative phosphorylation efficiency of artificially mixed mitochondria of certain inbred lines Mitochondrial heterosis and complementation are exhibited in various crops (Table 3)

Table 3. Mitochondrial heterosis and complementation

Parameters	Plant
Ribulose biphosphate carboxylase	Maize, sorghum, barley
Photophosphorylation	Maize, cotton
Hill reaction	Maize, cotton
Adenosine triphosphate	Wheat, maize, Rye, cotton

The more vigorous varieties of maize have more tightly coupled mitochondria than the less vigorous varieties. A correlation between mitochondrial complementation heterosis and grain yield had been interpreted to indicate that mitochondrial activity is rate-limiting for yield and enhanced mitochondrial efficiency, the biochemical basis of heterosis. Positive correlation between seed and oil yield and mitochondrial activity in oil palm had been observed. A relationship between mitochondrial efficiency and heterosis in Asparagus had also been demonstrated. Evidence showed that hybrid mitochondria possess an abundance of lipid-phospholipid, linoleic acid in their fatty acid fraction and a higher amount of internal 'bound' water which are indicative of the physiobiochemical changes in the biochemical systems of hybrid during heterotic expressions. All these observations are in accordance with the view that mitochondria play a significant role in the manifestation of heterosis.

The extensive studies of mitochondrial complementation and grain yield in wheat and maize, have given new clues to the detectable correlation between F_1 yield heterosis and mitochondrial efficiency (Zoble, 1972). Not only do mitochondria of heterotic hybrids exhibit superiority with respect to oxidative and phosphorylative activities, but they also are polymorphic or heterogeneous, as revealed by an elegantly designed experiment to separate the types of mitochondria on a linear sucrose density gradient. These studies on polymorphism of mitochondria in heterotic hybrids of maize and wheat indicated a biparental transmission of mitochondria, the presence of a new hybrid specific type indicated that mitochondria types are not determined only by direct genetic transmission (Jayamani, 2001).

6.4. Tolerance to high soil temperature

Mitochondrial functions contribute to the flexibility that is essential for plant cells in their highly variable environment. Temperature is one of the rapidly fluctuating parameters plants must adjust to studies in *Agrostis* species indicated that maintaining a higher proportion of alternative oxidase pathway at high soil

temperature contribute to root thermo-tolerance. Therefore the alternative pathway plays an important role in plant adaptation to high soil temperatures.

The alternative pathway branches from the main respiratory pathway at ubiquinone and the reduction level of the ubiquinone pool is one of the factors determining the activity of the alternative pathway (Figure 5).

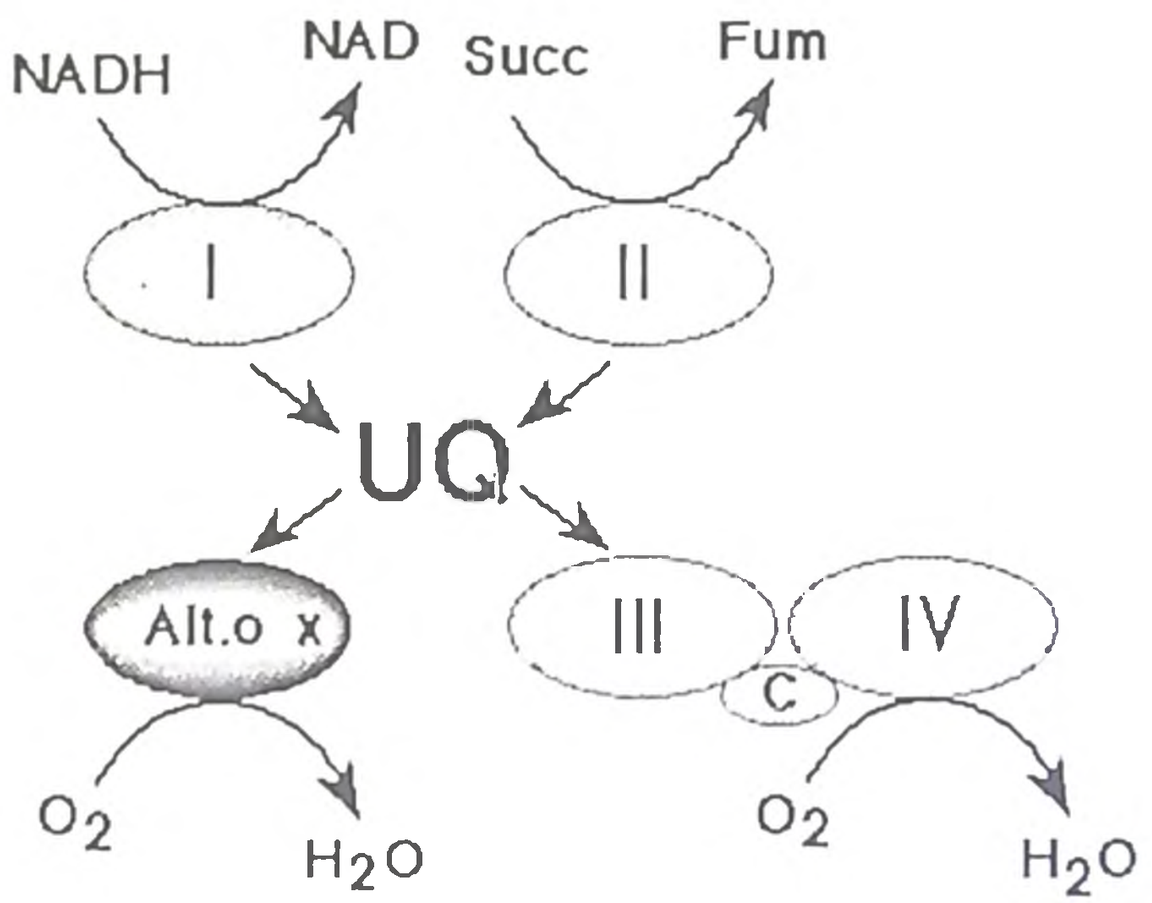

Figure 5. Alternative oxidase pathway

In alternative oxidase pathway, protein complex performing alternative respiration is alternative oxidase (AOX or Alt ox). For purpose of transporting electrons, AOX has a putative iron binding site at its C terminal and the cysteine peptide which enables the formation of active and inactive forms is located towards the N terminal. AOX is a dimer which is in inactive or oxidized form but dimer is split into two proteins at its active form. During alternative respiration process, oxygen is consumed and through a reaction with the electrons transported to the AOX and a proton, water is yielded. The alternative pathway is used to detoxify excess of oxygen produced in cell even if energy produced by this is low. When not many ubiquinones are free to transport electrons, then alternative respiratory pathway would proceed. Temperature-dependent variations in the ubiquinone reduction level are largely dependent on temperature - dependent

changes in adenylate ratios and that flux via the alternative pathway could in some circumstances help lower the maximum reduction level (Moller and Gardestrom, 2007).

6.5. Tolerance to stress

Ever since the introduction of molecular oxygen into our atmosphere, by O_2-evolving photosynthetic organisms, reactive oxygen species (ROS) or reactive oxygen intermediates (ROI) have been the unwelcome companions of aerobic metabolism. In recent years, a new role for ROS was identified : that is, stress tolerance. In stress, cellular production of ROS increases and the mitochondrial electron transport chain (ETC) is one of main sources of reactive oxygen species. ROS acts as signa ing compounds and by various signal transduction pathways, stress defense proteins are activated (Figure 6). Stress defense proteins include heat shock proteins, pathogenesis related proteins, chalcone synthase etc.

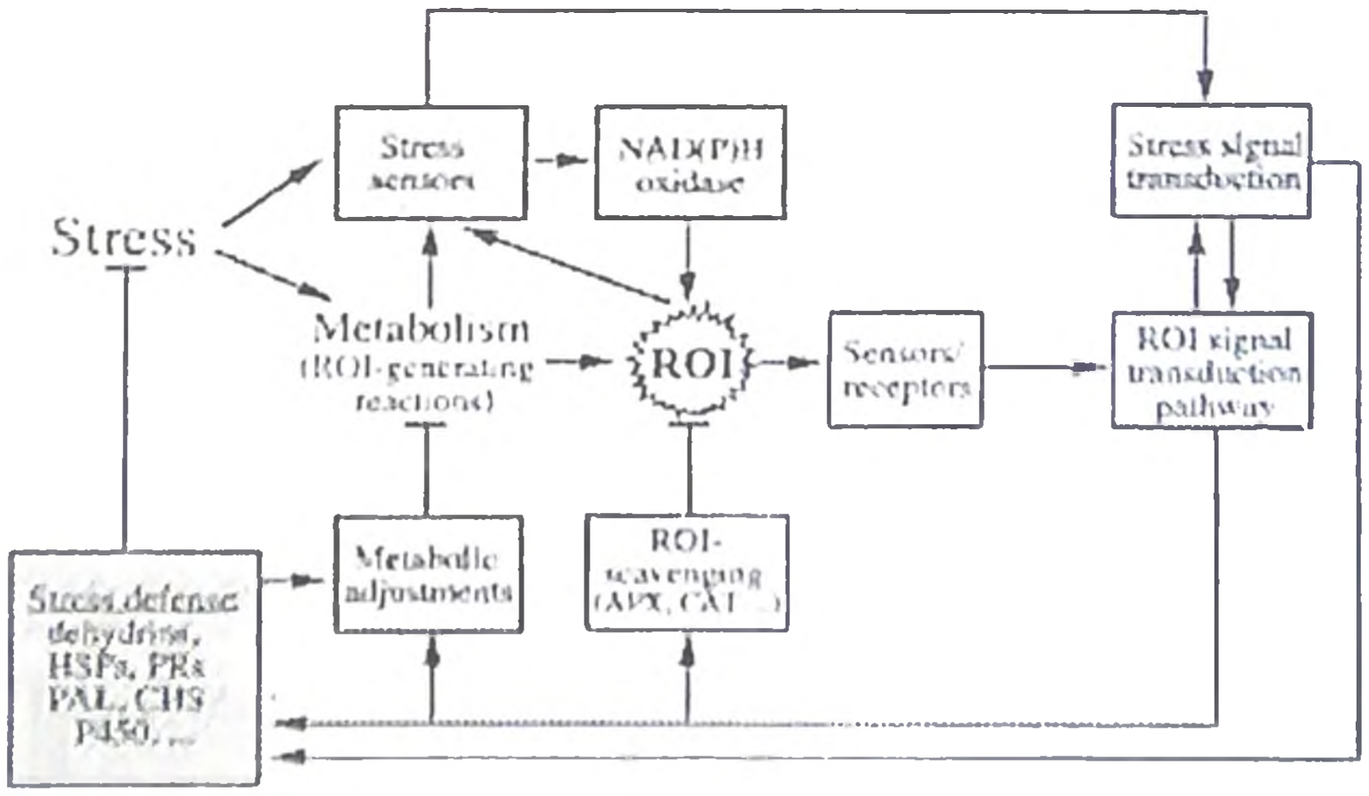

Figure 6. Reactive oxygen intermediates (ROI) cycle

ROS also react with and damage cellular components such as carbohydrates, DNA, lipids and proteins. The enhanced production of ROS during stress enhance the expression of ROS - scavenging enzymes. Major ROS

scavenging enzymes are enzymes of ascorbate biosynthetic pathway, that is, ascorbate peroxidase and catalase. The reaction of ROS with proteins are very complex and lead to a number of different products. Many oxidized mitochondrial proteins have been identified but as yet we know little about the effect of this oxidation on the properties of proteins. The last step in ascorbate biosynthesis is catalyzed by an enzyme located on the outer surface of the inner mitochondrial membrane. The ascorbate is then transported into the matrix where it is an important part of the defense against ROS. However, oxidized ascorbate, dehydroascorbate, needs to be reduced back to ascorbate. ETC of plant mitochondria also plays an important role in the regeneration of ascorbate from its oxidised form ROS induced defense pathways and ROS-scavenging enzymes are identified to be activated in biotic as well as abiotic stress conditions (Novrot *et al*., 2007)

6.6. Stress tolerance of seed mitochondria

The flexibility essential for plant cells in their highly variable environment is contributed by the mitochondrial functions. Seeds of higher plants are desiccation tolerant and mitochondria are functional at the onset of desiccation. The particular properties and functions of mitochondria during seed storage and germination had been studied. By this, it was identified that in dessication tolerance, accumulation of specific stress related proteins occurs in seed mitochondria. This is the major factor contributing to dessication tolerance in seeds. Gene expression studies have been used to identify seed-specific proteins accumulated (Macherel *et al*., 2007)

6.7. RNA editing

Central dogma of molecular genetics states that the information that is found in DNA is used to produce mRNA molecules that are instrumental in the production of proteins. Therefore, the information flows directly from DNA to protein, via the RNA intermediate molecule. Recently, it has been discovered that the information that is constained in the DNA is not always found in the RNA

products used to make proteins. One of the reasons for this is RNA editing. It is process in which information changes at the level of mRNA. Editing is reported in both mitochondria and chloroplast, but more editing is found in case of mitochondrial mRNA. More than 300 different editing events were detected in plant mitochondria. Majority of RNA editing events involve cytidine to uridine transitions. In some cases of RNA editing events, U to C transitions are also reported. RNA editing occurs random in any transcript. In a specific transcript, it modifies 8% to 5.8% of nucleotides. RNA editing are of two types - substitution editing and insertion/deletion editing.

6.7.1.Substitution editing

In this type of editing, chemical alternation of individual nucleotides occur. These alterations are catalyzed by enzymes that recognize a specific target sequence of nucleotides. Cytidine deaminases convert a C in the RNA to Uracil (U). Sometimes adenosive deaminases convert an A to inosine (I), which the ribosome translates as G. Thus a CAG codon (for glycine) can be converted to a CGG codon (for Arginine).

6.7.2.Insertion/Deletion Editing

Editing occurs by insertion or deletion of nucleotides in RNA. These alternations are mediated by guide RNA molecules that base pair with RNA to be edited and serve as a template for the addition (or removal) of nucleotides in the target. That is, the guide RNA contains a sequence complementary to the correctly edited mRNA (Figure 7).

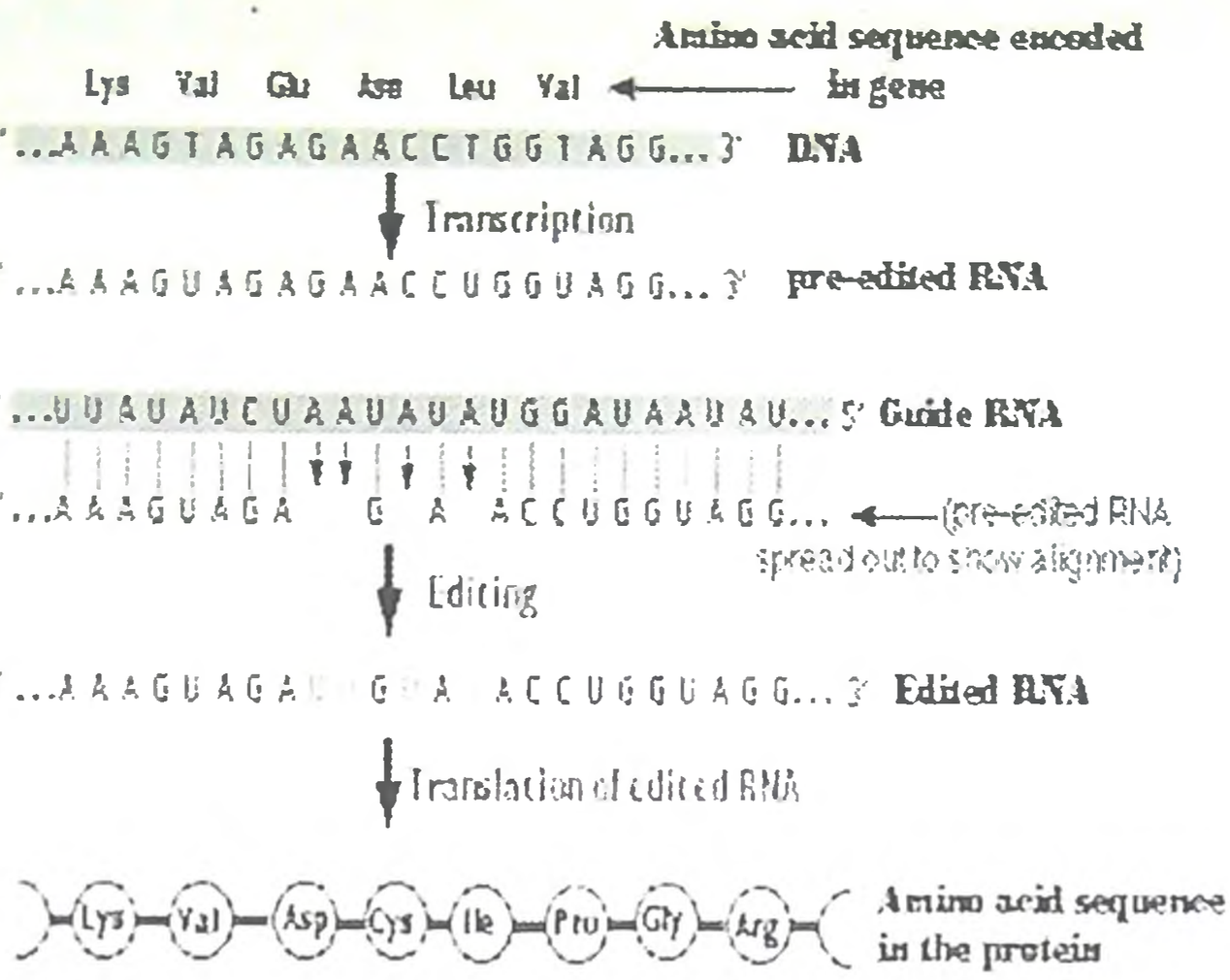

Figure 7. RNA editing by guide RNA

Consequences of RNA editing is the conversion of ammoacid in a polypeptide chain to another ammoacid. Argnmne codon (CGG) is edited to tryptophan codon (UGG). Threonine codon (ACG) is edited to produce start codon (AUG). Stop codons are produced by editing in CAG, CAA, CGA codons.

Importance of RNA editing is that it is a mechanism to increase the number of different proteins available without the need to increase the number of genes in genome. So it can create proteins with slightly different functions to use in specialized circumstances. RNA editing is needed to correct RNA transcripts for harmful mutations in DNA encoding them. There is also evidence that RNA editing of precursor mRNAs is a signal to retain them within the nucleus ready to be quickly exported if needed by the cell (Lewin, 2004).

7.Role of chloroplast genes

7.1.Chloroplast heterosis and complementation

Genetic interaction between cellular organelles and between organelles and their nucleus may be responsible for the integration of genetic information

available on different genomes, energy supply and substance flow in cellular functions. Several distinct lines of evidence from biochemical, physiological, ultra structural and restriction endonuclease DNA fragment analyses in a variety of organisms are available to show that all three genetic sources nuclear genome, mitochondrial genome and chloroplast genome and not just one of them are at work during the manifestation of heterosis. The results of complementary biosynthesis on the genome plasmone of key enzymes of organelle system, eg. Ribulose - 1, 5 biphosphate carboxylase/oxygenase in chloroplasts, provide view insights into the physiochichemical and genetic mechanisms responsible for heterotic expressions.

Chloroplast heterosis is higher photosynthetic rates in hybrids observed in artificially mixed chloroplast of certain in bred lines. Mechanism of heterosis is by complementation of polypeptide subunits of both chloroplasts. Resulting products due to intergenomic interaction in hybrids is expected to ensure enhanced structural, catalytic and regulatory functions leading to heterosis and adaptive advantage. Chloroplast complementation indicates greater activity of 1 1 parental mixture of isolated chloroplasts as compared to mid parental values (Table 4)

Table 4. Chloroplast heterosis and complementation

Parameter	Plant
Cytochrome & phospholipid content	Wheat
Chlorophyll content	Wheat Soyabean Maize

Electron microscopic studies have provided evidence that hybrids are characterized to posses highly developed fine structure of chloroplast than their respective parents. The increase in size of the lamellae and thylakoid membrane

structure in the chloroplast of the hybrid was directly correlated with their chlorophyll contents. In addition to the enhanced activities of key enzymes of the calvin cycle in hybrid chloroplasts, heterosis in chlorophyll content in maize and sorghum has been reported. Results of chloroplast complementation based on Hill's reaction and cyclic phosphorylation in maize showed 21% to 60% increased activity. The enhanced activities due to chloroplast complementation were also found to be closely associated with the degree of grain yield heterosis. These observations on chloroplast heterosis and complementation, just like mitochondrial complementation, suggest that hybrids are endowed with efficient conservation of energy in their organelles (Jayamani, 2001).

8.Chloroplast (plastome) Engineering

Chloroplast genomes defied the laws of Mendelian inheritance at the dawn of plant genetics, and continue to defy the mainstream approach to biotechnology, leading the field in an environmentally friendly direction. Recent development in manipulating the plastid genes is the introduction of foreign genes into chloroplasts and this technique known as plastome engineering. Recent success in engineering the chloroplast genome for resistance to herbicides, insects, disease and drought has opened the door to a new era in biotechnology (Bogorad, 2000). Transplastomic lines have already been synthesized in tobacco, soyabean, brassica, spinach, sweet potato, groundnut etc. plastome engineering certainly has mynad applications especially in crop improvement programmes. Chloroplast genetic maps of several crops including maize, rice, and tobacco have already been characterized.

Chloroplast transformation is an environmentally friendly approach to plant genetic engineering that minimizes out-crossing of transgenes to related weeds or crops and reduces the potential toxicity of transgenic pollen to non target insects. Because the plastid genome is highly polyploidy transformation of chloroplasts permits the introduction of thousands of copies of foreign genes per plant cell, and generates extraordinarily high levels of foreign protein. Chloroplast transformation vectors use two targeting sequences that flank the foreign genes

and insert them, through homologous recombination, at a precise, predetermined location in the organelle genome. This results in uniform transgene expression among transgenic lines and eliminates the 'position effect' often observed in nuclear transgenic plants. Gene silencing, frequently observed in nuclear transgenic plants has not been observed in genetically engineered chloroplasts (Decosa, 2001). Chloroplast and nuclear genetic engineering are compared in Table 5.

Table 5. Comparison of chloroplast and nuclear genetic engineering

Transgenic	Chloroplast proteome	Nuclear genome
Transgene copy no.	Many/cell	species specific
Gene expression level	Abundant transgene transcripts	Gene regulation- rate of transcription
Gene transcription	Transcribed into polycistronic RNA	Transcribed into monocistronic RNA
Gene silencing	Not reported	elimination of transgene expression
Gene containment	Natural gene containment (maternal)	Outcrossing among crops & weeds
Transgenic lines	Uniform gene expression	Highly variable gene expression
Homogenity at ploidy level	Mostly homoplasmic	Either heterozygous or homozygous

(Daniell *et al.*, 2002)

Recently, genes conferring agronomically valuable traits have been introduced via chloroplast genetic engineering. Chloroplasts have also been engineered recently to generate plants tolerant to bacterial and fungal diseases.

drought, herbicides. Transgenes engineered via the chloroplast genome are listed in Table 6.

Table 6. Foreign gene expression in chloroplasts of higher plants

Genes and use	Gene products
Herbicide resistance	
aroA	Glyphosate resistance
bar	Bialaphos resistance
Insect resistance	
Cry1Ac	Bacillus thuringiensis (Bt) toxin
Cry2Aa2	
Pathogen resistance	
msi-99	Bacterial, fungal resistance
Drought or salt tolerance	
ipsl	Trehalose phosphate synthase
BADH	Betaine aldehyde dehydrogenase
Aminoacid biosynthesis	
EPSPS	5-enol-pyruvyl shikimate-3-phosphate synthase
Phytoremediation	
mer A	Mercuric ion reductase
mer B	Organomercurial lyase

(Daniell *et al*.,2002)

8.1. Engineering for herbicide resistance

Engineering crop plants for resistance to herbicide is a strategy to overcome the lack of herbicide selectivity. However, this approach raises the concern that if engineered resistance gene escapes via pollen dispersal, it might result in resistant weeds or might cause genetic pollution among other crops

Engineering foreign genes through chloroplast genomes offers a solution to this problem. As chloroplast genes follows maternal inheritance, engineered resistance gene does not escape to weeds. In addition, the target proteins for many herbicides are compartmentalized within the chloroplast.

Chloroplast genome is engineered to confer herbicide resistance by expressing a petunia EPSPS nuclear gene via the chloroplast genome. The resultant transgenic plants are resistant to tenfold higher levels of glyphosate than the lethal dosage and the transgene is maternally inherited.

Glyphosate, is a potent, broad spectrum herbicide, that is highly effective against grasses and broad leaf weeds. Glyphosate works by competitive inhibition of an enzyme in the aromatic amino acid biosynthetic pathway, 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS). Resistance to glyphosate by chloroplast engineering is mainly by over production of herbicide target (Daniell, 1998).

Transgenic tobacco plants expressing bar genes via the chloroplast genome exhibited field level tolerance to phosphinothricin. Even plants with lowest levels of bar expression were resistant to the highest levels of phosphinothricin tested and no pollen transmission of the transgene was detected bar gene produce enzyme phosphinothricin by acetylation. However, high-level expression of bar genes in the chloroplast was not sufficient to allow direct selection of chloroplast transformants on medium containing PPT (Lutz, 2001).

8.2. Engineering for resistance to pathogens

As plant diseases have plagued global crop production, it is highly desirable to engineer plants that are resistant to pathogenic bacteria and fungi. Recently, a synthetic antimicrobial peptide (msi-99) was expressed via the chloroplast genome. msi-99 is an amphipathic α-helical molecule with affinity for the negatively charged phospholipids found in the outer-membrane of bacteria and fungi. Upon contact with these membranes, individual peptides aggregate to form pores, resulting in microbial lysis. Because of concentration - dependent action of antimicrobial peptides, msi-99 was expressed via the chloroplast genome to

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accomplish high-dose release at the point of infection. Leaf extracts from transgenic plants inhibited the growth of pre-germinated spores of fungal species *Aspergillus flavus*, *Fusarium moniliforme* and *Verticillium dahliae*. Importantly, growth and development of transgenic plants were unaffected by hyper expression of msi-99 within chloroplasts. Because the outer membrane is an essential and highly conserved part of all microbial cells, microorganisms are unlikely to develop resistance against these peptides (Degray, 2001).

8.3. Engineering for drought tolerance

Water stress caused by drought, salinity or freezing is a major limiting factor in plant growth and development. Trehalose is a non-reducing disaccharide of glucose whose synthesis is mediated by the trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase complex in *Saccharomyces cerevisiae*. Because it accumulates under stress conditions such as freezing, heat, salt or drought, there is a general consensus that trehalose protects against damage imposed by these stresses. Therefore, engineering high levels of trehalose in plants might confer drought tolerance.

Gene containment in transgenic plants is a serious concern when plants are genetically engineered for drought tolerance because of the possibility of creating drought-tolerant weeds and passing on undesired pleiotropic traits to related crops. To prevent these consequences, it is desirable to engineer crop plants, for drought tolerance via the chloroplast genome instead of the nuclear genome. Recently, the yeast trehalose phosphate synthase (tps1) gene was introduced into the tobacco chloroplast and nuclear genomes to study the resultant phenotypes. Although the chloroplast transgenic and nuclear transgenic plants expressed significant tps1 enzyme activity, chloroplast transgenic plants showed upto 25-fold higher accumulation of trehalose than nuclear transgenic plants. Nuclear transgenic plants with significant amounts of trehalose accumulation exhibited a stunted phenotype, sterility and other pleiotropic effects, whereas chloroplast transgenic plants grew normally and had no visible pleiotropic effects. Investigations have confirmed that trehalose functions by protecting the integrity

of biological membranes rather than regulating water potential. Therefore, this study shows that compartmentalization of trehalose within chloroplasts confers drought tolerance without undesirable phenotypes (Lee, 2002).

In several studies, a beneficial effect on abiotic stress tolerance against damage by drought and high salinity has been documented when transgenic plants accumulated a number of metabolites, such as fructan, trehalose and mannitol. These metabolites are called compatible solutes because they do not interfere with normal metabolic reactions even at high concentrations. The most obvious function of these compounds is in osmotic adjustment.

Bacterial mannitol-1-phosphate dehydrogenase gene, under control of a double cauliflower mosaic virus 35S promoter, was fused with pea Rbc5 transit peptide sequence to target the enzyme into the chloroplasts of tobacco. This enzyme catalyze the reaction from Fructose-6-phosphate to mannitol-1-phosphate. Mannitol-1-phosphate is then converted to mannitol by a nonspecific phosphatase in tobacco. Mannitol amounts in all of the transformants were dependent on plant and leaf age, with the highest amounts found in plants before flowering. Mature leaves accumulated more mannitol than young leaves. Chloroplast location of mannitol can supplement endogenous radical scavenging mechanisms and reduce oxidative damage of cells by hydroxyl radicals (Shen *et al*., 1997).

8.4. Engineering for increasing crop productivity

Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) is the most prevalent enzyme on this planet, accounting for 30-50% of total soluble protein in the chloroplast. It fixes carbondioxide, but oxygenase activity severely limits photosynthesis and crop productivity. Genetic manipulation of Rubisco to improve its function has involved both nuclear and plastid genetic engineering, mostly in tobacco. Synthetic Rbcs gene was integrated into chloroplast and nuclear genomes. Approximately 150 fold more Rbcs transcript obtained in chloroplast transgenic lines. This resulted in achieving efficient photosynthesis and increased crop productivity (Dhingra *et al*., 2004).

8.5.Limitations of chloroplast engineering

Chloroplast engineering is much more difficult than nuclear engineering, which is now more or less routine. Some of the difficulties encountered during chloroplast engineering are outlined below:

1. Lack of information on genome sequences for several important crop species to locate intergenic sequencies for integration of transgenes. Because of conservation of plastid genome sequence across many plant species, the concept of using targeting sequences from one species to transform the plastid genome of another unknown species was developed.

2. The methods of transgene transfer into chloroplasts are limited due to the double plastid membrane. Particle bombardment method is most efficient in transformation, but it is expensive.

3 The recovery of transplastomic cells is particularly dependent on efficient and prolonged selection procedure. Typically, 2-4 regeneration and selection cycles under the selection pressure are required for recovery of homoplastomic transplastomic shoots.

4 The transformation systems are far more successful with tobacco than with other plant species. In fact, few other plant species, eg. tomato, are at present amenable to chloroplast transformation. Thus there is an urgent need to develop more generally applicable and efficient chloroplast transformation protocols (Singh, 2006).

9.Conclusion

Significant roles are played by chloroplast and mitochondrial genome in crop plants. A single plant cell contains many molecules of ctDNA and mtDNA. These organelle genomes are packed into one or many organelles each of which may contain many DNA molecules. The DNA is localized into discrete regions which are called "nucleoids" by anlogy with similar regions in prokaryotes. Each chloroplast in leaf parenchyma cells of *beta vulgaris* contains 4-18 nucleoids, and each nucleoid is estimated to have 4-8 ctDNA molecules. The population of organelle genomes within a cell is divided into subpopulations in

individual organelles and in nucleoids, and these populations within populations complicate the analysis of organelle transmission genetics.Chloroplast or mitochondrial genes appear to be present in multiple copies distributed at a number of independent DNA containing nucleoids within the organelle, representing organelle genome polyploidy.

The nuclear DNA molecules are undoubtedly the major genetic determinants and are objects of intraorganism competition and natural selection, but it has not been generally appreciated that abundant and genetically distinct DNA in cytoplasmic organelles fulfill similar conditions of intra cellular competitive interactions. The co-operative intra organism genetic interaction between cellular organelles and between organelles and their nucleus may be responsible for the integration of genetic information available on different genomes, energy supply and substance flow in cellular functions. So extranuclear genes are considered as an accessory heredity source apart from nuclear genes. As individuals with extranuclear inheritance generally receive plasmagenes from one parent only, F_2 and subsequent generations do not show segregation for extranuclearly inherited trait. Therefore character is stable which offers scope for genome manipulation

Chloroplast transformation is an environmentally friendly approach to plant genetic engineering that minimizes out-crossing of transgenes to related weeds or crops and reduces the potential toxicity of transgenic pollen to non target insects. Because the plastid genome is highly polyploidy transformation of chloroplasts permits the introduction of thousands of copies of foreign genes per plant cell, and generates extraordinarily high levels of foreign protein. Gene silencing, frequently observed in nuclear transgenic plants has not been observed in genetically engineered chloroplasts. Instead of being enslaved by nucleus, the endosymbiont defiantly marches on, leading the crop plants in new directions

Discussion

1.Is *Agrobacterium* mediated transformation used for chloroplast engineering?

Agrobacterium mediated transformation is not used for chloroplast engineering due to the double plastid membrane. Particle bombardment method is most efficient in transformation, but it is expensive. Chloroplast transformation vectors use two targeting sequences that flank the foreign genes and insert them, through homologous recombination, at a precise, predetermined location in the organelle genome.

2.What is WA, Bt, Gam type in rice?

WA (Wild abortive) type, Bt (Chinsurah boro) type, Gam (Gambiaca) type are cytoplasmic male sterile type used for hybrid seed production in rice. Wild abortive type is the most commonly used type eventhough fertility restoration is less than others. This is because of the highest percentage of heterosis exhibited in hybrids. In Gambiaca type, fertility restoration is more

3.What method is used for chloroplast engineering?

The methods of transgene transfer into chloroplasts are limited due to the double plastid membrane. Particle bombardment method is most efficient in transformation, but it is expensive. Chloroplast transformation vectors use two targeting sequences that flank the foreign genes and insert them, through homologous recombination, at a precise, predetermined location in the organelle genome. This results in uniform transgene expression among transgenic lines and eliminates the 'position effect' often observed in nuclear transgenic plants

4. How development of superweeds avoided in chloroplast engineering?

Engineering crop plants for resistance to herbicide is a strategy to overcome the lack of herbicide selectivity. However, this approach raises the concern that if engineered resistance gene escapes via pollen dispersal, it might result in resistant weeds or might cause genetic pollution among other crops. Engineering foreign genes through chloroplast genomes offers a solution to this

problem. As chloroplast genes follows maternal inheritance, engineered resistance gene does not escape to weeds. In addition, the target proteins for many herbicides are compartmentalized within the chloroplast.

5. What are limitations in chloroplast engineering?

Lack of information on genome sequences for several important crop species to locate intergenic sequencies for integration of transgenes. The methods of transgene transfer into chloroplasts are limited due to the double plastid membrane.The recovery of transplastomic cells is particularly dependent on efficient and prolonged selection procedure. The transformation systems are far more successful with tobacco than with other plant species.

6 Why genetic engineering is not practiced in mitochondrial genes?

Lack of information on mitochondrial genome sequences for several important crop species to locate intergenic sequencies for integration of transgenes. Targetting sequences for targeting transgene into mitochondria has not been identified. The methods of transgene transfer into mitochondria are limited due to the double plastid membrane

7 Incorporation of gene into chloroplast is random or specific?

Chloroplast transformation vectors use two targeting sequences that flank the foreign genes and insert them, through homologous recombination, at a precise, predetermined location in the organelle genome. This results in uniform transgene expression among transgenic lines and eliminates the 'position effect' often observed in nuclear transgenic plants

8 What are pathogens controlled by chloroplast engineering?

Leaf extracts from chloroplast transgenic plants inhibited the growth of pre-germinated spores of fungal species *Aspergillus flavus*, *Fusarium monliforme* and *Verticillium dahliae*. Importantly, growth and development of transgenic plants were unaffected by hyper expression of antimicrobial peptide within

chloroplasts. Because the outer membrane is an essential and highly conserved part of all microbial cells, microorganisms are unlikely to develop resistance against these peptides.

9.How stability of trait maintained in extranuclear inheritance?

As individuals with extranuclear inheritance generally receive plasmagenes from one parent only, F_2 and subsequent generations do not show segregation for extranuclearly inherited trait. Therefore character is stable which offers scope for genome manipulation. Gene silencing, frequently observed in nuclear transgenic plants has not been observed in genetically engineered chloroplasts

10.What is role of RNA editing?

Importance of RNA editing is that it is a mechanism to increase the number of different proteins available without the need to increase the number of genes in genome. So it can create proteins with slightly different functions to use in specialized circumstances. RNA editing is needed to correct RNA transcripts for harmful mutations in DNA encoding them

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Abstract

Inheritance of certain characters in eukaryotes show maternal influence. Usually these characters indicate consistent reciprocal differences and lack of segregation. This was first reported by Correns in 1908 and this type of inheritance is referred to as non mendelian inheritance. Sum total of genes in the cytoplasm of a cell is known as plasmon and this include mitochondrial chondriom and chloroplast plastome (Singh, 2006).

Chloroplast and mitochondrial genes play significant role in different metabolic processes in plants such as nitrogen assimilation, maintenance of carbon and nitrogen balance etc. (Smith and Atkins, 2002). Mitochondrial genes play a key role in inducing male sterility. Both mitochondrial and chloroplast genes exhibit heterosis and complementation The mitochondrial genome in plants codes for only 30-40 of the 2000-3000 gene products present in the functional organelle(Moller and Gardestrom, 2007). DNA of organelles has been sequenced in *Arabidopsis*, rice, liverwort, maize, tobacco etc (Friso *et al* , 2004)

Introduction of foreign genes into chloroplasts known as plastome engineering was first reported in 1988 by Boynton and colleagues. Chloroplast transformation is an environmentally friendly approach in that it minimizes out-crossing of transgenes. As plastid genome is more in number, transformation of chloroplasts generates extraordinarily high levels of foreign protein which is important in genetic engineering. Limitations of chloroplast genetic engineering are lack of information on plastome sequences, difficulty in delivering foreign DNA through double plastid membrane etc. (Daniell *et al* , 2002)

Detection of quantitative trait loci (QTLS) for crop improvement

BY

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(2006-21-105)

Department of Plant Breeding and Genetics

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

PbGen. 752 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala

2007

DECLARATION

I hereby declare that the seminar report entitled '**Detection of quantitative trait loci (QTLS) for crop improvement**' is a record of the seminar presented by me during the course on 06.07.2007 and that this report has been prepared by me independently after going through the references cited herein

Vellanikkara
6.08.2007

Vidhu Francis Palathingal
(2006-21-105)

MS—

CERTIFICATE

Certified that the seminar report entitled 'Detection of quantitative trait loci (QTLS) for crop improvement' is a record of seminar presented by **Smt. Vidhu Francis Palathingal (2006-21-105)** under my guidance and that this report has been prepared by her independently

Vellanikkara
6.08.2007

Dr. V.V.Radhakrishnan
Major Advisor

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Detection of quantitative trait loci (QTLS) for crop improvement

1. Introduction

In the early history of genetics, it was recognized that some characters showed discontinuous variation, while a majority of biologically important traits exhibited continuous variation. Most of the traits of economic importance in all organisms show continuous variation. As a rule, characters showing continuous variation are subjected to measurement . Hence data on such traits are called as measurement or quantitative data and such characters are termed as quantitative characters or traits. The two characteristic features of quantitative characters are: 1)continuous variation and 2) a marked influence of the environment on their expression. The presence of continuous variation makes their genetic analysis through the classical Mendelian approach impossible.This may have been one of the reasons for the suggestion by a group of early workers that the inheritance of quantitative characters was nonmendelian. The large environmental effects on these traits makes it very difficult to determine, without appropriate trails if the expression of a character in an individual is due to heredity or environment. This may have prompted some of the early workers to suggest that continuous variation is nonheritable

2. Quantitative traits

2.1 Qualitative and quantitative traits

Different traits follow different inheritance pattern as it is simple Mendelian inheritance in case of qualitative traits while complex in quantitative traits. One or few oligogenes with large, easily detectable effects goven qualitative traits whereas quantitative traits are controlled by polygenes with small individual effects. Quantitative traits fall in to distinct classes and little affected by environment but quantitative characters show continuous distribution with marked influence of environment. Different traits like coluor and shape of flowers, pods, seeds, etc. are quantitative traits whereas complex traits like yield , agronomic traits, stress tolerance are grouped under quantitative traits Qualitative

traits are measured by typical Mendelian ratios and counts while quantitative traits are determined by chi-square analysis.

2.2.Multiple factor hypothesis

Primary study of quantitative traits was conducted by Yule in 1902. He suggested that quantitative characters are likely to be governed by many genes. Theoretically Yule demonstrated that if it was assumed that each of the several genes affecting a quantitative character had small and cumulative effect, and the genes did not show dominance, they would produce a continuous variation. Based on this, he proposed multiple factor hypothesis. He postulated that continuous quantitative variation was produced by multitude of individual genes, each with small effect on the measured traits. These genes with small effect on the measured traits are called polygenes. Yule failed in providing experimental evidence for multiple factor hypothesis. Later Johannsen published the results from selection for seed size in *Phaseolus vulgaris* and put forth the concepts of pure line , genotype and phenotype. These results clearly showed that a part of variation in quantitative traits was heritable, but a substantial portion of it was due to the environment and hence, nonheritable.

2.3.Experimental evidence for multiple factor hypothesis

In 1908, Nilsson-Ehle provided the experimental evidence for the existence of multiple factors by his studies in wheat. Therefore, he is credited with the concept of multiple factor inheritance. In studies on the inheritance of seed colour in wheats, Nilsson-Ehle obtained 3:1 , 15:1 and 63:1 ratios between coloured and white seeds from different crosses. It is clear from these ratios that in these crosses seeds colour was governed by one (3:1 ratios in F_2) ,two (15:1 ratio in F_2) or three (63:1 ratio in F_2) genes. Apparently, seed colour in wheat is produced by one,two or three genes (Figure 1)

When two or three genes for colour are present together, they seem to interact in duplicate manner so that white seed colour is produced only when all the genes are present in the recessive state. But on a close examination of the coloured seeds, Nilsson-Ehle found that there were marked difference in the intensity of their colour. Therefore, he further classified the coloured seeds on the basis of intensity of their red colour . These red seeds from crosses showing 15:1 red ratio could be classified in to four district classes on the basis of colour intensity. There classes were dark red, medium –dark red, medium red and light red and they were present in the ratio 1 4 6 4. This ratio was first clear- cut demonstration for the existence of multiple factors. Thus the positive alleles of all the genes governing the trait are similar to each other in their action (effect) and their effects are additive or cumulative in nature. This is the essence of multiple factor hypothesis (Singh, 2003).

2.4 Normal distribution of quantitative traits

Continuous variation is quantitative traits produces a bell shaped curve indicating normal distribution (Figure 2). From the curve, it is observed that extreme phenotypes among F_2 is quite rare while intermediate phenotypes those near average or mean is more frequent. Also the effect of environment produces a continuous phenotype. This differentiate quantitative traits from qualitative traits . This is indicated by frequency distribution of quantitative and qualitative traits (Figure 3)

In case of qualitative traits like stem colour. Different lines indicates two district classes, that is, purple and green. But is the case of quantitative traits like biomass, different lines show a continuous variation for the biomass. Thus the phenotypic variation of and conditioned by several polygenic, each with a relatively small effect. This indicates the complexity of quantitative traits (Singh and Narayanam, 2006).

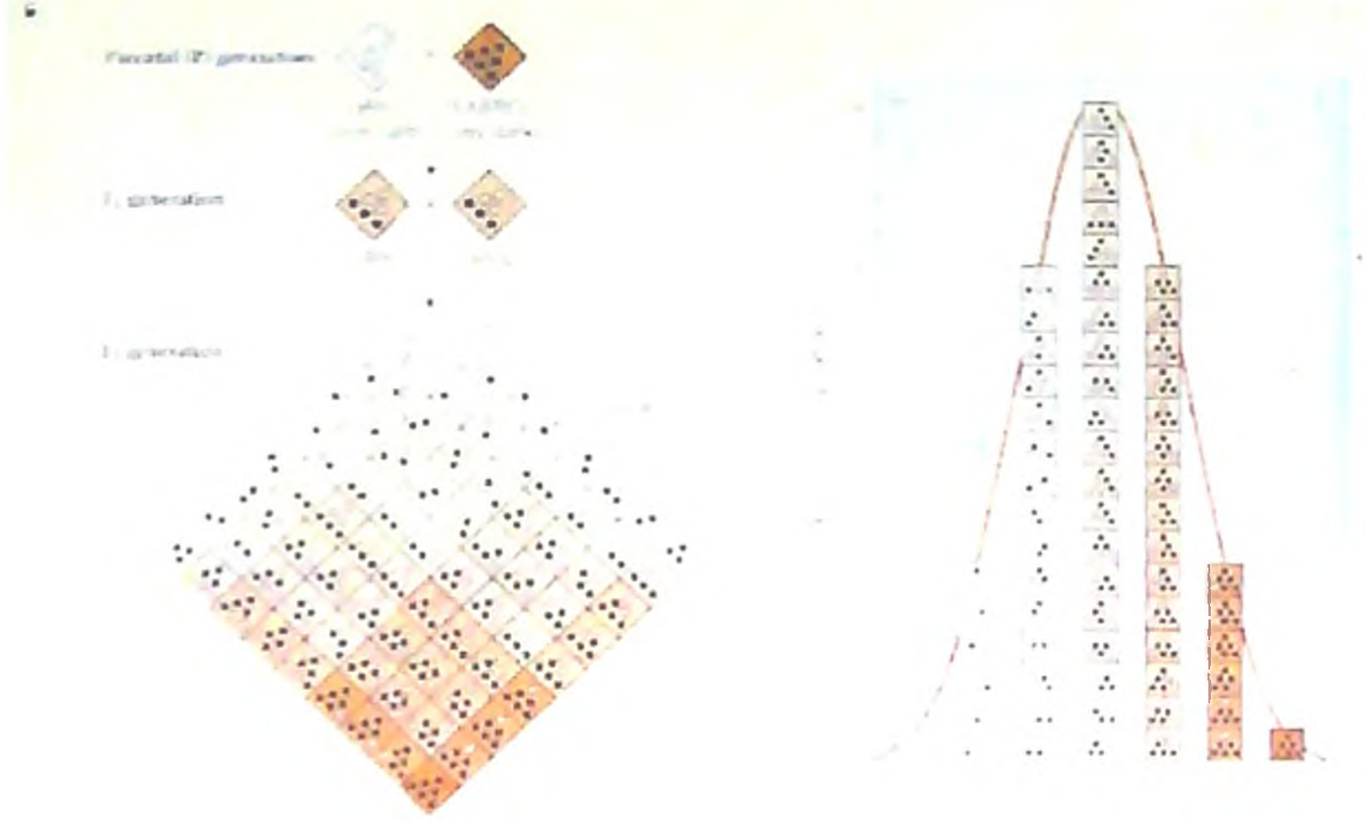

Figure1. Inheritance pattern of quantitative traits

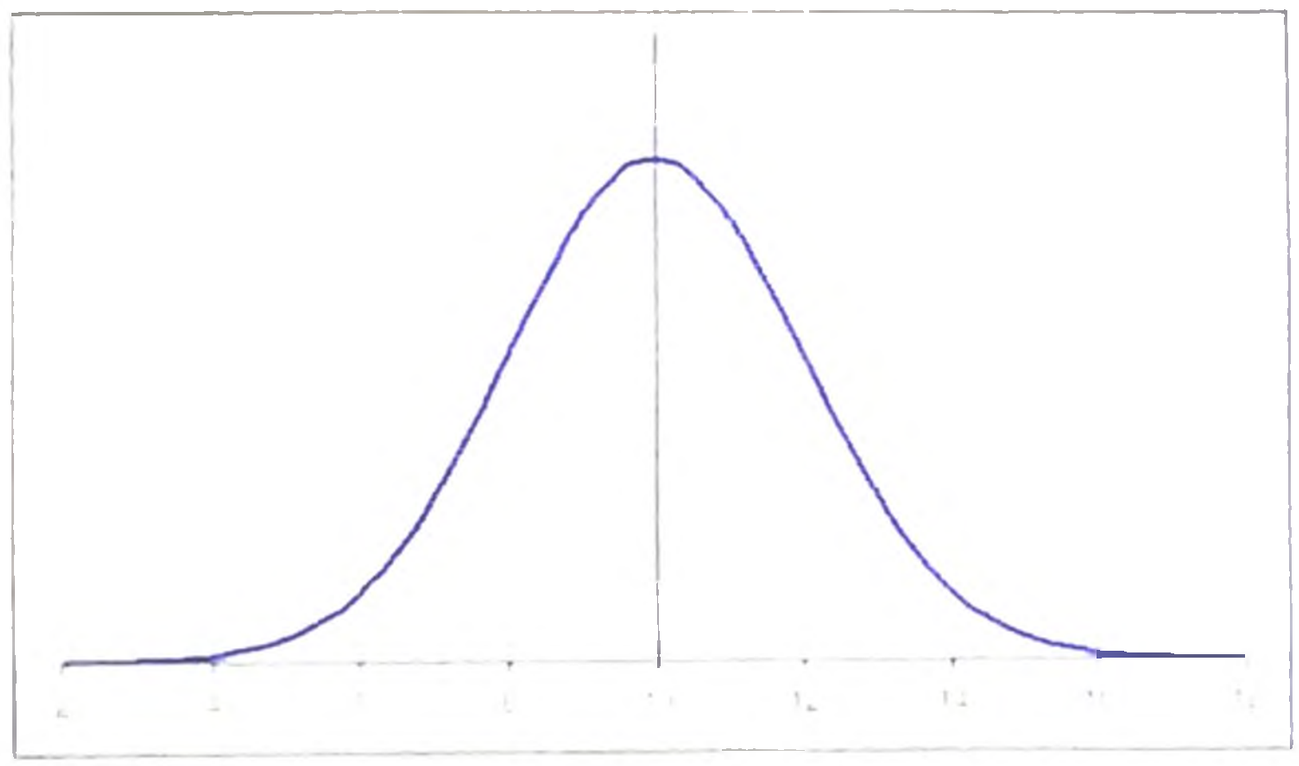

Figure 2. Normal distribution of quantitative traits

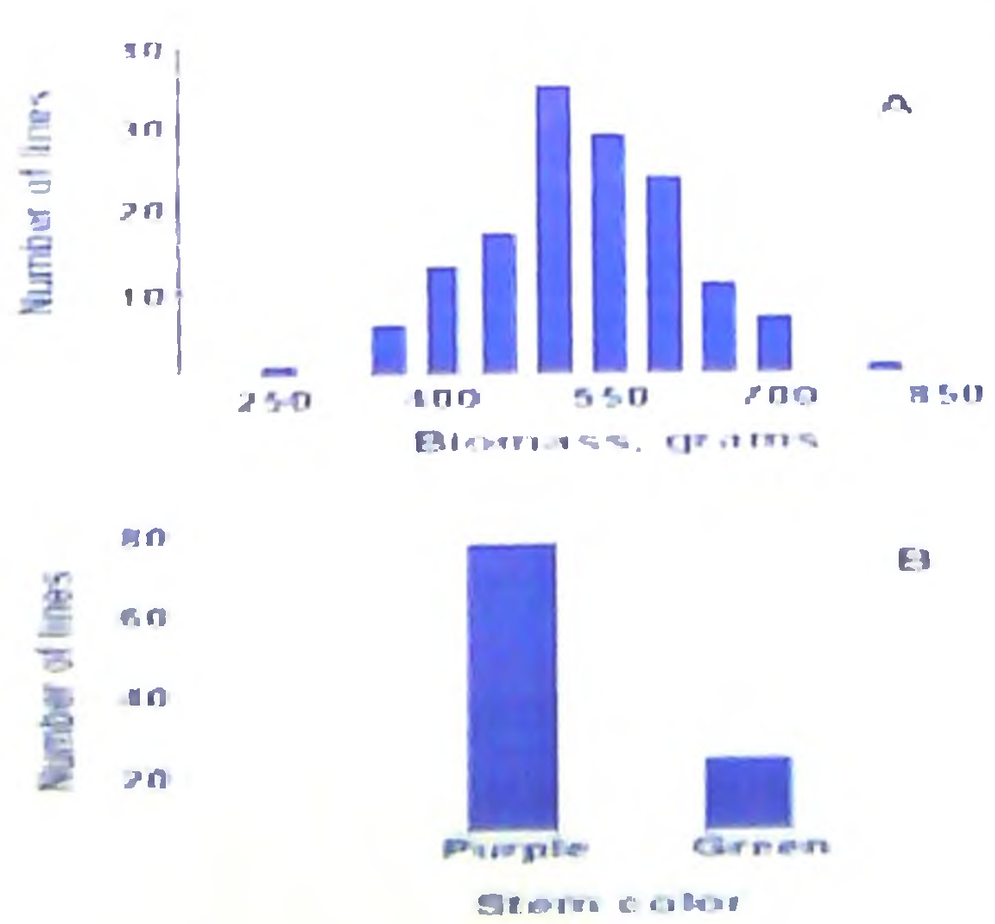

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Figure 3. Frequency distribution of quantitative and qualitative traits

3.Quantitative trait loci

Characters whose phenotypic variation is continuous and determined by the segregation of multiple loci hare often been referred to as quatitative traits and the inheritance as polygenic. The individual loci controlling a quantitative trait are referred to as polygenic or quantitative traits loci (QTL).

Different polygenes are unequal in magnitude of effects they exert on a character. Enough data has now been accumulated from marker studies to establish definitively that polygenes do vary widely in their effects and that the assumption of many polygenes with equal effects is not valid. More surprising has been the finding that in many instance , a large proportion of quantitative variation can be explained by the segregation of a few major QTL. QTL with major effects have been identified for different characters studies, but most by far of QTL reported are those of smaller effects. The smallest effect of a QTL can have and still be detected by the marker method depends on a number of factors

a) The map distance from nearest marker to QTL the closer a QTL is to a marker, the smaller the effect that QTL can have and still be detected statistically because the effects of QTL closer to the marker are less confounded by recombination events between marker and QTL

b) Size of segregating population the larger population size, the more likely the effects of lesser QTL reach statistical significance

c) Heritability of traits - the larger the environment effect on character, the less likely a QTL will be detected

d) Probability criteria used for declaring a QTL effect significant. Higher probability thresholds reduce the chance of spurious QTL being reported, but also reduces the chances of detecting QTL with smaller effects (Tanksley ,1993).

4.Detection of QTL

Detection of QTL has difficulty to determine how efficient an experiment has been in identifying the QTL responsible for a trait. One measurement of success has been the cumulative phenotypic variance attributable to the combination of all significant QTL. The bias towards detecting QTL with larger effects means that it is unlikely that one will ever defect, map, and characteringall of the polygenes affecting a character in any given segregating population . Using the marker approach, one is likely to identify and characteringQTL making the largest contribution to phenotype and it is likely to be these QTL that one would want to further characters and ultimately to clone For plant breeders, the major QTL are of greatest interest to manipulate in breeding schemes via association with molecular markers

4.1Conceptual basis of QTL detection

The theory of QTL detection was first described in 1923 by sax, where be noted that seed size in been (a complex trait) was associated with seed coat color (a simple monogenic trait) This concept was further elaborated by Thoday in 1961, who suggested that if the segregation of simply inherited monogenes cold be used to detect linked QTLS, then it should eventually be possible to map and characterise traits Modem QTL mapping is essentially the fulfillment of this idea, with the key innovation being that defined sequences of DNA act as the linked monogenic markers With the development of comprehensive DNA marker maps, it is now possible to search for QTL throughout the genomics of most crop species

QTL detection involves testing DNA markers throughout a genomic for the likelihood they are association with a QTL Individuals in a suitable mapping population are analyzed in terms of DNA marker genotypes and phenotype of interest . For each DNA marker , the individuals are split in to classes according to marker genotype Mean and variance parameters are calculated and compared among the classes suggests there is a relationship between the DNA marker and

the traits of interest. In other words, DNA marker in probably linked to a QTL. From the basis of QTL detection, it is clear that for any QTL detection there is a basic requirement of molecular markers and a mapping population (Young,1996).

4.2Advent of markers in quantitative genetics

The advent of markers has made it feasible to map and charactering the polygenes quantitative traits in natural populations (Martiney and Curnow,1992).

Morphological markers identified by macro-mutant alleles are rare in natural population. Without allele variation , there is no segregation, and without segregation no linkage tests can be performed to detect polygenes. Number of useful morphological markers for quantitative genetics was so limited that in most studies, only a few markers were used, representing only a small fraction of the genetic Most morphological markers loci segregate to dominant -recessive alleles In case of relationship between genotype and phenotype, that is, only homozygous recessive genotype can be deduced unambiguously from phenotype. With Morphological marker loci, strong epistemic interactions among loci limit the number of segregating markers that can be unequivocally scored in the save generation (Singh, 2006).

The discovery that allelic forms of enzymes or isozymes can be separated on electrophoresis gels and detected with histochemical activity stains heralded the era of molecular markers in genetics research By the early 1980s , isozyme markers were being employed as a general tool for mapping polygenes and these studies met with considerably more success them previous studies using morphological markers Eventhough the markers was limited by the number of available enzyme activity stains and in no instance were there enough informative isozyme markers cover entire genome

The next advance in molecular markers came with the introduction of DNA based genetic markers, the first of which was restriction fragment length polymorphism (RFLP) (Young and Tanksley, 1989) . In the past years, a new

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generation of DNA based genetic markers based on the polymerase chain reaction (RAPDs) has been developed. As with isozymes allelic variation for DNA based markers usually has no detectable phenotypic effect. But unlike isozymes, the genetic variation is surveyed directly at the DNA level and thus can reveal more polymorphism.

The key properties that differentiate molecular markers from other markers and have permitted the rapid advance of polygene mapping are

1 Phenotypic neutrality- problem with marker gene having a phenotypic effect than the linked polygene in largely overcome with molecular markers. Alternative alleles at molecular marker loci usually cause no obvious changes in the phenotype of an organism For DNA based markers, most of the allelic variation is the noncoding portion of the genome

2 High level of polymorphism – the level of polymorphism maintained at any gives locus in natural populations is determined by many factors, including population size, mating habit, selection, mutation rate and migration Two of these factors, relaxed selection pressure and higher mutation rates cause allelic variation to be higher at molecular marker loci In addition, the laboratory techniques used to monitor molecular markers eg gel electrophoresis , restriction enzyme analysis and polymerase chain reaction amplification are more sensitive in detecting existing variation

3 Abundance – If enough segregation marker genes are scattered throughout an entire genome, it is theoretically possible to detect and characterng all of polygene affecting a quantitatively inherited character. DNA based markers solved the problem of limited marker abundance. The availability of incomplete genome maps also opened up the opportunity for new statistical approaches for detecting polygene.

4. Codominance – For loci with codominance alleles , there is a one to one relationship between and codominant. Thus the advent of DNA-based markers has allowed straight forward polygene mapping in virtually any segregating generation.

5. Epistasis – Epistasis is a form of interaction between non allelic genes whereby one gene interferes with the phenotypic expression of another gene (Wade, 1992). With morphological marker loci, strong epistatic interactions among loci limit the number of segregating markers that can be unequivocally scored in the save generation . Because molecular marker loci do not normally exhibit epistastic or pleiotropic effects, a virtually limitless number of segregating markers can be used in a single genome (Singh, 2006)

4.3 Mapping population

The assumption underlying the use of marker loci to detect polygenes is that linkage disequilibrium exists between alleles at the marker loci and allele of linked polygene. Linkage disequilibrium can be defined as the nonrandom association of at different loci in a population and can be caused by a number of factors including selection and genetic drift. However in primary segregating generations (eg. F_2, F_1, or backcross population), the predominant cause of linkage disequilibrium is physical linkage of loci and this has formed the basis for classical linkage mapping for past century. Linkage disequilibrium due to physical linkage of loci is that its highest value in population derived from controlled mating and as a consequence the ability to map and characterizing polygene using marker loci is also at its highest in population from controlled matings

For most plants, it is possible to select any two plants and make controlled matings between them. Controlled mating have the advantage of allowing the investigator to pick individuals that differ significantly for the character of interest . The phenotypic difference between any individuals, the more likely one is to

detect significantly QTL controlling that character in a derived, segregating population. Controlled mating are also advantageous in that they results in maximum linkage disequilibrium for detecting QTL with molecular markers normally requires analysis of fairly large segregating populations (that is, > 100 individuals). Although most plants readily produces progeny in large numbers, not all species do. There for the types of modified population employed for QTL mapping are largely a function of the reproductive characteristics of the species under study (Paterson *et. al.*, 1988)

Back cross or F_2/F_3 population have been most commonly used for detecting linkage between molecular markers and genes controlling quantitative traits. Species where severe inbreeding is there modified populations are used or detecting QTL The modified populations used for QTL detection are

4.3.1 Recombinant inbred lines (RILs)

Recombinant inbred lines called F_2-derived lines, are homozygous lines derived from individual F_2 plants from a suitable cross. RILs provides an excellent mapping strategy and a permanent mapping source. RILs are created by single seed descent (SSD) from F_2 plants through atleast five or more (usually >8) generations of continued selfing (Figure) This process yields a set of lines each of which contain a different combination of linkage blocks from the original parents The different linkage blocks in these RILs provide a basis of linkage analysis RIL populations are, theoretically, immortal since they can be maintained for an indefinite period of time (Figure 4)

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A. Recombinant Inbred Lines (RIL)

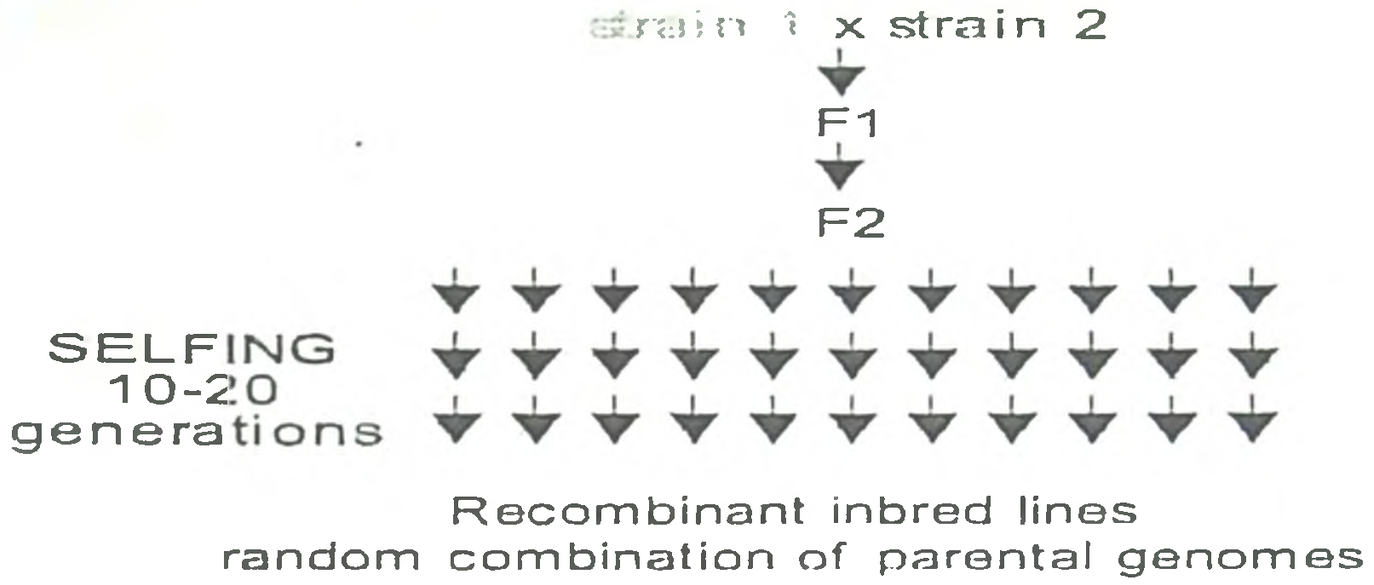


Figure 4. Production of Recombinant inbred lines

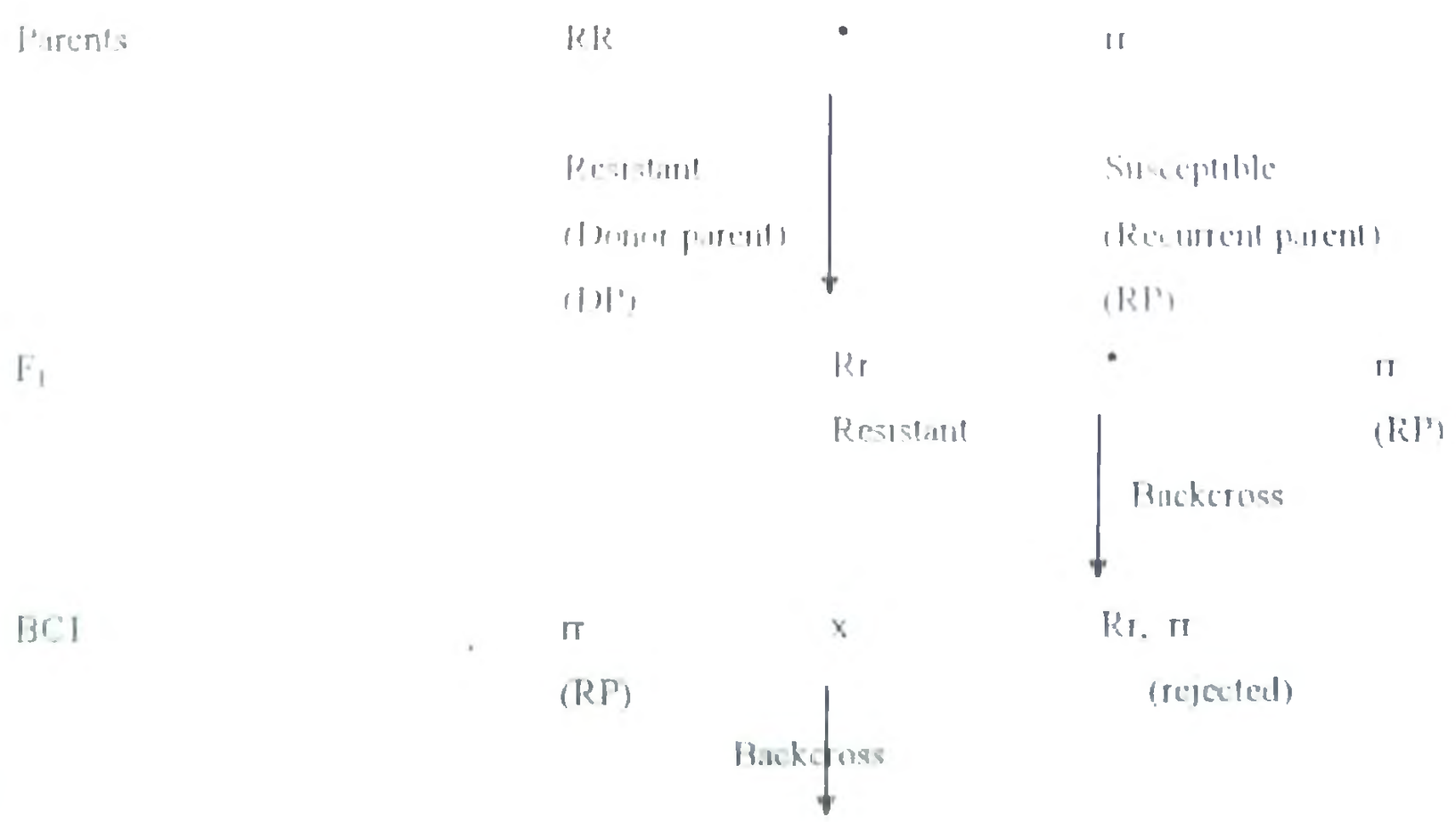
Dominant markers supply as much information as co-dominant markers in recombinant inbred lines, double haploids or backcross populations in coupling phase. Information obtained from dominant markers can be maximised by using RILs because all loci are homozygous or nearly so. Under conditions of tight linkage, that is, 10% recombination, dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations. However, backcross populations, are more (at low marker saturation) informative when compared to RILs as the distance between linked loci increases, that is, >15% recombination.

Generating RILs is quite time consuming. Moreover, some regions of the genome tend to stay heterozygous longer than expected from theory, and production of RILs in obligate outcrossing species is rather difficult. RILs have several uses. First they can be used to derive a map because it essentially is an eternal F_2 population with unlimited mapping possibilities. Additionally, these lines can be scored for morphological traits or quantitative traits, these data can then be used to integrate these traits with the molecular map being developed. RILs are especially powerful for analysing quantitative traits because they permit replicated trials necessary for the same. The quantitative trait data can then be

used to determine if any molecular markers are closely associated with these traits.

4.3.2. Near-isogenic lines (NILs)

Near-isogenic lines are those lines that are identical in their genotype, except for one gene. The first strategy used for molecular mapping was based on near-isogenic lines. Breeders have used recurrent backcross selection to introduce into cultivated lines, traits like disease resistance from their wild relatives. In such a programme, a donor parent (DP) which is homozygous for the allele for interest, is crossed with a recurrent parent (RP), which is homozygous for the wild type or standard allele of this locus. Resultant F1 individuals are backcrossed to RP to obtain backcross generation. In each backcross (BC) generation, only those BC individuals that have the introgressed allele and which are most similar to RP in phenotype, are selectively crossed with RP. An infinite number of backcross cycles would theoretically be required in order to ensure complete elimination of all DP derived genome from an NIL. However, breeders have used fewer than 10, typically only 5 or 6, backcrosses to generate most of the currently available NILs (Figure 5). Thus, the genome of any given NIL will most likely contain DP-derived alleles at several of its loci, which accounts for the use of the term near-isogenic to describe such lines.



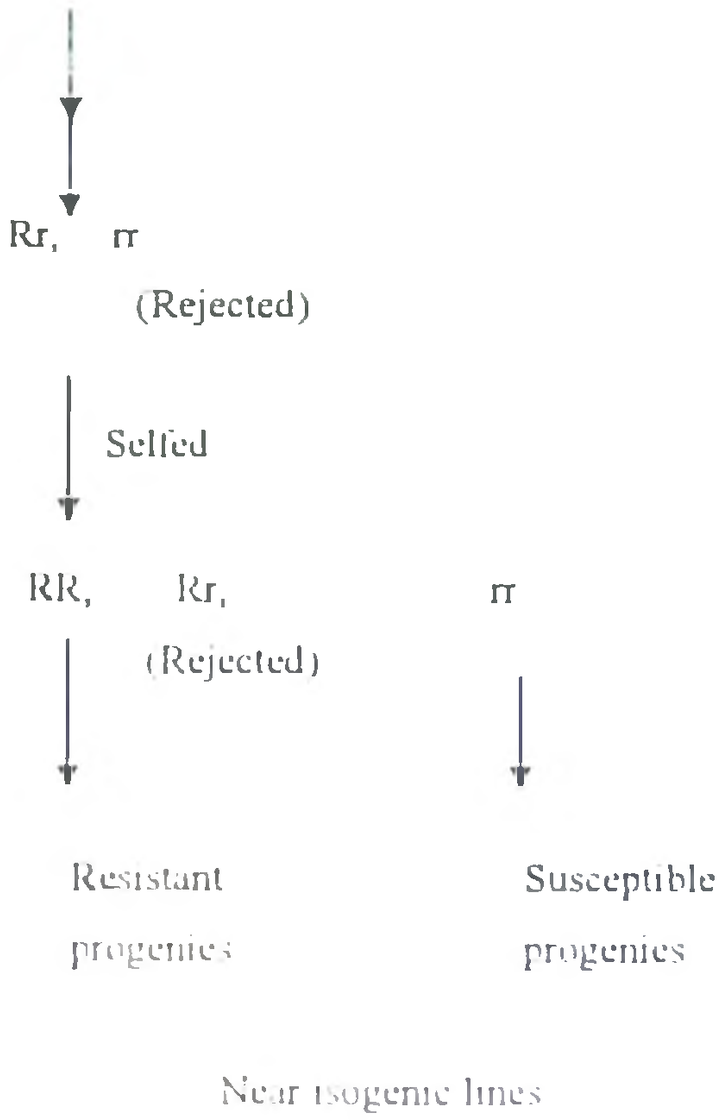


Figure 5. Production of near-isogenic lines (NILs)

Presumptive evidence of linkage between the introgressed conventional marker and the molecular marker would be obtained whenever an RP vs NIL allelic contrast, and corresponding NIL vs DP allelic equality, were detected for the molecular locus relative to any given set of RP/NIL and DP lines. When applied to a large number of available NILs, this near-isogenic gene mapping technique could very useful in detecting linkage between conventional markers and molecular markers.

Introgressed region is derived from the donor parent and is often highly polymorphic at the DNA sequence level. Therefore it provides a target for rapidly identifying clones/sequences located near the gene of interest. NILs make it easy to determine the location of a marker relative to the target gene, since only three DNA samples need to be analysed and any difference between them will be due to the sequence flanking the gene of interest. This is in contrast to the genetic

mapping based on RILs and BSA, where every clone/marker has to be tested with the complete mapping population to determine whether it maps near the gene of interest.

NILs have been extensively used to find molecular markers linked to such traits as disease resistance. If appropriate NILs have been developed, it appears that the technique has immense potential for mapping quantitative trait loci and molecular linkage maps. It also has the potential in locating quantitative trait loci on the genetic maps currently comprised solely of marker loci (Peleman *et al.*, 2005).

4.3.3 Bulked segregant analysis (BSA)

Bulked segregant analysis has been developed for the rapid identification of linkages. In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. Each of these bulks contains individuals that are identical for a particular trait or genomic region, but arbitrary at all unlinked regions. It is desirable to include only such plants that show the extreme phenotypes. The bulks are screened for DNA polymorphisms and these differences are compared against a randomised genetic background of unlinked loci. A marker that differs between two bulks is expected to be linked to the particular trait under investigation.

BSA overcomes several problems that are associated with the use of near-isogenic lines, which require many backcrosses for their development. Where only a portion of the polymorphic loci are expected to map to a selected region using NILs, regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA. Moreover, all loci detected during BSA will segregate and can be mapped, thus eliminating the linkage drag problems, that is, genes incorporated into the lines by backcrossing that are flanked by DNA segments introduced from the donor parent, associated with NILs.

4.3.4. Higher generation backcrosses

Breeders have used recurrent backcross selection to introduce into cultivated lines, traits like disease resistance from their wild relatives. In such a programme, a donor parent (DP) which is homozygous for the allele of interest, is crossed with a recurrent parent (RP), which is homozygous for the wild type or standard allele of this locus. Resultant F_1 individuals are backcrossed to RP to obtain backcross generation. In each backcross (BC) generation, only those BC individuals that have introgressed allele and which are most similar to the RP in phenotype, are selectively crossed with the RP.

Higher generation backcross populations are used in QTL mapping. As the distance between markers become larger, that is, loci become more independent and the information obtained per unit individual in RIL population for dominant markers decreases dramatically when compared to that for co-dominant markers. Backcross populations, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases, that is, >15% recombination

4.3.5. Doubled haploid lines

Doubled haploids improve plant breeding efficiency and effectiveness by generating inbred lines with 100% purity in just two generations. In more traditional breeding, it takes seven generations to do this – and the plants are still not 100% pure. Haploid plants are created with a special genetic process and have one set of chromosomes. They undergo chromosome doubling through a chemical process that produces a completely homozygous, fertile doubled haploid plant. The purity and genetic uniformity of doubled haploid lines make it easier to measure characteristics and reduce product development time(Primrose and Twyman, 2006).

4.4. QTL detection and mapping

A quantitative trait loci (QTL) is a position in a chromosome that contains one or more polygenes involved in the determination of a quantitative traits. It is impossible to follow the inheritance pattern of individual polygenes for obvious reasons . As a results these gene cannot be mapped using the conventional approaches . But is view of the importance of quantitative traits, scientists have been trying to find ways for mapping there genes, that is, QTL. Several characters of agronomic importance like yield and yield determining traits are difficult to study for their quantitative nature The recent breakthroughs in molecular biology have provided molecular markers that are helpful in identifying and tagging gene QTL QTL analysis enables to identify and dissect polygenes/QTL into individual Mendelian factors Detection of QTL helps in identifying stable QTLS irrespective of genetic backgrounds, locations and even across species Basic steps in QTL detection and mapping include

Evaluation of mapping population

Analysis to detect QTL

Creation of QTL likelihood maps

Permutation tests

Primary requirement of QTL mapping is a molecular markwer linkage map Therefore the construction of molecular marker linkage is primary step in QTL mapping This linkage map is then used in genetic loci that contribute to variation in the trait Different in constructing the marker linkage map are

1. Select a parent from each population
2. Find molecular markers that distinguish the two parents
3. Cross the parents to produce F_1 individuals
4. Inter cross F_1 individuals to produce an F_2 segregating generation
5. Score F_2 individuals for all molecular markers

6.Estimate pair wise linkage distance (centiMorgans) between markers by frequency of recombination

7.By using pairwise linkage map is constructed (Spencer and Fenster, 1995)

The map in conjunction with phenotypic measurements in the trait.

4.4.1.Evaluation of mapping population

Different mapping population is used to identify regions genome that are contributing to variation in trait of interest. Evaluation involves testing DNA markers throughout the genome for the likelihood that they are associated with a QTL individuals in a suitable mapping population are analyzed the tern of DNA marker genotype and the phenotype of interest For each DNA marker, the individuals are split classes according to marker genotype Mean and variance parameters for the phenotype of interest are calculate and compared among marker genotype classes. A significant difference between the DNA marker and the trait of interest indicates a linkage between DNA marker and trait of interest.

The mapping of a QTL is explained wring the example of AFLP marker. Two strains are selected ,which differ from each other for several RFLP marker and show significantly and markely different mean of values of many quantitative traits. Two strains are crossed to produce F_1, which is selfed to produce F_2, population For example if two strain (A and B) of a species (say rice) differ for two RFLP markers a (two alleles being G and g) and if alleles (H and h) Tthey also differ for protein content Strains A and B are crossed, F_2 population raised and individual plants evaluated for RFLP markers G and H, and for protein content. For RFLP marker G, F_2 plants are clarified into two groups those having (i) alleles G and (ii) allele g similarly, they are classified for RFLP marker H as well . the mean protein contents of the two groups for each, RFLP marker are now compared. A significant different is detected between the two groups for marker H. this indicates linkage between the RFLP marker H and the QTL

governing protein cotent in rice. A QTL detercted and mapped with an RFLP marker many effect consist of more than are tightly linked loci. The procedure is repeated throughout the genome to detect as many QTL as possible (Singh, 2006).

4.4.2.Analysis to detect QTL

It is not possible to determine whether the effect detected with a marker loci is due to one or more linked genes affecting the trait. For this reason, the term quantitative traits loci (QTL) was coined to describe a region of a chromo same usually defined by linkage to a marker gene that has a significant effect on a quantitative trait. Determining whether a QTL is comprised of one or more genes is still one of the most difficult aspects of quantitative genetics.

There are different analyses to determine whether the effect detected with a marker loci is due to one or more linked genes affecting the trait. They include single point analysis or point analysis, interval analysis and distributional extreme analysis

a) Single point analysis

The simplest approach for detecting QTL is to analysis the data using one marker at time. This approach is often referred to as single point analysis or point analysis and does not require a complete molecular marker linkage map. It is for this reason that point analysis were employed in the first molecular marker l quantitative genetic studies. The disadvantages of point analysis are

1) The farther a QTL is from the marker gene, the less likely it is to be detected statistically due to crossover events between the marker and QTL that result in misclassification

2) The magnitude of the effect of any detected QTL will normally be underestimated, due also to recombination between the marker locus and QTL.

Both problems are minimized when a large number of segregating molecular markers are used, covering the entire genome usually at intervals less than 15 cM . Under these conditions any potential QTL would be closely linked to at least one molecular marker (Edwards *et al.*, 1987).

b) Interval analysis

The availability of molecular linkage covering entire genomes has made it possible to overcome some problems with point analysis. To take the fullest advantage of linkage maps for quantitative studies, Lander & Botstein proposed a method called interval analysis. Instead of analysis the population one marker at a time, sets of linked markers are analyzed simultaneously with regard to their effects on quantitative traits. By using linked markers for analysis, it is possible to compensate for recombination between the markers and the QTL, increasing the probability of statistically detecting the QTL and effect on the character. Interval analysis, was first demonstrated on an interspecific backcross of tomato and has subsequently been used successfully for several quantitative trait linkage studies. The maximum benefits of interval analysis versus point analysis is realized when linked markers are fairly for apart (·20 cM) Under these condition there are likely to be many crossovers between the markers and QTL which can be compensated for with interval analysis Where the marker density is higher (markers ~ 15cM apart) point and interval analysis give nearly identical results When marker loci are very for apart (·35cM), even interval analysis is inefficient in detecting QTL in the interval between the loci

c)Distributional extreme analysis

Despite technological improvements in the speed and accuracy with which molecular markers can be assayed, it can still be time consuming and expensive to assay large populations. When the time and expensive of assaying molecular markers is significantly greater than measuring the quantitative character of

interest of on each individual , it is possible to use a modified approach to detect QTL. The approach also proposed by Lander & Botstein , starts with a large segregating population. A quantitative measure of the character of interest is taken on each individual in the population . Marker analysis is performed on individuals in the extreme tails of the distribution, that is those with the lowest and highest values for the character marker loces differs significantly at any two extreme subpopulations, it is interval that a QTL controlling the character of interest is located near the marker.

The benefit of distributional extreme analysis is in the savings of time and resource in assaying molecular markers. Given the same number of individuals assayed for molecular markers in total population analysis versus distributional extreme analysis, the statistical power or detecting QTL will be greater for the latter

The penalties for distributional extreme analysis are

1) More segregating individuals must be analyzed for the quantitative phenotype to collect enough individuals in distributional extremes.In same situations, the time and cost characterizing a large population phenotypic ally out weighs the advantages

2) While distributional extreme analysis is more efficient at detecting linkage between marker loci and QTL, it is less efficient in determining individual QTL effects. Individual in the extremes tend to have either a large number of positive or negative alleles at all QTL, depending on which extreme they represent. There is thus a deficiency of individual with a mixture of positive and negative alleles in the subpopulations being analyzed, which confounds the ability to individual measure the effects of any specific QTL.

3) It is often impractical to use distributional extremes to map more than one quantitative character, since the individual with extreme phenotypes for are characters are not likely to represent the extremes for other characters (Lander and Botstein , 1989).

4.4.3 Creation of QTL likelihood maps.

By the different analysis, likelihood or probability of each QTL in chromosome map position is detected. Basic principle is that for each alleles of given locus , there is a + or – correction with magnified of each, trait. For each marker locus, there are three different hypotheses-

Ho- 'A' and 'a' are not associated with the trait

Frequency 'A' ~ frequency 'a' for + and - phenotype

H1 - 'A' is positively associated with the trait

Frequency 'A' > frequency 'a' for + phenotype

Frequency 'A' < frequency 'a' for- phenotype

H1 - 'A' is negatively associated with the trait

Frequency 'A' < frequency 'a' for + phenotype

Frequency 'A' > frequency 'a' for- phenotype

The best hypothesis for a given locus (marker) is that hypothesis which maximizes the LOD score, where LOD is the log of the odds ratio between the two hypothesis

$$LOD = \log_{10}[\, P(H1)/\, P(Ho)]$$

LOD scores were plotted for each loces (marker) on chromosome . A curve fitting algorithm was used to make a continuous curve Thus QTL likelihood maps indicating LOD scores are constructed(Weller, 1986)

QTL likelihood maps indicating LOD scores were constructed in tomato on chromosomes 8,9,10,11 and 12 for three different character The traits are fruit mass, soluble solids and pH which are indicated by solid lines, dotted lines and hatched lines respectively(Figure 6) Chromosome 8 and 11 have likelihood curves for the characters fruit mass and soluble solids only Chromosome 9, 10 and 12 indicates QTL for three traits fruit mass, soluble solids, and pH which is depicted in the QTL likelihood maps of these chromosomes

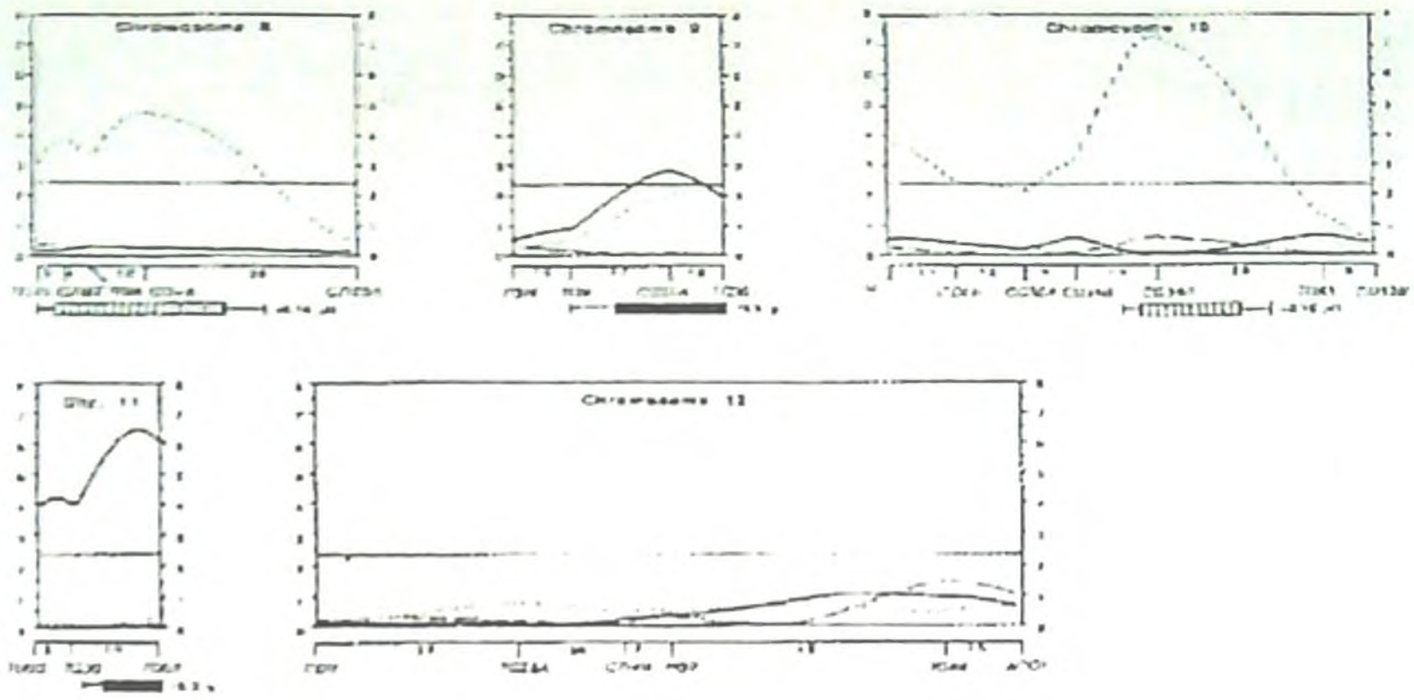


Figure 6. QTL likelihood maps indicating LOD scores

LOD score measures the strength or the evidence for the presence of a QTL at the location Larger LOD scores correspond to greater evidence for the presence of a QTL The LOD score is calculated at each position of the genome The probability of obtaining a LOD score as large or larger than that which was observed if there were no QTL is called the p value Large LOD scores give small P values . LOD curve for a chromosome indicates whether a QTL may be present and where it is likely to be located The region where the LOD score is within 1 5 of its maximum may be taken as the plausible region for the location of the QTL A plot of the LOD curve, re-centered so that its maximum is at O, is a valuable tool for depicting the the evidence for QTLs location adjustment must be made for the genome wide search for QTLs , so consider the distribution of the maximum LOD score genome wide Permutation test are valuable for score (Bromain, 2001)

4 4 4 Permutation tests

Permutation tests are tests for determining significant land works for LOD score. Deorge and Churchill (1996) gave permutation based tests to estimate a threshold value of QTL effects Quantitative traits data are permuted with respects to marker data a large number of times. Procedure – individuals in an experiment

are labeled I to n and each is scored at 'm' genetics markers selected from a known map. Also associated with each individual is a trait value Yi. A test statistic is computed at each a number of analysis points and its maximum value is an indicator of QTL effects in the genome. It is possible to use any test statistic in this procedure. Single marker F or t statistics or LOD scores from interval mapping are all reasonable choice. The trait values are shuffled N times among the 'n' individuals to create permuted data sets that have only random genotype-phenotype associations.

It is difficult to determine how efficient an experiment has been in identifying the QTL responsible for a trait. One measurement of success has been the cumulative phenotypic variance attributable to the combination of all significant QTL. Where complete molecular maps have been used for quantitative studies, the values have ranged from as high as 95% to less than 10%, with the average approximately 30-40%

The bias towards detecting QTL with larger effects means that it is unlikely that one will ever detect, map and characterize all of the polygenes affecting a character in any given segregating population. Theoretically, this may be a problem, but is only a minor practical limitation. Using the marker approach, are is likely to identify and characterize QTL waking the largest contribution to the phenotype and it is likely to be these QTL that one would want to further characterize and ultimately to clone. For plant breeders, the major QTL are of greatest interest to manipulate in breeding schemes via association with molecular markers (Zeng,1994)

5 Achievements

Different traits of agronomic importance like yield and yield determining traits have been studied for their quantitative nature in different crops. The recent breakthroughs in molecular biology have provided molecular markers that are

helpful in identifying and tagging QTL. QTL analysis has enabled to identify and dissect QTL into individual Mendelian factors. QTL comparison studies has provided an effective way to identify putatively orthologous QTLs across different species. QTL mapping works carried out in different crops are summarised below.

5.1.Tomato

Primary approach to QTL mapping was the detection of QTL on sugar content and fruit shape in tomato.

a) QTL for sugar contents

One of major objectives of tomato breeding is to increase the content of total soluble solids (TSS) in fruits to improve taste and processing qualities. TSS in fruits of wild tomatoes (Lycopersicon pennelli) is three times higher than in cultivated tomatoes. To resolve the genetic basis the variation, a set of 50 NILs was developed from a cross between L. pennelli and L. esculentum. Using these NILs it was possible to identify 23 QTLs tht increase brix. Key QTL (Brix 9-2-5) was located on a particular NIL that was defined by a 9 cM segment on chromosome 9. To map the Brix 9-2-5 QTL, 7000 F2 progeny of the NIL hybrid were subjected to RFLP analysis with two markers (CP 44 and TG 225) and this identified 145 recombinants. When the brix content of the recombination groups wee tested, the Brix 9-2-5 QTL was found to be associated with an 18 kb segment of the chromosome. Further mapping reduced the QTL to a 484 bp region of chromosome 9 and sequence analysis identified this as part of an invertase expressed exclusively in flowers and fruits of tomatoes

b) QTL for fruit size and shape

Two key morphological changes that accompanied tomato domestication were fruit size and shape. Wild tomatoes have small (~2g), round berries whereas commercial cultivars have fruit weighing 50-1000g that comes in a variety of

shapes (round, oval, pear-shaped etc.). Genetic crosses between wild and cultivated tomatoes have shown that most of the variation in size and shape is due to fewer than 30 QTLs. One of the key QTLs, FW 2.2, accounts for about 30% of the variance in fruit weight and was the first plant QTL to be mapped and cloned. comparative sequencing of FW 2.2 alleles suggested that the modulation of fruit size was attributable to 5' regulatory variation among the alleles rather than to differences in the structural protein. Large and small fruited tomatoes differed in peak transcript levels by one week and this was associated with changes in mitotic activity in the early stages of fruit development. (Paterson *et al.*, 1988).

5.2.Rice

QTL studies indicated sharing of common region of chromosomes for a particular trait/QTL irrespective of genetic background and environment. Comparison of QTLs became helpful in determining the origin and sharing of alleles between different cultivars and in identifying the chromosomal regions that harbour common and/or stable QTLs irrespective of the genetic backgrounds, locations and even across species (Septiningsih.2003)

a) QTL for grain yield

Several works on QTL analysis for grain yield have been reported in different mapping populations of rice eight double haloid lines of rice (*Oryza sativa* L.) derived from IR64/Azucena cross were used for mapping QTL underlying growth and yield traits plant height, number of tillers, days to first flowering, days to maturity and grain yield The composite interval mapping with a threshold LOD >2.5, revealed a total of nine QTLs distributed on chromosomes 3, 6, 8 and 10 A QTL having maximum LOD of 3.88 was detected for number of tillers, explaining 11% phenotypic variation Grain yield was controlled by three significant QTLs located on chromosomes 3, 6 and 8, which together explained 26% of phenotypic variation and the chromosomal regions associated with grain yield QTL were also associated with its contributing traits Four of the five selected traits viz., plant height, days to first flowering, days to maturity and grain

yield had a common QTL on chromosome 3 with a phenotypic variation ranging between 9-10% for these traits. This region indicated clustering of QTLs for these traits suggesting the scope for five mapping of common and/or correlated QTLs for yield and yield related traits. The mapping and defection of significant main effect QTL that have maximum phenotypic effect on a trait, helps in carrying out marker-assisted selection for that particular trait (Nagabhushana *et al.*, 2006).

b) QTL for seedling vigour

Cultivars having high seedling vigour are desirable for crop establishment. High seedling vigour helps the genotypes to suppress the weeds, thereby favour crop establishment. QTL underlying seedling vigour-related traits were studied using DH mapping population derived from a cross between a high vigour japonica cultivar CT9993 and a low vigour indica cultivar IR62266. Internal analysis was performed to detect QTLs. A locus with LOD>3.00 was declared a putative QTL. A total of 29 QTLs for 14 morphological and growth-related traits were tagged to molecular markers. significant QTLs were located on chromosomes 1 and 3. identification of QTLs for seedling vigour-related traits would help enhance the selection efficiency at an earlier stage of the crop, so that much of resources and time could be saved. If QTLs are found in the same genomic regions in both seedling and mature crop, screening can be done at an early stage with the QTL markers (Kanbar *et al.*, 2006).

c) QTL for salt tolerance

QTL for salt tolerance was evaluated in rice. This was evaluated in two separate heads, that is, reducing sodium uptake and maintining potassium uptake. Two stragies were followed because both rice has different uptake mechanisms for sodium and potassium uptake. Three QTLs were detected on chromosomes 4,6 and 1. Single point analysis was conducted to analyse the QTL for salt tolerance(Koyama *et al.*, 2001).

d) QTL for tiller number.

RILs were evaluated for QTL for tiller number. Strategy used was time related mapping. This is efficient because for each QTL, there is an appropriate time and time interval where maximum expression of QTL is produced. Time related mapping is more advantageous over time fixed mapping. In time fixed mapping, QTL effect of a trait is determined at a particular time. Interval analysis identified five QTLs for tiller number.QTL mapping revealed 5 QTLs on three chromasomes that is, chromosome, 1, 3 and 5. (Wu *et al.*, 1999).

5.3.Maize

1. QTL for grain yield

Advanced backcross breeding strategy was used to identify quantitative trait loci (QTLs) for grain yield in a cross between two elite inbreds of maize, RD6502 (Mo17-type recurrent parent) and RD3013 (Iodent donor parent). Two hundred and four BC(2) families were scored at 106 SSR, 15 AFLP, and 38 Heartbreaker (MITE) loci. BC(2) testcrosses (TC) with B73 were phenotyped at six locations in the Midwest and N.Y. Thirteen grain yield, six grain moisture, and three plant height QTLs were detected at which the RD3013 allele had a favorable effect ($p < 0.05$) (Austin and Lee, 1998).

2. QTL for downy mildew resistance

Quantitative trait loci (QTLs) of maize involved in mediating resistance to Peronosclerospora sorghi, the causative agent of sorghum downy mildew (SDM), were detected in a population of recombinant inbred lines (RILs) derived from the Zea mays L. cross between resistant (G62) and susceptible (G58) inbred lines. Field tests of 94 RILs were conducted over two growing seasons using artificial inoculation. Heritability of the disease reaction was high (around 70%). The mapping population of the RILs was also scored for restriction fragment length polymorphic (RFLP) markers. One hundred and six polymorphic RFLP markers were assigned to ten chromosomes covering 1648 cM. Thirty QTLs were detected

that significantly affected resistance to SDM combined across seasons (Bashir, 2003).

3. QTL for drought tolerance

Eighty-four RFLP markers were mapped in an F2 population of 81 plants from a cross between parents, Poj17 (drought resistant) and F-2 (drought sensitive), that differ markedly in many constitutive and adaptive responses to drought stress. In a soil glasshouse experiment, from which water was withheld for 3 weeks after anthesis, flowering time, stomatal conductance, tissue ABA contents, leaf water relations parameters and fluorescence characteristics, root pulling force, and nodal root number were measured. The minimum number and location of genes having major effects on the traits were determined and possible causal relationships amongst them tested. Comparing the coincidence of QTL for ABA content and stomatal conductance showed that xylem ABA content was more likely to have had a regulatory effect on the stomatal conductance of those plants than the whole leaf ABA content. However, both xylem and leaf ABA contents were significantly associated with root characteristics, suggesting that the rooting behaviour (either constitutive or adaptive) was important in regulating stress responses, particularly in determining xylem ABA contents (Lebreton *et al*., 2006).

4. QTL for flowering time traits

A set of 89 near-isogenic lines (NILs) of maize was created using marker-assisted selection. Nineteen genomic regions, identified by restriction fragment length polymorphism loci and chosen to represent portions of all 10 maize chromosomes, were introgressed by backcrossing three generations from donor line Tx303 into the B73 genetic background. NILs were genotyped at an additional 128 simple sequence repeat loci to estimate the size of introgressions and the amount of background introgression. Tx303 introgressions ranged in size from 10 to 150 cM, with an average of 60 cM. Across all NILs, 89% of the Tx303 genome is represented in targeted and background introgressions. The average

proportion of background introgression was 2.5% (range 0 to 15%), significantly lower than the expected value of 6.25% for third backcross generation lines developed without marker-assisted selection. The NILs were grown in replicated field evaluations in two years to map QTLs for flowering time traits. A parallel experiment of testcrosses of each NIL to the unrelated inbred, Mo17, was conducted in the same environments to map QTLs in NIL testcross hybrids. QTLs affecting days to anthesis, days to silking, and anthesis-silk interval were detected in both inbreds and hybrids in both environments. The testing environments differed dramatically for drought stress, and different sets of QTLs were detected across environments. Furthermore, QTLs detected in inbreds were typically different from QTLs detected in hybrids, demonstrating the genetic complexity of flowering time. NILs can serve as a valuable genetic mapping resource for maize breeders and geneticists (Szalma *et al.*, 2007)

5.4.Wheat

1. QTL for grain yield

Studies with 95 bread wheat doubled haploid lines (DHLs) from the cross Chinese Spring (CS)xSQ1 trialled over 24 yearxtreatmentxlocations identified major yield quantitative trait loci (QTLs) in homoeologous locations on 7AL and 7BL, expressed mainly under stressed and non-stressed conditions, respectively. SQ1 and CS contributed alleles increasing yield on 7AL and 7BL, respectively. The yield component most strongly associated with these QTLs was grains per ear. Additional results which focus on the 7AL yield QTL are presented here. Trials monitoring agronomic, morphological, physiological, and anatomical traits revealed that the 7AL yield QTL was not associated with differences in flowering time or plant height, but with significant differences in biomass at maturity and anthesis, biomass per tiller, and biomass during tillering. In some trials, flag leaf chlorophyll content and leaf width at tillering were also associated with the QTL. Thus, it is likely that the yield gene(s) on 7AL affects plant productivity. Near-isogenic lines (NILs) for the 7AL yield QTL with CS or SQ1 alleles in an SQ1

background showed the SQ1 allele to be associated with >20% higher yield per ear, significantly higher flag leaf chlorophyll content, and wider flag leaves. Epidermal cell width and distance between leaf vascular bundles did not differ significantly between NILs, so the yield-associated gene may influence the number of cell files across the leaf through effects on cell division. Interestingly, comparative mapping with rice identified *AINTEGUMENTA* and G-protein subunit genes affecting lateral cell division at locations homologous to the wheat 7AL yield QTL (Quarrie *et al.*, 2006).

2 QTL for resistance to Fusarium head blight

Wheat Fusarium head blight (FHB) may cause serious losses in grain yield and quality. Production of deoxynivalenol (DON) by Fusarium graminearum in infected grain is detrimental to livestock and is also a safety concern in human foods. Cultivation of cultivars with resistance to FHB and DON accumulation is the most effective strategy for disease control. Wangshuibai is a Chinese landrace with a high level of resistance to FHB and DON accumulation, and an F7 population of recombinant inbred lines (RILs) derived from the cross between Wangshuibai and Annong 8455 was developed for molecular mapping of quantitative trait loci (QTL) for FHB resistance. Proportion of scabbed spiklets (PSS) and DON content were assessed under the field conditions. Composite interval mapping (CIM) revealed that two and three QTL were significantly associated with low PSS and low DON content, respectively, over two years. QTL on chromosome 3B and 2A explained 17% and 11.5% of the phenotypic variance for low PSS, respectively, whereas QTL on chromosome 5A, 2A and 3B explained 12.4, 8.5 and 6.2% of the phenotypic variance for low DON content, respectively. The 3B QTL appeared to be associated mainly with low PSS, and the 5A QTL primarily with low DON content in Wangshuibai. The 2A QTL had minor effect to both low PSS and DON content. The SSR markers, linked to these QTL should be useful for marker-assisted selection (MAS) of QTL for low PSS and low DON content from Wangshuibai. (Ma *et al.*, 2006)

2. QTL for grain dormancy

Molecular markers were linked to a QTL (Quantitative Trait Locus) controlling grain dormancy on chromosome 4A of wheat. Seven Simple Sequence Repeat (SSR) and one candidate gene markers were linked within an interval of 110 cM. The most likely position of the QTL was estimated to reside within a 20 cM span between two SSR markers, GWM397 and WMC468. These markers explained 13 and 11 percent of the variation, respectively, for germination index (GI) scores, an indicator of dormancy and predictor of preharvest sprouting. The results suggest flanking markers for a QTL on chromosome 4A will facilitate the selection of less susceptible preharvest sprouting genotypes(Mares *et al.*, 2004).

5.5.Sorghum

1 QTL for grain yield

DNA markers were employed to determine if QTL affecting yield in inbred lines and hybrids are similar. It is generally believed that good inbreds make good hybrids, though the correlation between agronomic performance of lines and testcrosses can be poor. These differences are typically explained by differences in combining ability. Early reports on maize gave positive association between performance in inbreds and hybrids for phenological traits, but correlations for yield were weak. Evaluation of performance of recombinant inbred (RI) sorghum lines and their hybrids in five different environments were carried out. The correlation for grain yield between RI lines and hybrids were generally low, but significant associations were identified in high-yield environments. Genetic variation among RI lines and testcrosses was greatest in high yield environments and lowest in stress environments. We evaluated the genetic basis for the inbred-hybrid yield correlation through QTL analysis. We identified two QTL with similar effects on yield in both lines and hybrids. Several other QTL were identified for line or hybrid specific effects explaining perhaps differences in specific combining ability effects among inbreds. The results of this study indicated that QTL for grain yield of sorghum hybrids can be identified in

testcross populations; however, these QTL may differ from those associated with performance of lines per se. It also suggests that the power for QTL detection for yield in grain sorghum is the most favorable under high-yielding or low- stress environments (Ejeta *et al.*, 2000).

3. QTL for drought tolerance

Molecular markers linked to QTL for drought tolerance could be used in increasing efficiency of breeding efforts to select sorghum germplasm with enhanced drought tolerance.Further analysis of these traits could lead to better understanding of the biological basis of drought tolerance. In the last several years, a number of studies were undertaken towards this goal using a set of recombinant inbred (RI) sorghum lines especially developed for an array of interdisciplinary evaluation of the genetics and physiology of drought tolerance in sorghum. First, the RI lines were carefully evaluated for response to drought in a series of pre-flowering and post-flowering stress environments. Drought tolerance was estimated in several ways: evaluation of grain yield under drought, stability of yield, rate and duration of grain fill, seed weight, stay green and associated traits. Evaluation of the RI lines indicated segregation of drought tolerance during both developmental stages affirming its genetic basis and suggesting complementary interaction of loci from both parental sources. Second, the RI population was scored for the segregation of lines(Ejeta *et al.*, 2000)

5.6.Barley

a) Feed quality

Barley grain has been criticized as a cattle feed because of its rapid ruminal digestion, which may result in digestive disorders such as acidosis, laminitis, and frothy bloat. Low dry-matter digestibility (DMD), low acid detergent fiber (ADF) content, and high starch content are all important feed barley traits. There exists a great amount of genetic variation for these feed quality traits in the USDA barley world collection. PI370970, a 6-row accession of the

world collection, was crossed to the 2-row feed variety 'Valier' and a population of recombinant inbred lines was derived and subjected to QTL analysis, with the largest QTL for DMD being on chromosome 2 very near Vrs1. As Vrs1 impacts many aspects of plant development including head-type (2-row or 6-row), an interesting and useful question is whether the DMD variation attributed to the region around Vrs1 is due to pleiotropy or linkage. Our next objective is the QTL analysis of a very low DMD 2-row line, world collection accession PI28624. PI28624 has been crossed to a high yeilding dryland feed barley, 'Haxby.' QTL analysis will be based on an F2 generation, an advanced backcross population, and a recombinant inbred population (Jewel and Talbert, 2004).

5.7.Chilli

a)Resistance to anthracnose

Anthracnose fruit rot is an economically important disease that affects pepper production in Indonesia. Strong resistance to two causal pathogens, *Colletotrichum gloeosporioides* and *C. capsici*, was found in an accession of *Capsicum chinense*. The inheritance of this resistance was studied in an F_2 population derived from a cross of this accession with an Indonesian hot pepper variety *(Capsicum annuum)* using a quantitative trait locus (QTL) mapping approach. In laboratory tests where ripe fruits were artificially inoculated with either *C. gloeosporioides* or *C. capsici*, three resistance-related traits were scored: the infection frequency, the true lesion diameter (averaged over all lesions that actually developed), and the overall lesion diameter (averaged over all inoculation points, including those that did not develop lesions). One main QTL was identified with highly significant and large effects on all three traits after inoculation with *C. gloeosporioides* and on true lesion diameter after inoculation with *C. capsici*. Three other QTL with smaller effects were found for overall lesion diameter and true lesion diameter after inoculation with *C. gloeosporioides*, two of which also had an effect on infection frequency. Interestingly, the resistant parent carried a susceptible allele for a QTL for all three traits that was closely linked to the main QTL. The results with *C. capsici* were based on less

observations and therefore less informative. Although the main QTL was shown to have an effect on true lesion diameter after inoculation with *C. capsici*, no significant QTL were identified for overall lesion diameter or infection frequency (Voorrips *et al.*, 2004).

5.8.Potato

a) Tuber dormancy

The potential loss of chemical sprout inhibitors because of public concern over the use of pesticides underscores the desirability of breeding for long dormancy of potato (*Solanum tuberosum* L.) tubers. Quantitative trait locus (QTL) analyses were performed in reciprocal backcrosses between *S. tuberosum* and *S. berthaultii* toward defining the complexity of dormancy. *S. berthaultii* is a wild Bolivian species characterized by a short-day requirement for tuberization, long tuber dormancy, and resistance to several insect pests. RFLP alleles segregating from the recurrent parents as well as from the interspecific hybrid were monitored in two segregating progenies. We detected QTLs on nine chromosomes that affected tuber dormancy, either alone or through epistatic interactions. Alleles from the wild parent promoted dormancy, with the largest effect at a QTL on chromosome 2. Long dormancy appeared to be recessive in the backcross to *S. berthaultii* (BCB). In BCB the additive effects of dormancy QTLs accounted for 48% of the measured phenotypic variance, and adding epistatic effects to the model explained only 4% more. In contrast, additive effects explained only 16% of the variance in the backcross to *S. tuberosum* (BCT), and an additional 24% was explained by the inclusion of epistatic effects. In BCB variation at all QTLs detected was associated with RFLP alleles segregating from the hybrid parent, in BCT all QTLs except for two found through epistasis were detected through RFLP alleles segregating from the recurrent parent. At least three dormancy QTLs mapped to markers previously found to be associated with tuberization in these crosses(Berg *et al.*, 2004).

6. Conclusion

Quantitative trait loci (QTL) is a position in a chromosome that contains one or more polygenes involved in the determination of a quantitative traits. It is impossible to follow the inheritance pattern of individual polygenes for obvious reasons . As a results these gene cannot be mapped using the conventional approaches . But is view of the importance of quantitative traits, scientists have been trying to find ways for mapping there genes, that is, QTL. Several characters of agronomic importance like yield and yield determining traits are difficult to study for their quantitative nature. The recent breakthroughs in molecular biology have provided molecular markers that are helpful in identifying and tagging gene QTL QTL analysis enables to identify and dissect polygenes/QTL into individual Mendelian factors. Detection of QTL helps in identifying stable QTLS irrespective of genetic backgrounds, locations and even across species.

Determining whether the effect detected with a marker loci is due to one or more linked genes affecting the trait is difficult For this reason, the term quantitative traits loci (QTL) was coined to describe a region of a chromo same usually defined by linkage to a marker gene that has a significant effect on a quantitative trait Determining whether a QTL is comprised of one or more genes is still one of the most difficult aspects of quantitative genetics

The recent breakthroughs in molecular biology have provided molecular markers that are helpful in identifying and tagging QTL QTL analysis has enabled to identify and dissect QTL into individual Mendelian factors QTL comparison studies has provided an effective way to identify putatively orthologous QTLs across different species This helps in carrying out marker assisted selection for the particular trait

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Discussion

1.Is resistance to diseases quantitative or qualitative trait?

Disease resistance can be qualitative or quantitative trait. Different resistance traits follow different inheritance pattern as it is simple Mendelian inheritance in case of qualitative traits while complex in quantitative traits. Quantitative traits fall in to distinct classes and little affected by environment but quantitative characters show continuous distribution. Qualitative traits are indicated by typical Mendelian ratios and counts while quantitative traits are determined by chi-square analysis.

2 Explain epistatic interaction of markers?

Epistasis is a form of interaction between non allelic genes whereby one gene interferes with the phenotypic expression of another gene, so that inheritance pattern of traits will be different With morphological marker loci, strong epistatic interactions among loci limit the number of segregating markers that can be unequivocally scored in the save generation Because molecular marker loci do not normally exhibit epistastic or pleiotropic effects, a virtually limitless number of segregating markers can be used in a single genome

3 Is there any disadvantage for interval analysis?

A complete linkage map of the crop is essential for interval analysis Where the marker density is higher (markers < 15cM apart) point and interval analysis give nearly identical results When marker loci are very for apart (>35cM), even interval analysis is inefficient in detecting QTL in the interval between the loci

4. What is relevance of number of QTLs in plant breeding?

Recent breakthroughs in molecular biology have provided molecular markers that are helpful in identifying and tagging gene QTL. QTL analysis enables to identify and dissect polygenes/QTL into individual Mendelian factors. Detection of QTL helps in identifying stable QTLs irrespective of genetic backgrounds, locations and even across species. QTL comparison studies has provided an effective way to identify putatively orthologous QTLs across different species.This helps in carrying out marker assisted selection for the particular trait. Also this gives an idea of the QTLs which can be cloned.

5 What is advantage of using doubled haploids?

Doubled haploids improve plant breeding efficiency and effectiveness by generating inbred lines with 100% purity in just two generations. In more traditional breeding, it takes seven generations to do this – and the plants are still not 100% pure. Haploid plants are created with a special genetic process and have one set of chromosomes. They undergo chromosome doubling through a chemical process that produces a completely homozygous, fertile doubled haploid plant. The purity and genetic uniformity of doubled haploid lines make it easier to measure characteristics and reduce product development time.

6 What is use of bulked segregants?

BSA overcomes several problems that are associated with the use of near-isogenic lines, which require many backcrosses for their development. Where only a portion of the polymorphic loci are expected to map to a selected region using NILs, regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA. Moreover, all loci detected during BSA will segregate and can be mapped, thus eliminating the linkage drag problems, that is, genes incorporated into the lines by backcrossing that are

flanked by DNA segments introduced from the donor parent, associated with NILs.

7. Whether QTL analysis for two traits can be done simultaneously?

Quantitative traits are controlled by polygenes with small individual effects and show continuous distribution with marked influence of environment. QTL is governed by several genes with major and minor effects. To detect, map and characterize all of the polygenes affecting a character in any given segregating population is difficult. Because of this, it is impossible to do QTL detection of two traits simultaneously.

8 What are markers used in QTL analysis?

R FLP,RAPD and SSR are the commonly used markers for QTL analysis.

9 What is disadvantage of distributional extreme analysis?

More segregating individuals must be analyzed for the quantitative phenotype to collect enough individuals in distributional extremes. Time and cost characterizing a large population phenotypically out weighs the advantages. Distributional extreme analysis is less efficient in determining individual QTL effects. It is often impractical to use distributional extremes to map more than one quantitative character, since the individual with extreme phenotypes for are characters are not likely to represent the extremes for other characters.

10 What is advantage of QTL analysis?

QTL analysis enables to identify and dissect polygenes/QTL into individual Mendelian factors. Detection of QTL helps in identifying stable QTLS irrespective of genetic backgrounds, locations and even across species. QTL mapping helps to identify and characterize QTL making the largest contribution to the phenotype and it is likely to be these QTL that one would want to further characterize and ultimately to clone. For plant breeders, the major QTL are of

greatest interest to manipulate in breeding schemes via association with molecular markers

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Abstract

Genetically complex forms of inheritance are poorly understood. Classical quantitative genetics provides the tools for studying such inheritance. However quantitative genetics is unsuited for dissecting polygenic characters into discrete genetic loci or defining the roles of individual genes. An effective approach for studying complex and polygenic inheritance is known as QTL. Quantitative trait loci are genes which have relatively subtle quantitative effects on phenotypes.

The aim of QTL mapping is to identify chromosomal regions which contain genes that affect quantitative traits. First step in any QTL mapping experiment is to construct populations that originate from homozygous inbred parental lines. Resulting F_1 lines will be heterozygous at all markers and QTLs. From the F1 population a mapping population is generated such as F_2, backcross, doubled haploid, recombinant inbred lines, near isogenic lines etc.(Doerge, 2002). Next step involves molecular identification of genetic variants that are responsible for each QTL from mapping population individuals (Primrose and Twyman, 2006). The types of QTL analysis include single point analysis, interval analysis and distributional extreme analysis. Statistical methods like assigning probability, LOD (likelihood) scores, permutation tests etc. are utilized in QTL mapping.

One of the first published reports on QTL mapping with DNA markers involved fruit traits in tomato (Paterson *et al*., 1988). This was followed by several reports in other crops like stress resistance in rice (Kovama *et al*., 2001), chilli etc.

QTL comparison studies are an effective way to identify orthologous QTLs across species (Septiningsih *et al*., 2003). Conservation of QTLs among species may provide opportunities for plant breeders to use QTL mapping information from one species in designing and execution of breeding studies in another (Tanksley, 1993).

HARNESSING HYBRID VIGOUR IN CROP PLANTS

BY

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(2006-21-106)

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SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

PbGen. 751 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

I hereby declare that the seminar report entitled '**Harnessing hybrid vigour in crop plants**' is a record of the seminar presented by me during the course on 04.05.2007 and that this report has been prepared by me independently after going through the references cited herein

Vellanikkara
08.08.2007

Gayathri. G.
(2006-21-106)

CERTIFICATE

Certified that the seminar report entitled '**Harnessing hybrid vigour in crop plants**' is a record of seminar presented by **Smt. Gayathri. G. (2006-21-106)** under my guidance and that this report has been prepared by her independently

Vellanikkara
08.08.2007

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Major Advisor

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1. Introduction

Heterosis breeding is an important method of crop improvement for enhancing yield and other desirable traits. Hybrid vigour or heterosis is defined as the superiority of an F_1 hybrid over both its parents in terms of yield or some other character. Shull in 1914 referred to this phenomenon as the stimulus of heterozygosis and in his words it is the 'interpretation of increased vigour, size, fruitfulness, speed of development, resistance to diseases and insect pests or to the climatic rigours of any kind manifested by the outbreeding organisms as compared with the corresponding inbreds as a specific result of the unlikeness in the constitution of the uniting parental gametes'

F_1 hybrids is a term used in genetics and selective breeding. F_1 stands for *Filial 1*, the first filial generation seeds or plants resulting from a cross mating of distinctly different parental types. The offspring of distinctly different parental types produce a new, uniform variety with specific and/or desirable characteristics from either or both parents. In plant genetics parents usually are two inbred lines. Crossing specific parent plants produces a hybrid seed (plant) by means of controlled pollination. To produce consistent F_1 hybrids, the original cross must be repeated each season and hence F_1 seeds are expensive.

1.1. Advantages

- ✓ Homogeneity and predictability - because of the homozygosity of the parent pure lines, there is next to no genetic variation between individual plants. This makes their phenotype extremely uniform and thus attractive for mechanical operations. Once the characteristics of the cross are known, repeating this cross will yield exactly the same result.
- ✓ Higher yield

1.2. Disadvantages

- ✓ F_1 hybrid seeds have to be purchased every year.
- ✓ Both inbreeding and crossing both lines requires a lot of work, which translates in a much higher seed cost. In general, the higher yield offsets this disadvantage.

- ✓ F_1 hybrids usually give higher yields than traditional varieties, but only when they are grown using chemical fertilisers, pesticides, fungicides and herbicides. In contrast, traditional varieties usually have a higher natural resistance to disease. Therefore, large inputs of artificial chemicals are not usually necessary in order for them to grow well.
- ✓ F_1 hybrids lack genetic diversity, due to the inbred parental lines.

2. Basic requirements for large scale commercial hybrid seed production in crop plants

1. Availability of a proven heterotic hybrid combination which could distinctly and profitable surpass the yield levels of the commercial variety being grown
2. Availability of an easy, economic and effective means of eliminating or rendering functionless of the male part of a bisexual seed parent mechanically, genetically or even biochemically
3. Availability of a strong fertility restoration system in case of cytoplasmically governed male sterility system or availability of a tightly linked marker gene system in case of genetically governed male sterility
4. Full and detectable expression of self incompatibility and absence of modifier genes
5. Complete synchronization of flowering in both seed and pollen parents
6. Free unrestricted and natural pollen transfer from pollen to seed parent
7. Good seed setting on seed parent
8. A skilled and organised effort for large scale seed production, certification, processing and well knitted distribution channel of hybrid seeds

3. General methodology of hybrid seed production

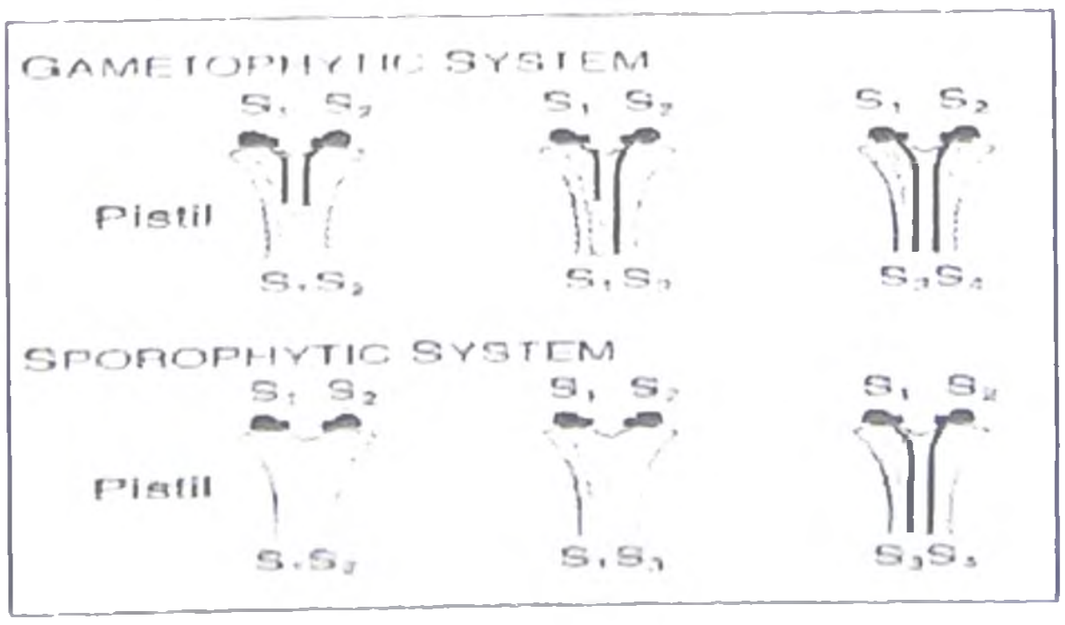
The main requirements for commercial hybrid seed production are easy emasculation of the female parent and effective pollen dispersal from the male parent to ensure a satisfactory seed set in female parent. Manual emasculation is followed in many crops like solanaceous vegetables and maize. However male sterility, self incompatibility and pistillate condition offer the means for genetic emasculation. It prevents self-fertilization by manipulating the genotype of the

plant. This is the basis for hybrid seed production in many plants. Male sterility is of different types like genetic male sterility, cytoplasmic male sterility and cytoplasmic-genetic male sterility. Novel type of genetic male sterility with the aid of recombinant DNA technology called transgenic male sterility is also reported. A non genetic type of male sterility system with the help of chemical hybridizing agents is also utilized in some crops like wheat.

3.1. Self incompatibility systems

Self-incompatibility (SI) is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and thus encourage outcrossing. In plants with SI, when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype, the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced. SI is one of the most important means to prevent selfing and promote the generation of new genotypes in plants, and it is considered as one of the causes for the spread and success of the angiosperms on our planet. The best studied mechanisms of SI act by inhibiting the germination of pollen on stigma or the elongation of the pollen tube in the style. These mechanisms are based on protein-protein interactions, each mechanism being controlled by a single locus termed S, which has many different alleles in the species population. Despite their similar morphological and genetic manifestations, these mechanisms have evolved independently, and are based on different cellular components (Charlesworth *et al*., 2005) therefore, each mechanism has its own, unique S-locus.

Figure 1. Systems of self incompatibility



In gametophytic self-incompatibility (GSI), the SI phenotype of the pollen is determined by its own gametophytic haploid genotype. This is the more common type of SI, existing in the families like Solanaceae, Rosaceae, Scrophulariaceae, Fabaceae, Onagraceae, Campanulaceae, Papaveraceae and Poaceae (Franklin *et al.* , 1995).

In sporophytic self-incompatibility (SSI), the SI phenotype of the pollen is determined by the diploid genotype of the anther (the sporophyte) in which it was created. This form of SI was identified in the families like Brassicaceae, Asteraceae, Convolvulaceae, Betulaceae, Caryophyllaceae, Sterculiaceae and Polemoniaceae (Goodwillie, 1997). Up to this day, only one mechanism of SSI has been described in detail at the molecular level, in *Brassica* (Brassicaceae). Since SSI is determined by a diploid genotype, the pollen and pistil each express the translation products of two different alleles, i.e. two male and two female determinants. Dominance relationships often exist between pairs of alleles, resulting in complicated patterns of compatibility/self-incompatibility. These dominance relationships also allow the generation of individuals homozygous for a recessive S allele (Hiscock and Tabah, 2003).

3.2. Pistillate condition

In case of monoecious crops like cucurbits and castor some mutants produce only pistillate flowers in place of both male and female flowers. This is manifested due to blocking of androecium development in male flowers. This system is commercially exploited in castor in India.

3.3. Male sterility

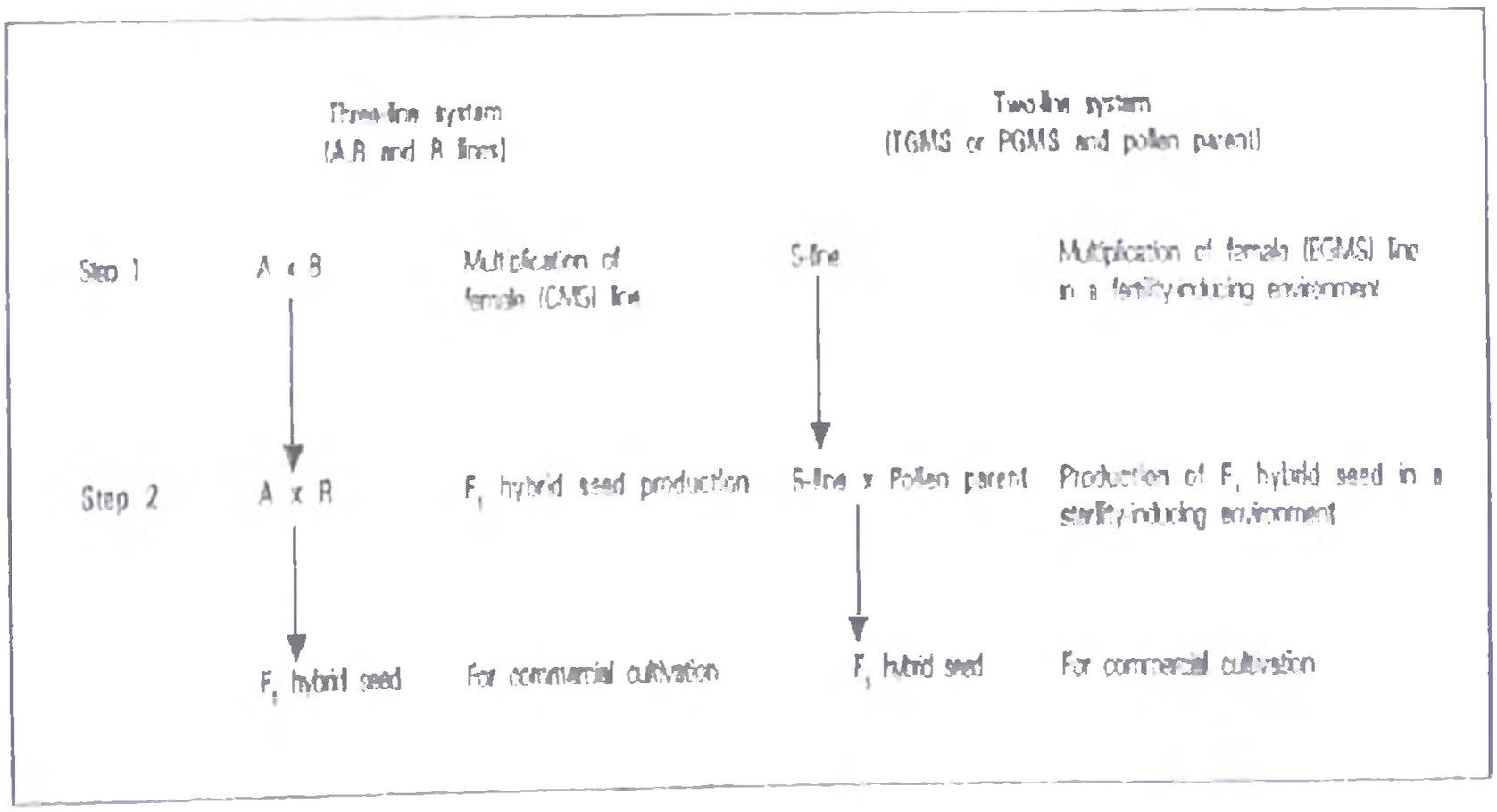
Male sterility is defined as the failure of plants to produce functional anthers, pollen, or male gametes. First documentation of male sterility came in 1763 when Kölreuter observed anther abortion within species and specific hybrids. It is more prevalent than female sterility, probably because, male sporophyte and gametophyte are less protected from environment than ovule and embryo sac. Male sterility is easy to detect because a large number of pollen are produced and hence can be easily studied. It can also be easily assayed through staining technique with the help of carmine, lactophenol or iodine while detection

of female sterility requires crossing. Male sterility has propagation potential in nature since it can still set seed and is important for crop breeding, while female sterility does not. Male sterility can be aroused spontaneously via mutations in nuclear and or cytoplasmic genes.

Among the two types of male sterility- genic and cytoplasmic- cytoplasmic male sterility is caused by the extranuclear genome of mitochondria and show maternal inheritance. Manifestation of male sterility in these may be either entirely controlled by cytoplamsic factors alone or by the interaction between cytoplamsic and nuclear factors.

Cytoplasmic male sterility as the name indicates is under extra nuclear genetic control. They show non-mendelian inheritance and are under the regulation of cytoplasmic factors. In this type, male sterility inherited maternally. This is a very common type of male sterile system in the plant kingdom. In general there are two types of cytoplasm *viz*., N (normal) and the aberrant S (sterile) cytoplasms. These types exhibit reciprocal differences. This may be utilised for producing hybrid seed in ceram ornamental species or in species where a vegetative part is of economic value. But in those crop plants where seed is the economic part it is of no use because the hybrid progeny would be male sterile.

Figure 2 General methodology for three line and two line system of hybrid seed production



3.3.1. Cytoplasmic- Genetic Male Sterility (CGMS)

It is the most widely used system for commercial exploitation of hybrid seed production in many crops. It is also called as nucleoplasmatic male sterility. It is based on a cytoplasm that produces male sterility and on a gene that restores fertility in the presence of male sterile cytoplasm.

When nuclear genes for fertility restoration (Rf) are available for CMS system in any crop, it is called as Cytoplamic Genetic Male Sterility (CGMS). This type of male sterility system is common in many plant species across plant kingdom. The sterility is manifested by the influence of both nuclear and cytoplasmic genes. There are commonly two types of cytoplasms, N (normal) and S (sterile). There are also restorers of fertility (*Rf*) genes, which are distinct from genetic male sterility genes. The *Rf* genes do not have any expression of their own unless the sterile cytoplasm is present. *Rf* genes are required to restore fertility in S cytoplasm which causes sterility. Thus a combination of N cytoplasm with rfrf and S cytoplasm with *Rf*- produces fertiles, while S cytoplasm with *rfrf* produces only male steriles. Another feature of these systems is that *Rf* mutations (*i.e.*, mutations to *rf* or no fertility restoration) are frequent, so N cytoplasm with *Rfrf* is best for stable fertility.

The CGMS system is used commercially to produce hybrid seed in maize, bajra, cotton, sunflower, sorghum etc. This system involves the use of three different lines to produce a hybrid. The female parent, called A-line is male sterile and this male sterilty is maintained by crossing it with another line called B-line which is similar to A-line in all respects except that it is male fertile. A third line called R-line is used to cross with A-line to produce hybrid seed which is male fertile. A generalised scheme for three line breeding is given in Figure 2.

Because of the convenience to control the sterility expression by manipulating the gene – cytoplasm combinations in any selected genotype, cytoplasmic genetic male sterility systems are widely exploited in crop plants for hybrid breeding. Incorporation of for male sterility evades the need for emasculation in cross pollinated species, thus encouraging cross breeding producing only hybrid seeds under natural conditions.

3.3.2. Genetic Male Sterility

This is of wide occurrence in plants. It is ordinarily governed by a single recessive gene *ms* which can arise spontaneously or can be artificially induced. GMS is subdivided into two groups (1) environmental insensitive (*ms* gene expression is much less affected by the environment) and (2) environmental sensitive (*ms* gene expression occurs within a specified range of temperature and or photoperiod regimes). The environmental sensitive male sterility (EGMS) is further divided into two groups (a) Temperature- sensitive GMS (TGMS) and (b) Photoperiod- sensitive GMS (PGMS). Both EGMS types of male sterility are identified and utilized for hybrid rice production in China where variation in temperature and photoperiod is available. The system of hybrid seed production is called two line system as it involves the use of only two lines ie. A-line and R-line. A generalised scheme for two line breeding is given in Figure 2.

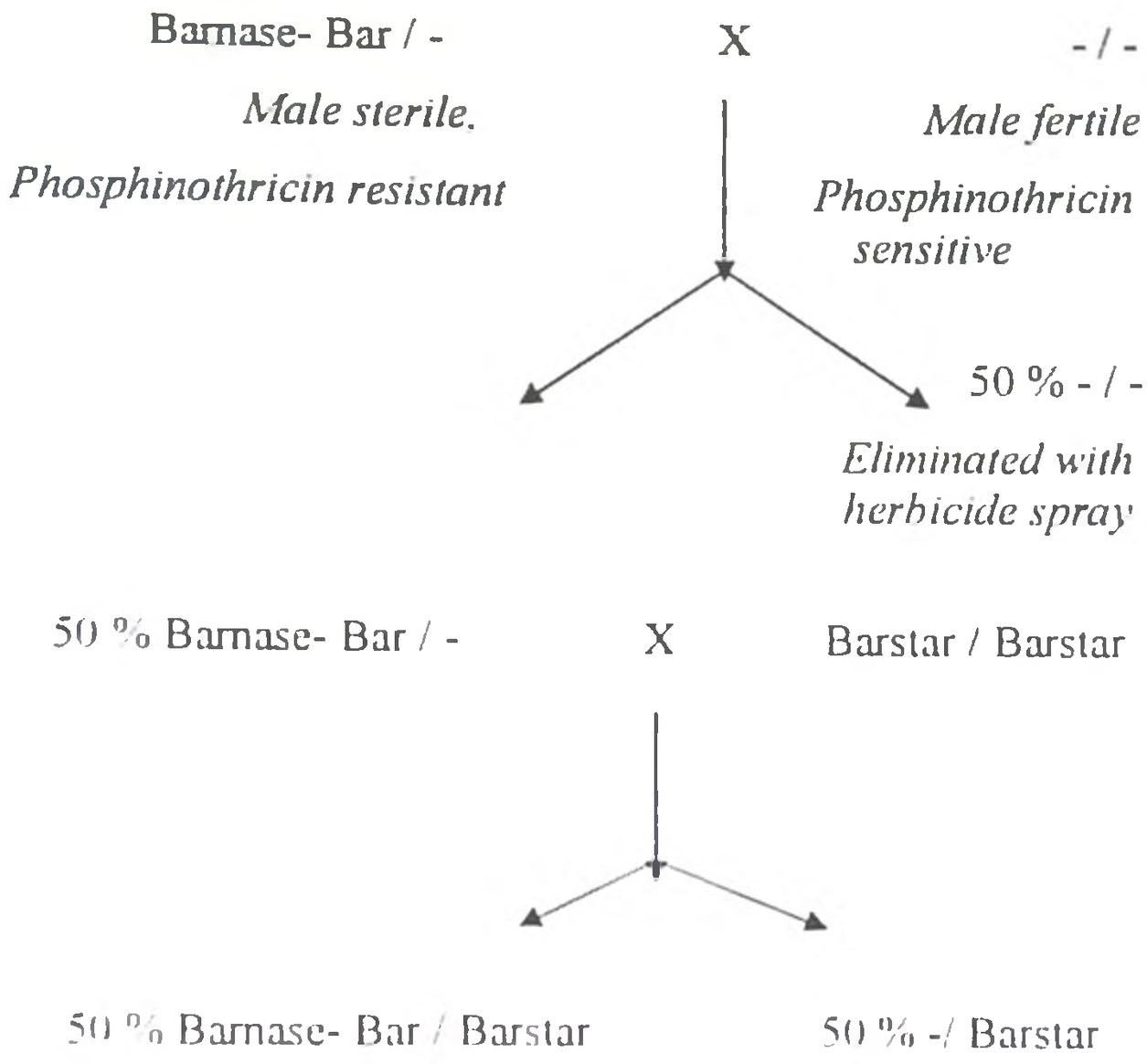
3.3.3. Chemically induced male sterility

Many chemical saffect the function of male reproductive organs in plants to induce artificial, non genetic male sterility. They are called as male gametocides, pollenocide, chemical hybridizing agents etc. Eg. maleic hydrazide (MH), ethrel, RH531, zinc methyl arsenate, sodium methyl arsenate etc. This system is used for hybrid rice production in China with arsenical CHA's MG1 and MG2. It is also used for hybrid wheat production in USA with CHA called RH0007 or hybrex.

3.3.4. Transgenic male sterility

A gene introduced into the genome of an organism by recombinant DNA technology is called a transgene. Many transgenes have been shown to produce genetic male sterility which is dominant to fertility. But effective fertility restoration systems are to be discovered if these are to be utilized for hybrid seed production. These techniques follow principles of genetic ablation and or gene suppression. An effective restoration system is available in one case called *Barnase-Barstar* gene system (Figure 3)

Figure 3. Barnase-Barstar gene system



The sterility of the MS line can be attributed to a newly introduced gene derived from the bacterium *Bacillus amyloliquefaciens*, whose activity leads to cell death. This toxic effect is caused by a special enzyme (barnase), which cleaves the RNA molecule in the cells. *Barnase* expressed in tapetum encodes RNAse (Mariani *et al*., 1990). To ensure that only the male flower parts are affected, the toxin gene is linked to a genetic switch, or promoter. This switch activates the gene only in specific cells that are needed for the development of the male inflorescence. As a result, no pollen is produced on the filaments.

The RF lines contain a gene for an active substance (barstar), which neutralises the RNA-cleaving barnase. The gene for this inhibitor was also isolated from *B. amyloliquefaciens*. *Barstar* protein inhibits RNAse restoring fertility (Mariani *et al*., 1992). In the offspring that result from crossbreeding the MS and RF lines, although the barnase and barstar genes are both active, they cancel each other out. Breeders obtain "normal", viable seed. System used in cauliflower, chicory, lettuce, oilseed rape and tobacco (Reynaerts *et al*.,1993).

Another genetically engineered system of inducing male sterility is the antisense RNA approach. Antisense RNA is single-stranded RNA that is complementary to a messenger RNA (mRNA) strand transcribed within a cell. Antisense RNA may be introduced into a cell to inhibit translation of a complementary mRNA by base pairing to it and physically obstructing the translation machinery. This effect is therefore stoichiometric. It helps in inactivating specific genes without interfering with others.

Flavanoid biosynthesis gene arrested when antisense construct of Chalcone synthase gene (*Chs A*) was used to induce male sterility in Petunia hybrida (Van der Meer *et.al.*, 1992). RolC gene when present induces male sterility and the antisense construct of RolC restores male fertility in tobacco (Schmulling *et al.*, 1993)

In the present era of biotechnology and recombinant DNA technology, private companies are conducting lot of research on inducing and restoring male sterility so that it can be utilized for hybrid seed production. Some patents issued in USA related to male sterility include

- Patent No. 6072102 entitled 'Reversible nuclear genetic system for male sterility in transgenic plants' which was issued on June 6, 2000 relates to the use of dominant negative genes and an anther-specific promoter. Male sterility is reversed by incorporation into a plant of a second genetic construct which represses the dominant negative gene
- Patent No. 6207883 entitled 'DNA sequences coding for a protein conferring male sterility' was issued on March 27, 2001. This invention relates to a gene associated with male fertility, labeled Ms41-A, and a recessive mutant form thereof, labelled ms41-A, which confers male sterility. The Ms41-A gene is isolated from Arabidopsis, while the related gene Zm41-A is isolated from maize
- Patent No. 6603064 entitled 'Nuclear male sterile plants, method of producing same and methods to restore fertility' which was issued on August 5, 2003 is directed to the production of male sterile plants by providing them with a recombinant DNA capable of specific expression in the male reproductive system of a plant of the enzyme trehalose phosphate (TPP). Resotration of the fertility can

be established either by providing said male sterile plants with a recombinant DNA capable of expression of trehalose phosphate synthase (TPS) under control of an inducible promoter or with a recombinant DNA capable of expression of a suppressor protein which suppresses expression of TPP under control of an inducible promoter. This inducible restoration possibilities enable the maintenance of a homozygous male sterile line. Restoration can also be done by spraying the male sterile plants with gibberellic acid. For production of hybrids or hybrid seed a site-specific recombination system is provided, by inserting two site specific recombination sites flanking the recombinant DNA coding for TPP and crossing the male sterile lines with lines expressing the corresponding recombinase. When the two plants are crossed the recombinase will excise the gene coding for TPP and fertile hybrids are produced.

- Patent No. 6852911 entitled 'Method of producing a male sterile plant by exogenic allelism' which was issued on February 8, 2005 is drawn to a method of producing a male-sterile plant by using two different recombinases to generate different deletions in a transgenic construct in a plant, thereby resulting in a male-sterile plant with two different constructs in an allelic relationship to one another. Exogenic allelism is based on the idea that two genetic components, positioned precisely across from each other on homologous chromosomes, interact to generate male sterility in the female parental generation. In meiosis, during gamete formation, the male sterility trait disintegrates by natural segregation. Subsequent pollination by any pollen donor of choice thus results in fully fertile F_1 hybrid seed. Currently transformation of commercial crops such as cotton and rice are under way. Transformed cotton plants containing essential components of this technology are now available

4. Hybrid seed research and development in India

a. Rice

The successful development and use of hybrid rice technology in China during 1970's led the way for development and release of rice hybrid varieties elsewhere. India has made good progress in this regard and it is expected that

hybrid varieties of rice shall be soon available for cultivation to the Indian farmers.

Hybrid-rice can be produced in the following ways:

a) Three-line system : The hybrid seed production involves multiplication of cytoplasmic-genetic male sterile line(A line), maintainer line (B line) and a restorer line (R line); and production of F_1 hybrid seed (AxR). Different cytoplasms have contributed male sterility to cultivated rice and they are listed in Table 1.

Table 1. Cytoplasms contributing male sterility to rice.

Cytoplasm	Restorer genes	Remarks
CMS- WA (Wild abortive strain)	Four genes, *Rf –WA 1* strongest	> 95 percent hybrids based on this
CMS-bo	Single dominant gene	
CMS- DA (Dwarf abortive strain)	-	
CMS-ARC (*O sativa* ARC13728-16 strain)	-	Usable
CMS- W180 (*O rufipogon* W1080 strain)	-	
O *glumaepetuleae* X O *sativa*	-	Stable system

b) Two-line system The hybrid seed production involves the use of photo-period sensitive genetic male steriles (PGMS) or temperature sensitive genetic male steriles (TGMS) Any normal line can serve as a restorer Seed production in this case is simpler than three line system (Aananthi and Jebaraj. 2006)

c) By using chemical emasculators Chemicals that can sterilize the stamen, with little or no effect on the normal functioning of the pistil, can he used to emasculate female parents for hybrid rice production The advantages are obvious, no special development of male sterile or restorer lines is required, and extensive varietal

resources are available. Chemical emasculators such as male gametocide1(MF1) and male gametocide2(MG2) were developed in China and have been successfully used in hybrid rice production. In chemical emasculation, physiological male sterility is artificially created by spraying the rice plant with chemicals to induce stamen sterility without harming the pistil. In hybrid seed production, two varieties are planted in alternate strips, and one is chemically sterilized and pollinated by the other.

Table 2. Hybrid rice varieties from India

Sl No.	Name	Parentage	Year
1	KRH 1	IR 58025 A X IR 9671 R	1994
2	CORH-1	IR 62829 A X IR 10198-66-2	1994
3	APHR 1	IR 58025 A X Vajram (R)	1994
4	APHR 2	IR 62829 A X MTU 992 (R)	1994
5	CNRH 3	IR 2829 A X Ajaya (R)	1995
6	DRRH 1	IR 58025 A X IR 40750 R	1996
7	KarnatakaRiceHb-2	IR 58025 A X KMR 3R	1996
8	PantSankarDhan- 1	IR 58025 A X UPRI-93-133	1997
9	UPRH- 27	IR 58025 A X UPRI-92-133	1997
10	CORH 2	IR 58025 A X C 20 R	1998
11	ADTRH-1	IR 58025 A X IR 66R	1998
12	Sahyadri	IR 58025 A X BR-827-35-3-1-1-1 R	1998
13	Narendra Sankar Dhan-2	IR 8025 A X NDR 3026	1998
14	Hybrid 6444	6CO2 X 6MO 5	2001
15	Pusa RH-10	Pusa 6A X PRR 78	2001
16	KRH 2	IR 58025 A X KMR 3R	2002
17	DRRH 2	IR 68897A X DR 714-1-2 R	2005

b. Wheat

Three mechanisms have been used to facilitate hybrid wheat production namely cytoplasmic male sterility (CMS), genetic male sterility (GMS) and chemical hybridizing agent (CHA). Still, a fully satisfactory system for mediating male sterility is yet to be introduced into large scale commercial production. CMS lines have been mostly developed from a related wild species of cultivated wheat *Triticum timopheevi*. Good fertility restorers are yet to be found out for the male sterile lines developed which show appreciable hybrid vigour. Presently emphasis is on CHA approach (Mahajan, 2006) with the collaboration of NCPL, Pune. They have come up with certain combinations of CHA namely CH9832 and CH9701. National Hybrid Wheat Trials going on and some superior hybrid combinations have been identified (Rai, 2006)

c. Maize

The development of hybrid breeding methodology and its successful application to exploit heterosis is considered as a significant achievement of plant breeding in the present century. In India, double crosses and double top crosses were first released in early 1960's. However the first single cross hybrid was released in 1996 named Paras. Presently more than 12 SCH are available in maize (Dhillon and Prasanna, 2001). Hybrids are produced by manual emasculation method called detasselling followed by pollination and use of CGMS. Different cytoplasms used in induction of male sterility in maize are listed in Table3.

Table 3. Cytoplasms inducing male sterility in maize

Cytoplasm	Restorer genes	Remarks
CMS- T (Texas)	Rf1, Rf2	Most commonly used, Susceptible to *Helminthosporium* leaf blight
CMS-S (USDA)	Rf3	Commercially used
CMS- C (charrua)	Rf4	Spontaneous reversion high

d. Sorghum

The discovery of cytoplasmic genic male sterility in Sorghum (Stephens and Holland, 1954) and its subsequent development for hybrid seed production made possible the mass production of F_1 hybrids and basic revolution in sorghum cultivation. All commercial hybrids of sorghum developed to date are based on *milo-kafir* system. Milo cytoplasm remains the principal means of inducing male sterility in sorghum.

The development of male sterile CK60 A and maintainer CK 60B lines based on *milo-kafir* system is a landmark in the history of commercial sorghum hybrid seed production. As other cytoplasmic genetic male sterility systems were discovered later in sorghum, the *milo-kafir* system was named as A_1 system. Other CMS sources in sorghum include A_2, A_4 and GE with restorer genes from Guinea . A_2 with restorer genes from *S. caudatum*, A_5 with restorer genes from *S. bicolor* and A_6 with restorer genes from *S. durra*. Popular hybrids in sorghum include CSH 1,5,6,9 for *kharif* season and CSH 12R for *rabi* season.

e. Pearl Millet

The first commercial use of CMS pearl millet was based on the development at Tifton, Georgia (Burton, 1958). Using this source the first five hybrids HB1 to HB5 were released commercially for cultivation during 1960's. Before the discovery of male sterility, chance hybrids like Hybrid X_1, Hybrid X_2 were developed in pearl millet that was facilitated by protogyny and the time lag between anther dehiscence and stigma emergence. But these hybrids were not much popular due to its nonuniformity in phenotypic appearance.

The A_1 cytoplasm has been a valuable tool for producing many commercial F_1 hybrids. But these hybrids were highly susceptible to downy mildew and caused severe reduction in grain yield. Hence the need arose to widen the genetic as well as cytoplasmic base of male sterility to induce high level of downy midew resistance. The different sources of male sterile cytoplasms characterised in pearl millet are given in Table 4

Table 4. Cytoplasms inducing male sterility in pearl millet

Cytoplasm	Remarks	Reference
A_1	Backcrossing of Tift 23 to Gahi, Most common source of male sterility	Burton, 1958
A_2	Selection from genetic stock of IP 189	Athwal, 1961
A_3	Population of natural stock with pearly amber grains	Athwal, 1961
A_4	Selection from *P.glaucum* ssp.*monodii*	Hanna, 1989
A_5	Selection from large seeded gene pool, restorers hard to find	Rai, 1995
A_{ICRP}	Derived from ICRISAT early gene pool	Rai, 1995

About 107 hybrids have been released in pearl millet from public sector until now. PHB10 and PHB13 were the first downy mildew resistant hybrids released in India

f. Pigeon pea

The discovery of stable genetic male sterility in redgram (Reddy et al. 1978) coupled with its outcrossing nature opened up the possibility of exploiting hybrid vigour in this crop. 1st hybrid in pigeon pea from ICRISAT - ICPH 8 is a cross between ms Prabhat (DT) and ICPL 161 was released in 1991 which was produced based on GMS system. Recently a CMS system involving a wild relative of pigeon pea *Cajanus cajanifolius* was identified in ICRISAT that is reported to be more stable and produces high frequency of fertility restorers (Tikka *et al*., 2006). A CMS line- GT-288A was developed in Gujarat and the first CMS based hybrid GTH- 1 was released by GAU in 2004.

g. Castor

India is the only country in the world to exploit hybrid vigour in castor on a large scale and more than 95 percent of area under irrigated castor crop in

Gujarat, Rajastahn and Maharashtra is dominated by hybrids. The first commercial hybrid GCH-3 was released in 1968 using TSP 10R, an exotic pistillate line introduced from USA. Later, an indigenous stable pistillate line VP 1 was developed and several hybrids like GCH 2, GAUCH 1 and GCH 4 were released from Gujarat. GCH-4 is the first wilt resistant hybrid which was released in 1986. Sevearal stable indigenous stable pistillate lines like LRES 17, NES 6, NES 15, NES 19, SKP 24, SKP 52, SKP 93, 240, JP 65 have now been developed in India and many hybrids have been released (Pathak *et. al.*, 2006).

h. Sunflower

Important landmark in the development of commercial sunflower hybrids was the discovery of cytoplasmic male sterility by Leclercq in 1969 in the progeny of a cross between in *H. petiolaris* and cultivated *H. annuus*. This is named as PET 1 cytoplasm and its restorer genes Rf1, Rf2 are available. BSH 1 was the first sunflower hybrid released in India in 1980 and it was a cross between CMS 234 A X RHA 274.

i. Rapeseed

Hybrid research in *Brassica* species is of recent origin in India. Cytoplasmic male sterility called *tour* system developed from *Brassica tournifortii* and *ogura* system developed from *Raphanus sativus* is used in India for hybrid seed production. India is thus on the threshold of releasing hybrids in *Brassica* after China and Canada.

j. Cotton

Cotton is one of the oldest groups in which hybrid vigour was observed in interspecific crosses of *G hirsutum X G barbadense* in the earlier parts of the last century. Both intraspecific and interspecific hybrids are present in cotton. India is the pioneer in commercial exploitation of heterosis in cotton. The first Intra *hirsutum* hybrid H4 was released in 1970 which was developed by Dr C.T.Patel from Gujarat Agricultural University by crossing Gujarat 76 and American Nectariless. For his outstanding contribution, Dr C.T.Patel is called the father of hybrid cotton. In 1972, an interspecific *G hirsutum X G barbadense* hybrid named Varalaxmi was released which was also a pioneer in the field. The first cotton

hybrid using genetic male sterility (source Gregg) was released in 1978 under the name Suguna. The first diploid hybrid DH7 was released from Gujarat Agricultural University in 1988. Diploid hybrids are not popular among farmers due to difficulty in their seed production. The first CGMS based hybrid PKVHy3 was released in1993.

Male sterile line Gregg is used extensively. Some of the cytoplasms that induce stable male sterility include *G.harkensii* (Meyer, 1973), *G.aridum* (Meshram *et al.*, 1994) and *G.trilobum*. Some cytoplasmic male sterile lines developed in cotton in India include C 9 using *G.anomalum*. P 24-6A using *G.arboreum*. HAMS 16,277 using *G.harkensii* and 104-7A using *G.hirsutum*.

k. Vegetable Crops

The cultivation of hybrid varieties in vegetable crops is comparitively of recent origin in India. Tomato, brinjal, chilli, cucumber, muskmelon, watermelon, cabbage, cauliflower, carrot, onion etc. are important crops in which hybrids are available and farmers are adopting them to a considerable extent. Tomato hybrids cover more than fifty per cent area under tomato cultivation and so is the case with cabbage hybrids. In brinjal, sweet pepper, cucurbits etc., potential hybrids have been developed through manual emasculation and pollination. Male sterility systems have been exploited to develop hybrids of cauliflower, cabbage, onion, carrot, radish and chilli. Gynoecious lines are used in cucumber. Limiting factors in popularisation of hybrid vegetable seeds are extremely high cost of hybrid seeds. Still in the past few years hybrid seed cost has gone down because considerable number of seed companies have initiated production and marketing of vegetable hybrid seeds. In okra hand emasculation and pollination is the technique adopted to produce hybrids. Some of the nationally released hybrids of okra in the private sector include AOH 262 and AOH 263 from GAU, Anand; DVR-1 and DVR-2 from IIVR, Varanasi, Varsha, Vijay and Vishal from IAHS, Bangalore.

In solanaceous vegetables like tomato, brinjal and chilli hand emasculation and pollination is the main method used to produce hybrids. In bell pepper, GA_3 is used as male gametocide. Some genetic male sterility mechanisms have been

reported in tomato (Crane,1915) brinjal and chilli. Male sterile lines ms-12, ms-13 and ms-41 have been developed in chilli and two hybrids CH-1 and CH-3 have been produced in chilli at PAU with ms-12 as female parent (Rai, 2006). In potato, vegetative propagation has been mainly utilised in raising F_1 hybrid populations to select new genotype or clone showing maximum hybrid vigour in yield, quality and other desirable characters.

Cucurbitaceous vegetables are monoecious and hence there is no need of emasculation. Manual pollination alone is sufficient to produce hybrids. Male sterility has been reported in crops like pumpkin, bottle gourd and musk melon (Bohn and Principe, 1962 ; McCreight and Elmstrom, 1984; Lecouviuor *et al.*, 1990 and Pilrat, 1990)

Use of chemical hybridizing agents have been advocated in some cases for hybrid seed production. In pumpkin, ethephone *a* 600ppm at two and four leaf stages is applied to induce male sterility (Shannan and Robinson, 1979). Ethrel *a* 200-300ppm at two and four leaf and flowering stages is used in bottle gourd (Rai and Rai, 2006). Use of $AgNO_3$ *a* 100-200ppm in musk melon is reported by Rai and Rai (2006) for inducing male sterility.

In cabbage and cauliflower free insect pollination is allowed in between self incompatible but cross compatible lines. Nieuwhof (1971) has reported the presence of genetic male sterility in these crops. Use of dominant marker gene for hybrid seed production has been illustrated by Swarup and Gill (1964). Presence of cytoplasmic male sterility has been reported by Ogura (1968). Some of the hybrids developed in cabbage include H-113, H-64, BRH-5 and Pusa Hybrid 2, Pusa Hybrid 3 and Snow Crown in cauliflower

Another cool season vegetable where heterosis is utilized is carrot. In carrot hand emasculation and pollination and use of male sterility is tried to develop hybrids. Two types of cytoplasmic male sterility systems are reported in carrot namely brown anther type and petalloid type (Cross, 1970)

In onion hand emasculation and pollination is a tedious work as the flowers are very small and the inflorescence bears many flowers. Hence use of male sterile lines with CMS-S cytoplasm and CMS-T cytoplasm are advocated.

Both cases are thermolabile and commercially used. In CMS-T cytoplasm two systems of restorer genes namely gene A and complimentary genes B and C are recognized. Nationally released hybrids in Onion include Arka Kirtiman and Arka Lalima from IIHR, Bangalore.

l. Ornamentals

Floriculture is now considered as a well established industry in India. The production of F_1 seeds of annual ornamental crops is considered as the extreme focused area in floriculture industry. Heterosis has played a prominent role especially in flowering annuals or seasonal flowers because they can be conventionally grown from seeds. Of these petunia, pansy, marigold and antirrhinum are considered to be important annual flower crops. The first hybrid in *Begonia semperflorens* was developed in 1906, a couple of years before Shull presented hypothesis on heterosis. The first F1 hybrid petunia was developed in early 1940's. Actual breakthrough in hybrid seed production came from 1950 onwards. Hybrid in single geranium was developed in 1960. Hybrids of zinnia, pansy, marigold and antirrhinum were developed in 1965. Hybrid of ageratum was developed in 1966. Geranium double hybrid was initially produced in 1970. Hybrids of dianthus, begonia, balsam and portulaca were developed during 1976-77. Gerbera was developed in 1980 and carnation hybrid in 1981. Self-incompatibility, Genic male sterility, Cytoplasmic male sterility and manual pollination are utilized for developing hybrids in petunia. In pansy, self incompatibility and manual pollination are used for producing hybrid seeds. Genic male sterility, manual pollination are used in producing antirrhinum hybrids. In marigold, apetalous and or double flowered male sterility and manual pollination are adopted. Though research institutions initiated the work in this direction, private sector pioneered and advanced the hybrid flower seed production through latest technology and took lead in export of these seeds (Janakiram, 2006).

m. Perennial crops

In perennial crops like fruits, many hybrids have been developed. As most of the fruit trees can be vegetatively propagated, once a hybrid is identified, it is easier to maintain the vigour unlike in seed propagated crops. In mango hybrids

like Mallika, Amrapali, Manjeera, Ratna, Arka Aruna, Arka Puneet, Arka Anmol, Neeleshan, Neeluddin, Neelgoa, AU-Rumani etc. are popular. Papaya hybrids developed at TNAU include Co-3 and Co- 4 and another hybrid papaya Surya was developed at IIHR. IIHR has also released hybrids in Guava (Arka Amulya and Arka Mridula), Acid lime (Rasraj), Grapes, Annona (Arka Sahan) and Pomegranate (Ruby and Amlidana).

In plantation crops like coconut many types of hybrids like Tall X Dwarf, Dwarf X Tall Dwarf X Dwarf and Tall X Tall are produced. 12 hybrids have been released in India from 1985 (Kumaran *et al.*, 2006). They include hybrids ChandraSankara, ChandraLaksha and KeraSankara from CPCRI; VHC-1, VHC-2 and VHC-3 from TNAU and GodavariGanga from APAU.

Cashew is yet another crop where hybrids are available. H 46 and H 32/4 were released from NRC on Cashew, Puttur. From CRS, Bapatla the hybrids include BPP-1, 2 and 8. Vengurla 3,4,5,6 and 7 were released from RFRS, Vengurla.

Hybrids are also available in Rubber which are produced by utilizing both indigenous and exotic clones. Some of them include RRII 105, RRII 414, RRII 430, RRII 203, RRII 417, RRII 422, RRII 118, RRII 176, RRII 208 , RRII 300 and RRII 429

Table 5. Hybrids from Kerala Agricultural University

Sl. No.	Hybrid	Year of release	Parents
Coconut			
1.	Lakshaganga	1989	LO x GB
2.	Keraganga	1989	WCT x GB
3.	Anandaganga	1989	AO x GB
4.	Kerasree	1992	WCT x MYD
5.	Kerasowbhagya	1993	WCT x SSA

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Cashew			
1.	Kanaka	1993	Anakkayam 1 x H-3-13
2.	Dhana	1993	LGD 1-1 x K 30-1
3.	Dharasree	1996	Tree No. 30 x BRZ-18
4.	Priyanka	1996	BLA 139-1 x K 30-1
5	Amrutha	1998	BLA-139-1 X K-30-1
6	Akshaya	2002	H-4-7 X K-30-1
7	Anagha	2002	T-20 X K-30-1
8	Damodar	2002	Anakkayam 1X H-3-13
9	Raghav	2002	ALGD-1-1 X K-30-1
Cocoa			
1	CCRP 8	2002	CCRP 1 X CCRP 7
2	CCRP 9	2002	CCRP 1 X CCRP 4
3	CCRP 10	2002	CCRP 3 X G VI 68
Black Pepper			
1	Panniyur 1	1967	Uthirankotta x Cheriyakaniyakadan
2	Panniyur 3	1990	Uthirankotta x Cheriyakaniyakadan
Sugarcane			
1	Madhuri	1990	CO 740 X CO 775
2	Thirumadhuram	1992	CO 740 x CO 6806
3	Madhurima	1996	CO 740 x CO 7318
4	Madhumathi	1996	CO 63X CO 740
Banana			
1	BRS-1	1998	Agniswar X Pisanglilin
2	BRS-2	1998	Vannan x Pisanglilin

Pineapple			
1	Amrita	2004	Kew X Ripley Queen
Brinjal			
1	Neelima	1998	Surya X SM 116
Okra			
1	Manjima	2006	Gourisapattom local X IC 48053

5. Conclusion

India is pioneer in developing and releasing hybrid varieties of pearl millet, cotton, castor and pigeonpea. In India, the adoption of pearl millet hybrids in the desert of Western Rajasthan, sorghum in most of the rainfed areas and castor I marginal rainfed lands, could dispel the doubts that hybrid technology is suited for rich farmers and for irrigated ecosystems only. The ground truth is that resource poor farmers have adopted the hybrids of these crops on an extensive scale and majority of the area under these crops is rainfed.

But the intensification of hybrid technology is slow mainly due to high cost of seed production and poor transfer of technology to farmers. Hence the present day need is to intensify research and use resources from various gene pools to develop feasible protocol for hybrid production so that the seed cost will be economical

Innovative research would be required for exploiting apomictic genes for hybrid development as inbred varieties, diversification of male sterility systems, identification of effective areas for quality seed production in varying seasons and situations, enhancing capabilities for seed certification and effective arrangement for seed production and its timely availability to farmers. The availability of hybrids for a wide range of crops would provide impetus to the efforts to enhance productivity and thereby production. An effective planning supported with policy initiatives in relative sectors is desirable to profitably harness the potential of hybrid technology.

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DISCUSSION

1. How much area is cultivated for different crop hybrids in India?

   ☑ The area under cultivation depends on particular crop species. For eg. In pearl millet, >90 per cent area under cultivation are hybrids. This is also true for cotton. For more remunerative crops like vegetables, many farmers cultivate hybrids

2. How can we undertake more cost effective methods of hybrid seed production?

   ☑ Though hand emasculation and pollination is practiced commonly for the commercial production of hybrid seeds, it is better to search for genetic methods of emasculation which are heritable and hence can be transmitted from parents to progeny ie. Once identified, it can utilized for hybrid seed production forever. Hence heritable methods of emasculation will definitely aid in cost effective hybrid seed production.

3. Can we provide artificial atmospheric conditions for utilizing EGMS?

   ☑ The main aim of utilizing EGMS is to reduce the cost and labour involved in emasculation which is the most crucial step in hybrid seed production. When we go for providing artificial environmental conditions, it will increase the cost of seeds. Hence for less remunerative crops like rice, providing artificial conditions to induce male sterility is not advisable

4. What is the difference between a hybrid and a hybrid derivative?

   ☑ A hybrid is the F1 generation of a cross between two genetically dissimilar parents while a hybrid derivative is obtained by raising the segregating generations produced by selfing of hybrid seeds.

5. Why are varieties referred as hybrid seeds and not as hybrids?

   ☑ When a particular variety is referred to as hybrids alone, it will not come under the seed bill as a variety as under this bill only seeds and other

propagules can be considered as varieties. In order to include the hybrids under Seeds Bill, it is designated as hybrid seed.

6. What is the role of KAU in harnessing hybrid technology?

☑ As already mentioned, KAU has developed and released hybrids mostly in perennial crops like coconut, cashew etc. KAU has entered vegetable hybrid seed production recently with one variety each of brinjal and okra.

7. How are hybrids produced in coconut?

☑ Male and the female parent palms of the already developed hybrid are identified at the farmers field level with the help of krishibhavans following the principles of mother palm selection. The farmer is trained to emasculate the mother palm and pollinate it with pollen collected from the male palm. When the seed nut is formed, it is germinated and evaluated for seedling vigour. Seedlings showing appropriate vigour are identified as hybrid seedlings and distributed to farmers. Unlike vegetable crops, here seedlings are distributed to farmers and not seeds. Another difference from other perennials is that, in coconut, seed nuts have to be produced afresh each time as it cannot be vegetatively propagated.

8. What is the parentage of ICPH 8?

☑ ICPH 8 is a cross between ms Prabhat (DT) and ICPL 161 was released in 1991 which was produced based on GMS system at ICRISAT, Hyderabad

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KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara
PbGen 751 Seminar

Topic: HARNESSING HYBRID VIGOUR IN CROP PLANTS

Name: Gayathri, G. (06-21-106)

Time: 04/05/07 (11.15 am)
Venue: A.V. Lab,
Pomology and Floriculture

The production of hybrid plants has been practiced ever since Thomas Fairchild attempted artificial plant hybridization in 1717 to produce the Fairchild's mule by crossing sweet william (*Dianthus barbatus*) with carnation (*Dianthus caryophyllus*). Plant breeders discovered that crossing two distinct parental lines often resulted in a hybrid plant that exhibited hybrid vigour or heterosis. Hybrid vigour is defined as the superiority of an F_1 hybrid over both its parents in terms of yield or some other character. In world agriculture, hybrid varieties are available in about fifty field and horticultural crops (Rai, 2006).

The crucial step for the production of hybrid is emasculation as most of the crop plants are bisexual or monoecious in nature. Emasculation can be done manually by mechanical removal of anthers or by spraying male gametocides. Exploiting genetic tools like self-incompatibility and male sterility also aids in emasculation. Currently innovative techniques have been used to develop male sterility in plants like use of *Barnase- Barstar* system and antisense RNA approach. At present patents have been issued in the Unites States of America for developing transgenic male sterility.

The Indian scenario of hybrid development in different crops can be broadly divided into two sections a) successful crops where hybrids have already become popular like maize (Dhillon and Prasanna, 2001), vegetables (Rai and Rai, 2006) and cotton (Khadi *et.al.*, 2003) and b) potential crops where hybrids have either just begun to be cultivated or would become popular among the growers in the near future like rice (Aananthi and Jebaraj, 2006) and wheat (Mahajan *et.al.*, 2000).

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SEXUAL POLYPLOIDISATION IN CROP IMPROVEMENT

BY

Gayathri, G.

(2006-21-106)

Department of Plant Breeding and Genetics

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

PbGen. 752 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

I hereby declare that the seminar report entitled '**Sexual polyploidisation in crop improvement**' is a record of the seminar presented by me during the course on 07 07 2007 and that this report has been prepared by me independently after going through the references cited herein

Vellanikkara
08 08 2008

Gayathri. G.
(2006-21-106)

CERTIFICATE

Certified that the seminar report entitled **'Sexual polyploidisation in crop improvement'** is a record of seminar presented by **Smt. Gayathri, G. (2006-21-106)** under my guidance and that this report has been prepared by her independently

Vellanikkara
08 08 2007

Dr Djee Bastian
Major Advisor

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1. Introduction

It was almost a century ago that the phenomenon of polyploidy was discovered in plants. This was important for plant evolutionary biologists as well as plant breeders in view of the widespread occurrence of polyploids in the wild as well as in cultivated plants. Polyploidy is a characteristic feature in plants, and yet it is relatively rare in animals and fungi. There is, however, some evidence that the genomes of animals, fungi, and some plants are the products of polyploidy, but had later become diploidized. In angiosperms, estimations of polyploid abundance range from 47 per cent to 70 per cent (Masterson, 1994).

Two types of polyploids are recognized based on the chromosome constitution of the individuals, viz., autopolyploids and allopolyploids which are often referred to as polysomic and disomic polyploids respectively. Autopolyploids are polyploids with chromosomes derived from a single species. Autopolyploids can arise from a spontaneous, naturally-occurring genome doubling (for example, the potato) Bananas and apples can be found as triploid autopolyploids. Allopolyploids are polyploids with chromosomes derived from different species. Triticale is an example of an allopolyploid. Autopolyploids typically have multivalent chromosome pairing at meiosis and polysomic inheritance patterns, while allopolyploids typically exhibit bivalent pairing and disomic inheritance. Segmental allopolyploids are essentially intermediate forms between auto- and allo-polyploids. Allopolyploids are generally considered to be much more common than autopolyploids, however, estimates of autopolyploid abundances may be greatly underestimated (Soltis and Soltis, 2000). Many crop species (e.g. *Brassica, Coffea, Glycine, Oryza, Saccharum, Triticum, Zea*) are polyploids (Soltis, 2005), therefore understanding polyploid evolution could be useful in agricultural settings.

The frequencies of polyploids in angiosperms have been estimated to be somewhere from 47 per cent to 70 per cent (Grant, 1981), depending on the criteria used and regarded as an important mechanism of speciation and adaptation in plants. Ancient genome duplications probably characterize all life. Duplication events that occurred long ago in the history of various evolutionary lineages can

be difficult to detect because of subsequent diploidization such that a polyploid starts to behave cytogenetically as a diploid over time.

In many cases, these events can be inferred only through comparing sequenced genomes. Examples of unexpected but recently confirmed ancient genome duplications include the baker's yeast (*Saccharomyces cerevisiae*), mustard weed (*Arabidopsis thaliana*), rice (*Oryza sativa*), and an early evolutionary ancestor of the vertebrates (which includes the human lineage) and another near the origin of the teleost fishes. Angiosperms or flowering plants have paleopolyploidy in their ancestry (Paterson *et al.*, 2005). All eukaryotes probably have experienced a polyploidy event at some point in their evolutionary history. Among the cultivated plants, some of the important crops such as wheat, potato, cotton, oat, sugarcane, banana, groundnut, tobacco and numerous horticultural crops are all polyploids.

2. Induction of polyploidy

With a hope that induced polyploids might be a shortcut for crop improvement, numerous attempts were made during the early part of the last century to synthesize both autopolyploids and allopolyploids, mostly through colchicine treatment. The most important crops synthesised by man artificially were Raphanobrassica and Triticale

Raphanobrassica includes all intergeneric hybrids between the genera Raphanus (radish) and Brassica (cabbages, etc). The name comes from the combination of the genus names for radish and cabbage. Some botanists prefer the alternative name Brassicoraphanus. The first *Raphanobrassica* or "rabbage", an allopolyploid cross between the radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*), was obtained by the Soviet agronomist Georgi Dmitrievich Karpechenko in 1928, who hoped to create "a Vegetable of the Proletariet," that combined the nutritious root of the radish with the flavorful leaves of cabbage, with very little to go to waste. A meiotic accident occurred, allowing Karpechenko to produce fertile hybrids. Unfortunately for farmers, his resulting hybrid of this crossing had radish leaves and cabbage roots.

Triticale (x *Triticosecale*) is an artificial or man-made hybrid of rye and wheat first bred in laboratories during the late 19th century. Triticale combines the high

yield potential and good grain quality of wheat with the disease and environmental tolerance including soil conditions of rye. Earlier work with wheat-rye crosses was difficult due to low survival of the resulting hybrid embryo and spontaneous chromosome doubling. To improve the viability of the embryo and thus avoid its abortion, in vitro culture techniques were developed. Colchicine was used as a chemical agent to double the chromosomes. After these developments a new era of triticale breeding was introduced. Triticale is essentially a self-fertilizing, naturally inbred crop. This mode of reproduction results in a more homozygous genome.

Emphasis on the usefulness of synthetic polyploids was overly optimistic and these have contributed little, if any, to crop improvement directly. Unlike synthetic ones, the natural polyploids have been quite successful in view of their predominance among flowering plants as well as their success of being important crops

The probable evolutionary history of some of the important cultivated crops is given in figures 1-4. Maize is thought to be an ancient segmental allotetraploid (Gaut and Doebley, 1997). The evidence for this is based on chromosome number and molecular analysis. Maize has n = 10 chromosomes, compared with $n = 5$ for species in its tribe Andropogoneae. Moreover, sequence analysis of 14 pairs of duplicated genes revealed two sets of gene pairs: those that diverged approximately 20 mya and those that diverged approximately 11 mya. Sequence analysis by Gaut (2001) reveals that up to 80% of the maize genome remains organized in colinear regions. Rice is considered to be the best characterized paleopolyploid in the grasses (Goff et al., 2002). The polyploidy origin of the rice genome has been a longstanding hypothesis with little supporting evidence (Nayar, 1973, Levy & Feldman, 2002). To clarify the date and extent of the duplication in rice genome, Vandepoele *et al* (2003) further analyzed 2897 rice (ssp. *japonica*) bacterial artificial chromosome (BAC) sequences generated by the International Rice Genome Sequencing Project, and discovered that approximately 15 per cent of all rice genes are in duplicated segments, with a major fraction of the duplication associated with chromosome 2. On this basis,

they proposed that rice is an ancient aneuploid that has experienced duplication about 70 million years ago. Maize and rice were probably formed approximately 11 million years ago and approximately 40 to 50 million years ago, respectively. Altogether, these data indicate that polyploidy is an ongoing process that has been occurring millions of years ago and is still occurring now, giving rise to new species at a rapid rate (Wang *et.al.*, 2005)

3. Chromosome-Doubling Mechanisms

There are two major chromosome-doubling mechanisms that are involved in polyploid evolution: 1) somatic doubling, and 2) unreduced gamete formation. Somatic doubling refers to chromosome doubling in 'somatic' (or body) cells undergoing mitosis, while unreduced gamete formation occurs through meiosis. Somatic doubling occurs in cells which do not make gametes. Some failure in mitosis prevents normal cell division after the chromosomes have doubled. In plants, this can occur to yield an entire plant that has double the chromosome number of its parent, or it can occur in a single part of the plant (e.g. a stem or flower bud). For example, in a diploid plant, the somatic doubling of a bud can give rise to a tetraploid branch. Crossing two different (but perhaps closely related) plant species will give rise to hybrid offspring -- offspring with two different genomes. Diploid hybrids are typically sterile or 'mostly sterile'. Tetraploid hybrids, on the other hand, are often fertile. The mechanism for somatic doubling in nature is uncertain, but certain chemicals (e.g. colchicine) which inhibit spindle formation during cell division can be used in the lab to create polyploid plants.

Unreduced gamete formation is the second major chromosome doubling mechanism. Typically, gametes (e.g. pollen and ovules) are haploid. Gametes are formed by meiosis and are typically haploid. Gametes may then be represented by *n*. Meiosis is a process which divides the chromosome number of the parent in half (fertilization then restores the original somatic number). Sometimes, however, failure in meiosis can produce what are known as 'unreduced gametes', or gametes with the somatic chromosome number (i.e. a diploid organism would produce diploid or 2*n* gametes). Unreduced gamete formation is considered to be

Figure 1. Evolution of bread wheat

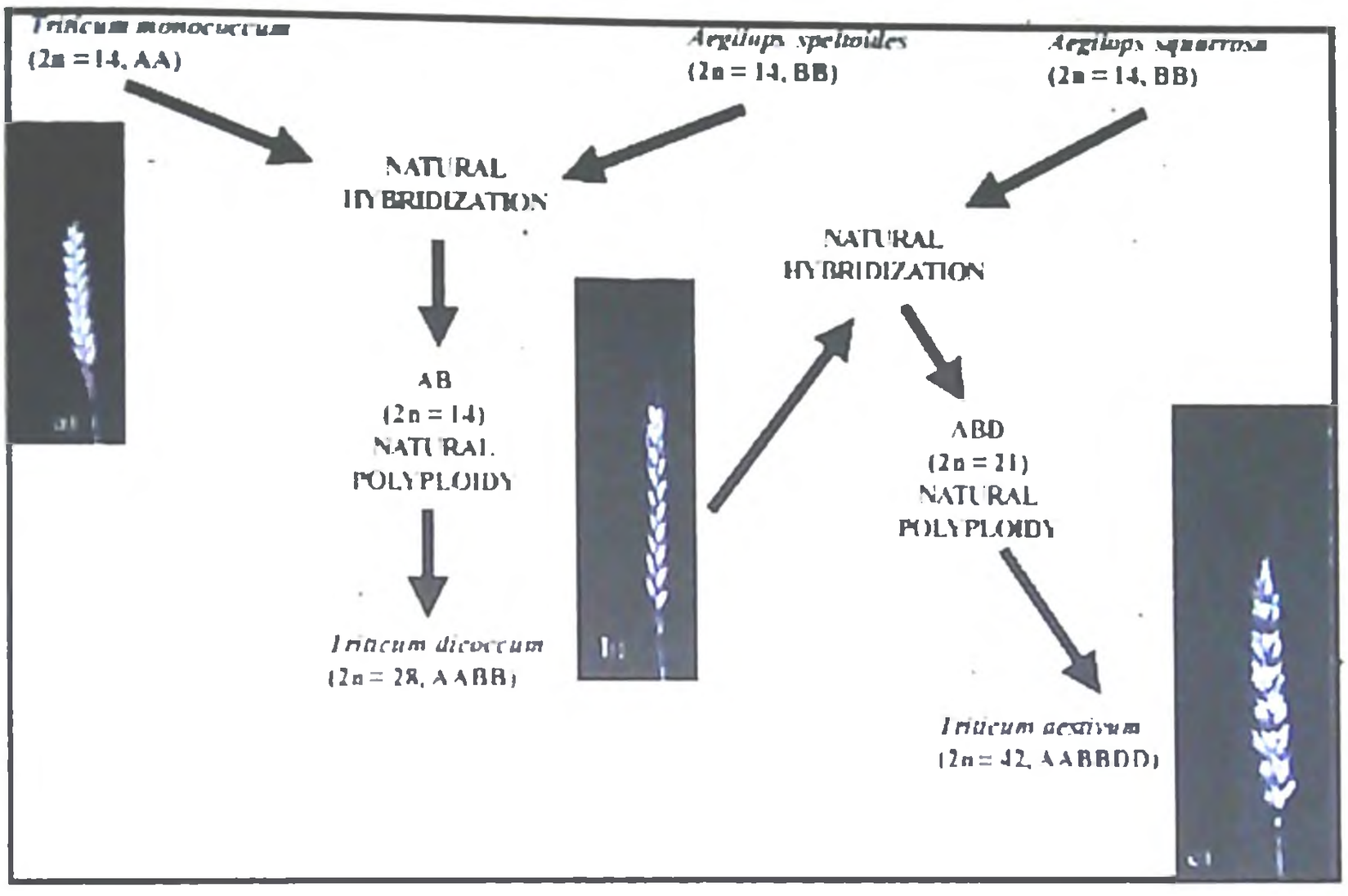

Figure 2 Evolution of *Brassica* sp.

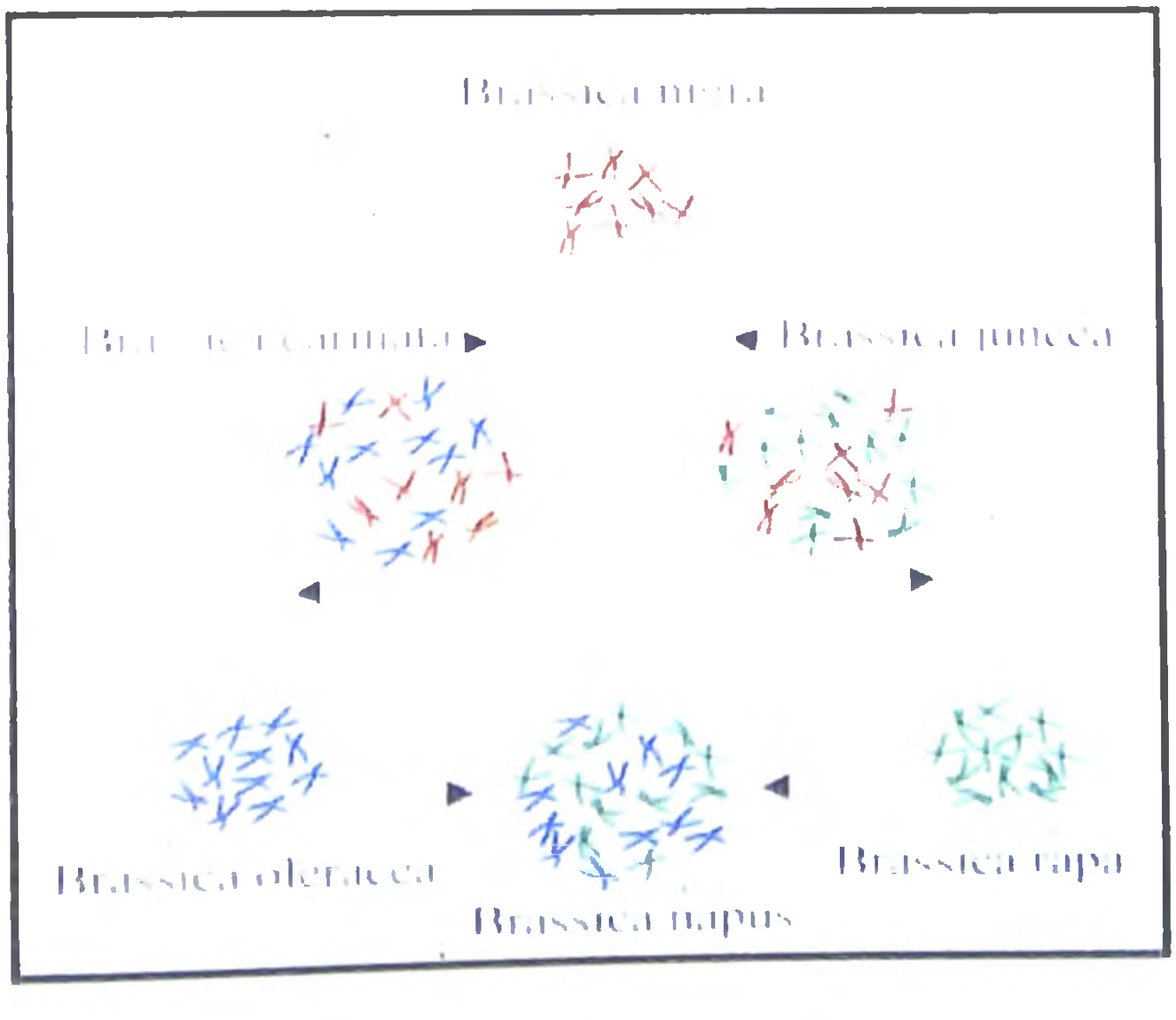

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Figure 3 Evolution of Cotton

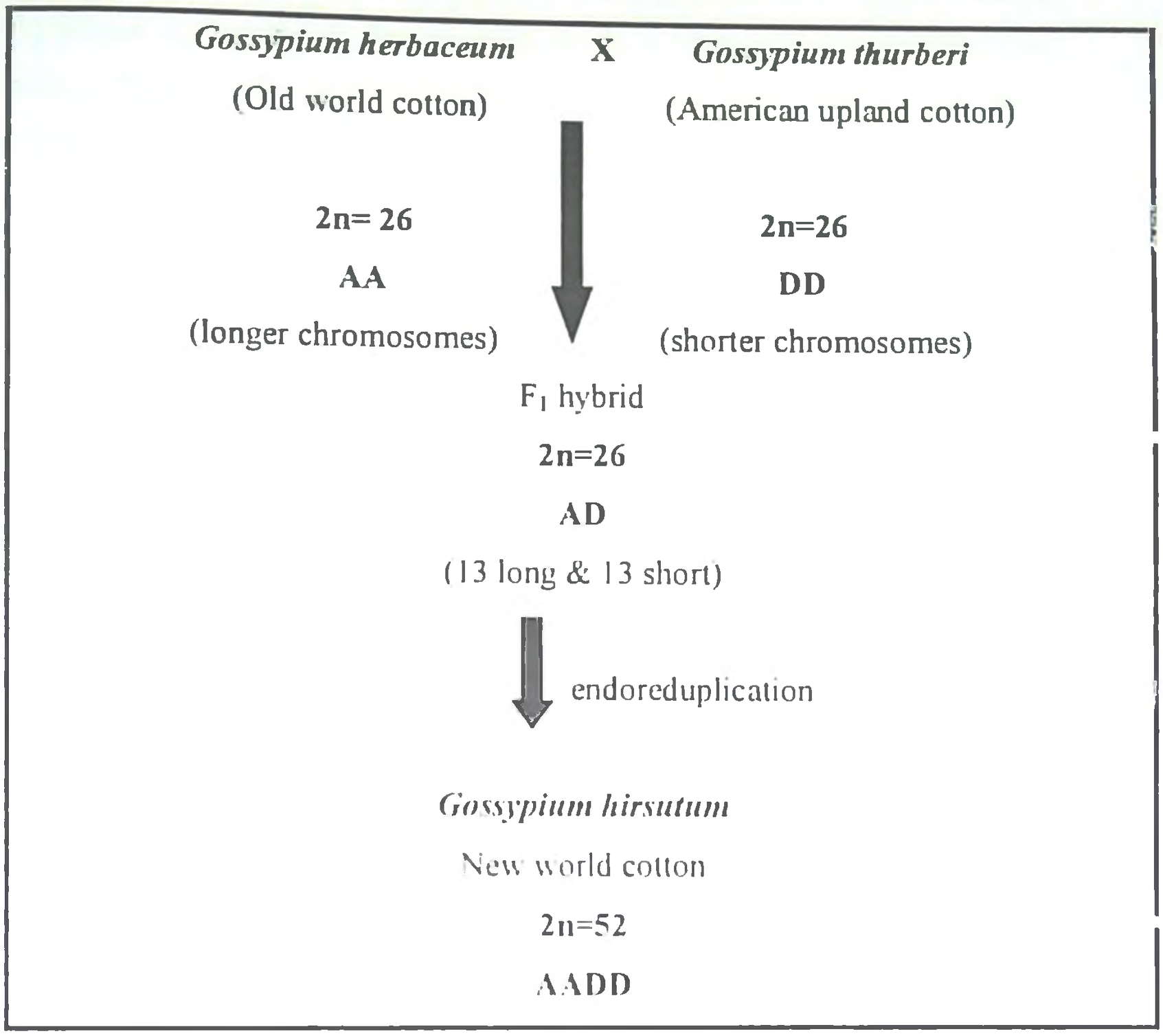
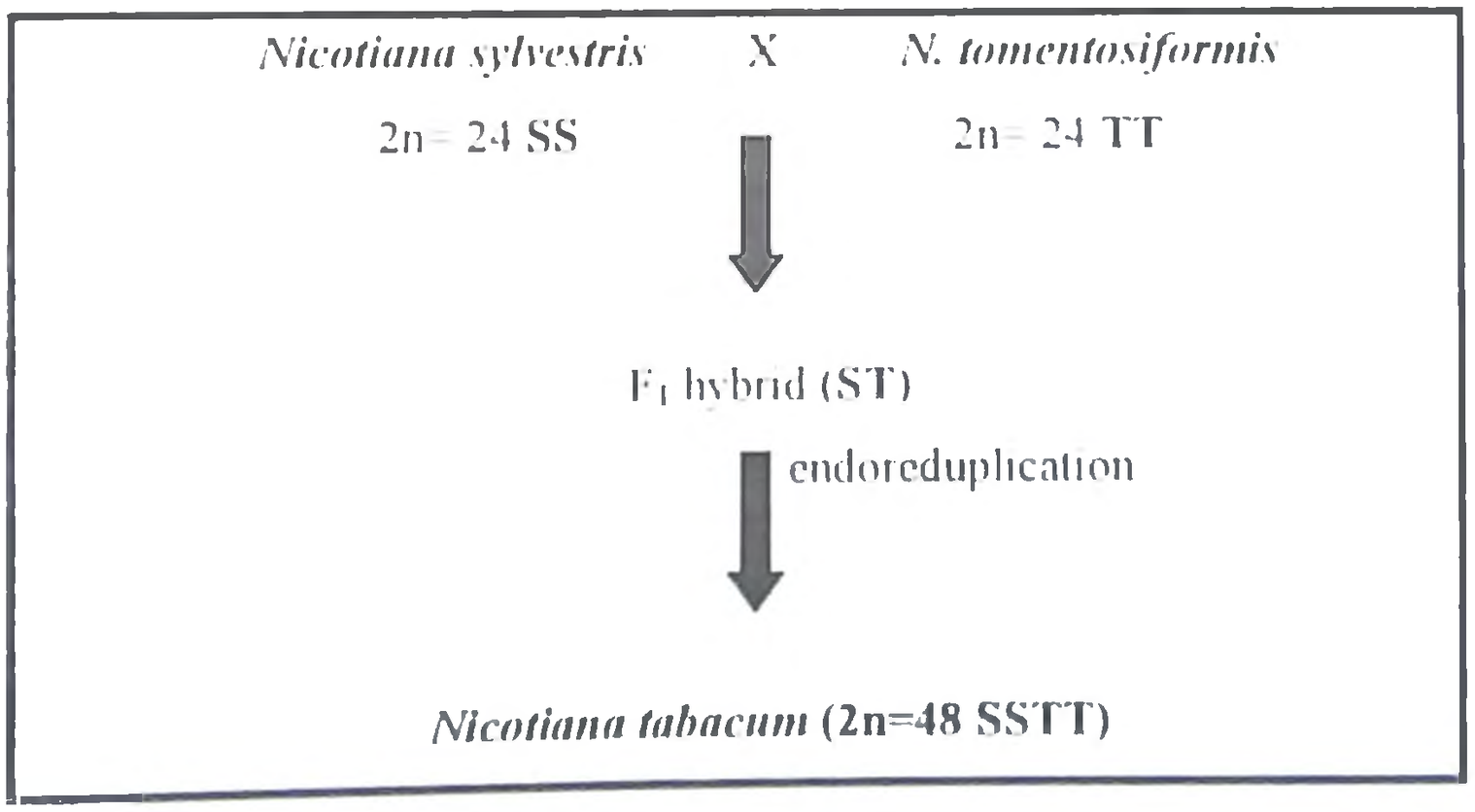


Figure 4 Evolution of tobacco



rare, therefore a diploid plant may produce low frequencies of 2*n* gametes along with the normal *n* gametes. Plants generally produce much more pollen than they do of ovules; therefore 2*n* pollen is probably more likely than 2*n* ovule formation. Polyploidy originating through functioning of 2n gametes is called sexual polyploidisation.

After recognizing the distinction between the synthetic and natural polyploids, Skiebe (1958) made the first systematic effort to synthesise polyploids through the use of numerically unreduced (*2n*) gametes. He assumed that natural polyploids might have originated through the functioning *2n* gametes and demonstrated their superiority in the case of *Primula melocoides* Franchet.

Although the origin of polyploids through *2n* gametes was recognized much earlier, the importance of such polyploids was underestimated. This was because of the assumption that the occurance of *2n* gametes in plants is rare and sporadic, and therefore, might have contributed little to the origin of polyploids. Contrary to this assumption, almost all plant species produce *2n* gametes in some frequencies and all polyploids in plants have originated through functioning *2n* gametes (Ramanna and Jacobsen, 2003)

Polyploids that originate through the functioning of *2n* gametes are called sexual polyploids and their usefulness for crop improvement has been demonstrated in some of the autopolyploid crops like potato (Carputo and Barone, 2005), red clover, blueberry and alfalfa (Veronesi *et al*., 1986). But it is apparent that sexual polyploidization is as important in the case of allopolyploids as they have been in autopolyploids.

In the case of autopolyploid crops, sexual polyploidization has been useful for the maximization of heterozygosity in the sexual polyploid progenies and secondly for the germplasm enhancement through analytic breeding method. In allopolyploids apart from these objectives, introgression of genes and chromosomes from alien species into the cultivars is also important (Bingham *et al*., 1994)

3.1. Meiotic nuclear restitution

Generally, *2n* gametes originate due to deviating meiosis in plants. Deviations can occur in plants with normal chromosome pairing as well as in those with disturbed chromosome pairing as, for example, in distant interspecific hybrids or synaptic mutants. The process that leads to *2n* gamete formation is called meiotic nuclear restitution that occurs either during micro- or megasporogenesis. Depending on the particular meiotic stages at which nuclear restitution occurs, different restitution mechanisms have been recognized, namely first division restitution (FDR), second division restitution (SDR). Besides these, other mechanisms such as intermediate meiotic restitution (IMR) and post meiotic restitution (PMR), have also been recognized (Lim *et.al.*, 2001).

Production of genetically identical *2n* gametes in diplosporic apomictic plant species has been recognized for a long time. Two important characteristics may be noted in diplosporic apomictic species 1) the occurrence of *2n* gametes is not sporadic but regular, and 2) the process is genetically determined. Extensive cytological investigations in various apomictic plant species have revealed that there are variations with regard to the cytological events that lead to the formation of restitution gametes. Here the entire chromosome complement in the megaspore mother cell divides as in mitosis (i.e., centromeres divide and the two sister chromatids of each chromosome separate to two poles during the modified process of meiosis) and gives rise to two genetically identical *2n* megaspores. In apomictic species, this is confined to *2n* egg formation during megasporogenesis.

Apart from apomictic species, meiotic nuclear restitution and *2n* gamete formation are also observed in several sexual species. Some differences are observed in the case of sexual species as compared to apomictic species. a) Meiotic nuclear restitution can occur in both micro- and megasporogenesis. b) Formation of *2n* gametes is highly sporadic instead of being regular and highly dependent on environment. c) Mostly heterogeneous populations of *2n* gametes are formed depending on the cytological mechanisms.

Nuclear restitution depends on various meiotic events such as chromosome pairing, chiasma formation and cytokinesis during the first meiotic division, and

spindle abnormalities during metaphase II, cytokinesis and cell wall formation during telophase II of the second division. In most of the dicotyledonous plants cytoplasmic division during microsporogenesis occurs at telophase II giving rise to a tetrad. This is called 'simultaneous type' of cytokinesis. But in most monocotyledonous plants cytokinesis is of the successive type that is cytokinesis and cell wall formation occurs at telophase and the second meiotic division proceeds separately in two different cells within a pollen mother cell. In this case, following the second division, cytokinesis and cell wall formation again occurs at telophase II giving rise to a tetrad.

3.2. First division restitution

First division restitution (FDR) in a strict sense, occurs due to and equational division of the entire chromosome complement (as in mitosis) before telophase I stage. The two *2n* nuclei of a dyad will be genetically identical to each other as well as to the mother cell. This type of division occurs in plants in which the homologous or homoeologous chromosome pairing is completely absent, as for example, in synaptic mutants or distant hybrids. Essentially, this is a modification of meiosis in which the centromeres divide already during the first meiotic division rather than later. Examples of such modified division has been observed in *Aegilops squarrosa* x *Triticum durum*, emmer wheat x *A squarrosa*, durum wheat x *A squarrosa*, and *rye* x *A squarrosa*, wheat x barley, haploids of durum wheat, *Alstroemeria* interspecific hybrids and *Lilium* interspecific hybrids (Jauhar *et al*., 2000). Complete absence of chromosome pairing in the meiotic mutants and distant hybrids can occur only rarely. In those cases where a limited amount of crossing-over occurs, FDR can give rise to *2n* gametes that are not identical, but differ with respect to recombinant segments.

Failure of chromosome pairing is not a precondition to FDR *2n* gamete formation. Indeed, in spite of normal microsporogenesis, with normal chromosome pairing FDR occurs in a large number of cases. In these cases, meiosis proceeds normally during the first division, i.e., anaphase and telophase I stages will be normal, but during metaphase II stages the spindles 'fuse' and give rise to dyads instead of tetrads.

FDR in a strict sense produces identical *2n* gametes whose genotypes are the same as those of the parent, the division equivalent to FDR produces *2n* gametes that are not identical to each other nor to the parent genotype. A notable feature of the equivalent of FDR is that it an occur only in plants that have the so-called 'simultaneous type' of cytokinesis during microsporogenesis. This type of FDR is not observed in the case of 'successive type' of micro- and megasporogenesis where cytokinesis and cell wall formation occurs at the end of the first meiotic division.

Besides spindle abnormalities, abnormal cytokinesis following anaphase II can also lead to FDR *2n* gamete formation as in orchid (Storey, 1956). In this case a triad is formed in such a way that the *2n* microspore possess the fusion product of two non-sister nuclei during telophase II giving rise to FDR gametes. An important feature of the FDR mechanisms is that, with the exception of crossover segments, the chromosome sets of the parental genomes remain intact in the resulting *2n* gametes

Traditional cytogenetic methods based on chromosome orientation, spindle abnormalities and chromosome division can be fairly reliable for detecting FDR mechanism, especially in the case of synaptic mutants and distant hybrids. Nevertheless, when more than one mechanism leads to *2n* gamete formation in one and the same plant, conclusions can be misleading. This is overcome by analyzing the chromosomes of the parent and sexual polyploid progenies through molecular cytological techniques (GISH and FISH) or mapping methods (half-tetrad analysis) through the use of molecular genetic markers. Both of these methods have been demonstrated to be effective for detecting the mechanism of restitution and the extent of crossing-over

3.3. Second division restitution

Second division restitution involves the disruption of parental genomes due to independent assortment of chromosomes as well as the segregation of the recombinant segments. In the case of typical SDR, homologous or homoeologous chromosomes pair completely and the half-bivalents disjoin normally at anaphase I of meiosis. The resulting haploid products do not divide further but restitute by

division of centromeres without chromatid separation. As a consequence, the regions between the centromere and the first cross-over point in each chromosome pair remain identical and homozygous whereas the segments distal to the cross-over point will be heterozygous in the resulting *2n* gametes (Mok and Peloquin, 1975).

When both the nuclei restitute at two poles they give rise to a dyad, and when only one of the nuclei restitutes, a triad is formed. Although the formation of a dyad or a traid can be used as a criterion for the detection of SDR, conclusive proof for SDR mechanism can be established only through genetic mapping of the sexual polyploid progenies. This is because SDR occurs only in hybrids or genotypes in which the genomes are closely related so that they pair normally during metaphase I. In the absence of chromosome differentiation, DNA in situ hybridization methods are ineffective for the elucidation of the chromosome constitution in the sexual polyploid progenies. Therefore, genetic mapping methods (half tetrad analyses) using genetic markers such as morphological, isozyme, RFLP and RAPDs have been used for the elucidation of SDR. SDR gametes constitute a highly heterogeneous population of *2n* gametes due to random assortment of chromosome pairs and a high degree of homozygosity due to chromosome doubling.

3.3. Special cases of restitution

A mechanism that is neither FDR in strict sense nor SDR has been reported in a *Lilium* interspecific hybrid. In this, a part of the chromosome complement can divide equationally as in FDR and simultaneously another part of the complement in the same cell can disjoin normally as in anaphase I and restitute as in SDR. When both univalents and bivalents are formed during meiosis in a distant hybrid, univalents divide equationally whereas bivalents disjoin and restitute giving rise to two euploid *2n* gametes. This novel type of restitution is termed as 'indeterminate meiotic restitution' (IMR) (Lim *et al*., 2001).

There are several instances among plants where meiosis proceeds to completion and yet produce *2n*, instead of *n* or the haploid spores. One of the well-known examples is that of sugarcane interspecific hybrids where the

chromosome numbers of the haploid egg cells are doubled giving rise to *2n* eggs (Price, 1963). In *Rubus laciniatus*, apomictic progenies have been reported to arise from diploidization of the reduced egg cells (Dowrick, 1966). These events are post-meiotic chromosome doubling events and involve genetic recombination and chromosome assortment that characterize normal meiosis. A salient feature of post meiotic doubling is that the resulting *2n* gametes are expected to possess 100 per cent homozygosity for the genetic loci. Cytological demonstration of PMR can be proved indirectly from the fact that meiosis in such genotypes will be completely normal and produce normal *n* gametes but give rise to polyploid progeny (Bastiaanssen *et.al*, 1998).

4. Factors Influencing Unreduced Gamete Formation

Although the formation of unreduced (2n) gametes is considered to be rare in general (McCoy, 1982), 2n gamete production is likely to play a major role in polyploid origins (Harlan and deWet, 1975; Vorsa and Bingham, 1979). A number of factors both genetic and environmental have been shown to influence the frequency of 2n gamete formation (Sax, 1937; Thompson and Lumaret, 1992).

Genetics is a major factor in unreduced gamete formation. Genetic control of 2n gamete production has been demonstrated in a few species (Mok and Peloquin, 1975; Veilleux et al., 1982; McCoy, 1982; Qu and Vorsa, 1999). In *Solanum*, three mechanisms (one for parallel spindle formation and two for premature cytokinesis) of 2n pollen (or "diplandroid") formation were found to be each controlled by simple recessive genes, although the frequency of diplandroid production had wide-ranging variability (Mok and Peloquin, 1975). Certain genotypic clones of potato (Veilleux et al., 1982), alfalfa (McCoy, 1982), and blueberry (Qu and Vorsa, 1999), have been observed to consistently produce high frequencies of 2n pollen, while others are more variable.

In plants mutant genes can affect meiosis in various ways and some of these can lead to the formation of *2n* gametes. In diplosporic apomictic species, *2n* egg formation is a regular feature and it is likely to have a genetic basis. Single recessive genes have been shown to determine *2n* eggs and *2n* pollen formation

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in some plants. Polyhaploids mostly produce *2n* gametes, e.g., potato dihaploids; *Triticum durum: Rosa* hybrids.

There are instances in which one and the same genotype produces both *2n* eggs and *2n* pollen simultaneously, but such cases are generally rare. Commonly, plants produce either only *2n* eggs or *2n* pollen indicating that these genetic traits are independent from each other. For example, in a desynaptic mutant of potato, genotypes that produced high frequencies of *2n* pollen failed to produce *2n* eggs in high frequencies. On the other hand, those genotypes that produce high frequencies of only *2n* eggs have been reported in potato and alfalfa. The trait of *2n* gamete formation is supposed to be controlled by single recessive genes. The genes that induce meiotic nuclear restitution are highly influenced by environment and, therefore, it is difficult to establish whether it is a genetic trait or solely influenced by the environment. Nevertheless, evidence for genetic control of meiotic nuclear restitution exists from the fact that through genetic selection the frequencies of *2n* gamete production can be significantly enhanced. Therefore, it is most likely that there might be major genes that are influenced by numerous modifier genes

Single genes or individual chromosomes controlling *2n* gamete formation have been reported in some of the distant hybrids. For example, in wheat, rye and *Aegilops* hybrids a single gene has been reported to determine FDR *2n* gamete formation (Xu and Joppa, 1995). In oat-maize chromosome addition lines, highest 'fertility' has been reported in F1 plants (haploid oat genome with an addition of a maize chromosome) with maize chromosome 2 addition, indicating the effect of a particular chromosome for FDR *2n* gamete formation. A recessive gene (*Tnc 1*) that determines the *2n* egg formation in alfalfa has been localized with respect to a DNA marker (CA)8-GC. Although there is evidence for the monogenic inheritance of *2n* gamete formation, the trait is highly influenced by the environment. Genetic controls of unreduced gamete production is important to polyploid evolution, since such genes could become fixed in populations which would enable rare polyploids to become more frequent and possibly overcome the minority cytotype disadvantages in diploid populations.

Temperature has been demonstrated to increase 2n pollen formation (Sax, 1937; McHale, 1983). In *Tradescantia*, plants that had been grown at a relatively low temperature (18 ^{0}C) and then transferred to an environment with a higher temperature (38^{0}C), showed meiotic irregularities (e.g. spindle disruption and failure of segregation) in pollen mother cells (Sax, 1937). This sudden change in temperature somehow prevents the nuclear division of a single pollen mother cell and can lead to the formation of two diploid pollen grains (e.g. dyads), each of which are larger than normal haploid pollen grains (Sax, 1937). Low temperature has been shown to have an effect on the formation diploid pollen grains in other species (McHale, 1983). In *Solanum phureja*, plants that had been grown in cool environments consistently produced a higher frequency of dyads and large pollen grains than plants grown in a warmer environment (McHale, 1983). The effects of temperature in producing diploid pollen grains has been demonstrated in a number of different plant species (Sax, 1937).

Dehydration inhibits spindle formation, therefore preventing normal segregation during meiosis, and it can also inhibit cell division, generating bi- and quadri-nucleate cells (Giles, 1939). Other factors that can induce chromosome doubling, besides temperature and the degree of hydration, include X-rays, ultraviolet radiation, mechanical injury, certain chemicals, infection by certain viruses or mites, and genetics (Sax, 1937).

Many of these factors appear to have similar effects, as they disrupt the synchronization of nuclear and cellular division (Giles, 1939). Under natural conditions, sudden changes in temperature, water abundance, and (now, with the ever decreasing ozone layer) UV radiation, can be expected to be the major environmental factors which could influence the population dynamics of polyploids through unreduced gamete formation.

5. Detection of *2n* gametes

Several criteria can be used for the detection of *2n* gametes in plants. The simplest way is by staining the pollen grains with traditional staining reagents, such as acetocarmine or lacto phenol acid fuchsin, the size differences can be recognized. This method is applicable in genotypes with normal meiosis and

generally the larger pollen grains represent *2n* pollen and the smaller ones the *n* pollen. When meiosis is abnormal as in distant hybrids synaptic mutants or odd polyploids (such as triploids), the presence of stainable pollen is an indication for the occurance of *2n* pollen although aneuploid pollen grains may also be stained. In both instances, the progeny will also possess plants with higher ploidy levels than the parent (Bretagnole and Thompson, 1995).

Unlike *2n* pollen, the detection of *2n* eggs in plants is much more difficult. This requires chromosome counting in the progenies, which is a laborious task. However, the formation of *2n* eggs can be more easily detected without chromosome counting in the progenies by using 'triploid block' that occurs in several plant species. In these cases when 2x-4x crosses are made, the expected triploid embryos resulting from the union of a haploid egg with a 2x male gamete do not survive because of the embryo-endosperm imbalance of the chromosome numbers. When such triploid block is effective in a cross, the only sporophytes that survive are tetraploids resulting from the union of *2n* eggs with 2x male gametes in a 2x-4x cross. This means, by simply making a 2x-4x cross, the frequencies of the occurrence of *2n* eggs in a diploid can be quantified on the basis of seed set. This method has been successfully used in potato, alfalfa, *Brassica*, *Dactylis* and several other plants.

Besides using pollen size and 2x-4x crosses as criteria for the assessment of *2n* gamete formation, there are certain other methods that are less commonly used but have the potential for application in some of the crops. These include the production of 'metamorphic progeny' in *Brassica* intergeneric or interspecific hybrids, occurrence of diploid plants through anther culture from diploid genotypes in tuberous *Solanum* species and DNA measurement of pollen grains through flow cytometry in *Lilium* interspecific hybrids (Vantuyl et al., 1989).

6. Analyses of polyploids

In order to evaluate the advantages of sexual polyploidization, both traditional as well as molecular techniques have been used for analyses. Analysis is mainly done for assessing a) the degree of heterozygosity transferred through *2n* gametes, b) the amount of genetic crossing-over and c) transfer of alien chromosome

segments. The analystical techniques used in the case of auto- and allopolyploids differ. In autopolyploids the main objective is the assessment of the degree of homo- or heterozygosity that occurs in the sexual polyploidy progenies and in allopolyploids it is the detection of genomes, intergenomic recombination and introgression of alien chromosomes or their segments.

For the analysis of autopolyploid progenies, both traditional as well as molecular genetic markers have been used. For example, in potato, morphological markers such as yellow tuber flesh colour (*Y*), crumpled (*cr*), desynapsis (*ds*), yellow cotyledon (*yc*) amylase free (*amf*) have been used for the half-tetrad analysis. Besides these, isozyme markers have also been used. However, because the genetic markers in these cases are highly restricted, numerous molecular genetic markers (RFLP, AFLP, RAPDs) have become available for the purpose of evaluating the autotetraploid progenies. Some examples are potato, alfalfa and *Vaccinium*.

Unlike in autopolyploids, mostly molecular cytological approaches involving GISH and FISH have been successfully used in the case of allopolyploids. An important advantage of allopolyploids for molecular cytogenetic analysis is that the constituent genomes in these can be clearly discriminated through DNA *in situ* hybridization methods. This includes the identification of not only genomes and individual alien chromosomes but also the recombination segments in the sexual polyploid progenies (Lim *et al*., 2003).

GISH and FISH analyses of the polyploidy progenies have been confined so far to only monocotyledonous taxa like *Festuca-Lolium* hybrids, *Gasteria-Aloe* hybrids, *Alstroemeria aurea* x *A. modora* and other interspecific hybrids, *Lilium* interspecific hybrids, *Musa* hybrids and sugarcane (Cantor *et al*., 1999).

It is now generally assumed that almost all polyploids in nature have originated through sexual polyploidization. The recent molecular cytogenetic data have revealed new information especially on allopolyploids. For example, GISH and FISH analyses of natural allopolyploid taxa have revealed the following three aspects: viz., a) genome constitution, b) intergenomic recombination or 'translocations' and c)multiple origins of allopolyploid species. Traditionally,

genome constitutions of numerous allopolyploid species were determined on the basis of their taxonomic affinities to the suspected putative diploid parents as well as meiotic chromosome pairing. Through DNA *in situ* hybridization, the genomes of allopolyploids like bread wheat, oat, cotton, sugarcane, tobacco, *Festulpia*, banana and *Crocus* have been assigned to the diploid putative parental species. Differences have been observed among the related polyploidy species regarding the chromosomes involved in translocations.

On the assumption that sexual polyploidization in distant F1 hybrids might be a recurrent event, the occurrence of both intergenomic translocations, or recombinants, as well as multiple origins of polyploids can be explained. This further supports the hypothesis of multiple origins of polyploid species advocated on the basis of genetic evidence. Intergenomic translocations are more likely to occur in the F1 hybrids of distant species because the homoeologous chromosomes are 'forced' to pair and the *2n* gametes resulting from such meiosis are most likely to transmit recombinant chromosomes to the sexual polyploid progenies. This has been shown to occur in the progenies of hybrids of *Gasteria-Aloe*, *Alstroemeria* species and *Lilium* species (Lim *et al.*, 2001).

7. Steps in polyploid evolution

A problematic and critical step in polyploid evolution is the establishment, and subsequent persistence, of the polyploid (Fowler and Levin, 1984). The predominance of one cytotype excludes other cytotypes from reaching high frequencies in a randomly mating population due to the ineffective matings of the rare cytotype (Husband, 2000). This is known as the minority cytotype exclusion principle. A new, and therefore rare, polyploid in a diploid population would be at a major fertility disadvantage, since most pollinations of the polyploids will involve pollen from diploids. Rare polyploids will be less fit, since mostly sterile or inviable triploid progeny would be produced (Fowler and Levin, 1984).

Niche separation, caused by a change in ploidy level, allows for the coexistence of diploids and tetraploids. Polyploids are known, in general, to have higher stress tolerances and therefore may occupy separate habitats from their diploid ancestors (Levin, 1983). An important feature of polyploidization is that

entire genomes are duplicated in the process and not just particular genes. This would potentially allow for a higher genetic diversity in a polyploid than in its diploid progenitor, since more than two alleles would be present per gene loci in the polyploid while the diploid would have only two alleles per loci. Chromosome doubling has consequences at cytological, biochemical, physiological, developmental levels and it can bring about adaptive changes which may cause ecological differentiation between cytotypes.

Roose and Gottlieb (1976) suggested that polyploids have the capacity for greater physiological buffering than their diploid progenitors due to enzyme multiplicity. Increased enzyme activity, novel enzymes and metabolites, and increased metabolic regulation may enable polyploids to invade new habitats that are not occupied by their diploid progenitors.

Small population size would also play an important role in polyploid evolution, since chance events could allow the minority cytotype (i.e. the rare polyploid) to gain a frequency advantage necessary to replace the parental diploid cytotype (Fowler and Levin, 1984). Once 2n gamete production exceeds a certain threshold, tetraploids replace diploids

8. Relevance of *2n* gametes for breeding

Before the discovery of colchicine, *2n* gametes were used for inducing polyploids in plants. This method of meiotic doubling was, undoubtedly, very inefficient for inducing polyploids at will. Although colchicine induced polyploids were produced in large numbers in several crops, none of the so-called synthetic crops, which were multiplied by seed, was successful, with the rare exception of *Triticale*. For seed propagated polyploids, the production of balanced gametes and high seed set are the prerequisites. In the case of *Triticale*, a broad-based breeding program was required to achieve success. This does not mean that all new polyploids require such efforts. In many horticultural crops like Rose, Narcissus and Alstroemeria polyploids have originated spontaneously in the breeders nursery through the functioning of 2n gametes. Systematic work on sexual polyplidisation has progressed in some crops like potato, alfalfa and Vaccinium (Carputo and Barone, 2005). Initially, sexual polyploidisation in any

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crop can be difficult and laborious because of the non availability of desirable diploid genotypes that produce either 2n pollen or 2n eggs consistently that can be used in breeding programme. There are several autopoluploids like sweet potato, cassava, taro, yams etc. which are vegetatively propagated and are amenable to sexual polyploidisation. In triploid crops like banana, sexual polyploidisation might be the only way for improvement. In sugarcane, nobilisation of wild canes was thought to be rapidly possible due to the 2n egg production in noble cane *Saccharum officinarum*.

9. Conclusion

Although the importance of polyploidy has been widely recognized, the reasons for its success are not fully understood. Polyploidy does not merely result in additivity for all traits from the progenitors, but often produces novel phenotypes that are not present in the parents or exceed the range of the parents. This phenomenon is analogous to heterosis, in which hybrid genotypes often have phenotypes that exceed those of their inbred parents. A systematic cytogenetic knowledge of some of the cultivars or synthetic sexual polyploids can yield even more valuable knowledge that might be potentially useful for breeding of polyploidy crops

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DISCUSSION

1. Name a polyploid leafy vegetable.

   ☑ *Amaranthus dubious* is a tetraploid vegetable.

2. Which are the cotton species cultivated in India?

   ☑ Desi types *Gossypium arboreum* and *G. herbaceum* and New World types *G. hirsutum* and *G. barbadense* are the four commonly cultivated types in India

3. Which is considered to be Egyptian cotton?

   ☑ Egyptian cotton arose from a single plant selected from a garden in Cairo. It is actually a descendant of *Gossypium barbadense* and is valued for its fine fibre

4. What are polyhaploids?

   ☑ Polyhaploids are the gametes produced by polyploids. ie. a tetraploid plant AAAA will normally produce a gamete AA which is considered to be a polyhaploid as it contains two sets of genome.

5. Can we consider banana as an autopolyploid?

   ☑ Banana has two genomes A from *Musa acuminata* and B from *Musa balbisiana*. There are varieties which are autopolyploids of A genome like BodlesAltaFort (AAAA) but majority of table varieties are allopolyploids with both A and B genomes

6. Why was triticale produced?

   ☑ Triticale was produced as an allopolyploid from wheat and rye to incorporate the biotic and abiotic stress resistance present in rye to wheat. Triticale is mainly intended to be cultivated in such areas where wheat cultivation is not possible due to stress

7. Are there any varieties produced using this technique?

   ☑ At present no varieties are available in any crops using this technique

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KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE
PBGen. 752– Seminar

SEXUAL POLYPLOIDISATION IN CROP IMPROVEMENT

Name: Gayathri, G. (2006-21-106)

Venue : Conference hall
Time: 10.15 am, 07.07.07

ABSTRACT

Polyploidy breeding is a non-conventional tool in crop improvement. Individuals carrying chromosome numbers other than the diploid number are referred to as polyploids. Polyploidy is artificially induced by the application of colchicine on growing tips of plants. Triticale and Raphanobrassica are the examples to denote the success and limitations of this technique respectively.

Sexual polyploidisation is a term used to indicate polyploidy breeding through the utilisation of 2n or unreduced gametes. Evolutionary pattern of polyploidy formation has been reported to be due to sexual polyploidisation in many crops (Paterson *et al*., 2005). An interesting fact is that most of the commonly cultivated crops like wheat, cotton and tobacco are allopolyploids while potato, coffee and alfalfa are autopolyploids (Soltis, 2005). Studies on the evolution of these plants reveal that polyploidy occurred through the functioning of 2n gametes

Occurrence of 2n gametes is usually due to cytological abnormalities during meiosis. Genotype of the plant and environmental conditions greatly affect their formation. Improvement of autopolyploid crops like potato, alfalfa and some fodder grasses has been attempted with the use of unreduced gametes (Carputo and Barone, 2005). In allopolyploid crops, 2n gametes can be more useful for introgression of alien genes and chromosomes into cultivars (Ramanna and Jacobsen, 2003). Introgression can be achieved through recombination due to genetic crossing-over between alien chromosomes. The methods for the analysis of 2n gametes and sexual polyploid progenies include use of DNA *in situ* hybridization and molecular mapping (Lim *et al*., 2003). A neopolyploid establishes and replaces diploids when the 2n gamete production exceeds a certain threshold level

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PLANT VIRUSES AS TOOL FOR GENETIC ENGINEERING

By

S.M.Purushothaman
(2006-21-110)

SEMINAR REPORT

Submitted in partial fulfillment for the requirement of the
Course No. Pl. Path.751, Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
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THRISSUR, KERALA, INDIA

CERTIFICATE

This is to certify that the seminar report titled "**PLANT VIRUSES AS TOOL FOR GENETIC ENGINEERING**" has been solely prepared by S.M.Purushothaman, (2006-21-110) under my guidance, and has not been copied from any seniors, juniors or fellow student's seminar reports.

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DECLARATION

I, S.M.Purushothaman (2006-21-110) hereby declare that the seminar entitled

"PLANT VIRUSES AS TOOL FOR GENETIC ENGINEERING"

has been prepared by me, after going through the various references cited here and has not been copied from my fellow students.

Vellanikkara

S.M. Purushothaman

(2006-21-10)

PLANT VIRUSES AS TOOL FOR GENETIC ENGINEERING

1. Introduction

Recombinant DNA technology has made significant advancement that has applications in several branches of biological science. The recombinant DNA technology basically involves cutting a molecule of DNA specifically at a defined site using restriction endonuclease enzymes and joining it to another DNA molecule that can replicate and can be used as a cloning vector. The process of joining the DNA molecules is known as ligation. If the cloning vehicle is a bacterial plasmid or bacteriophage DNA, it has to be copied into cDNA which is a complementary copy of RNA, using reverse transcriptase.

The primary requirement to initiate the experiments, is to prepare a restriction enzyme map to cut DNA at known sites and choose fragments for cloning or deletion. If the restriction enzymes cut, has overlapping complementary cohesive or sticky ends with several nucleotides, ligation is relatively easy and bacteiophage T4 DNA ligase used for achieving ligation of desired DNA fragments. When cloning at a specific site is attempted, the gene or virus genome from which a fragment is cut, has an identical, preferably unique site on he vector. Another important prerequisite is the information on the nucleotide sequence of viral nucleic acid and its cloning vector to know exactly the location of a restriction enzyme site, the orientation of the insert, the flanking sequences and also the possible functional consequences. The knowledge of the nucleotide sequences is necessarily desirable, when a complete gene is cloned and its expression is required. The gene function can be investigated using the data on nucleotide sequence and it will be useful for bulking the product of a gene in a prokaryote as in the case of plasmid vector in *Escherichia coli*. Again it will be of great significance, while attempting to insert foreign genes into plant virus vectors.

The availability of a means of identifying and selecting the recombinant molecules is another important requirement. Plasmid vectors that can carry genes conferring resistance to antibiotics such as tetracyclines or ampicillin with cloning sites in these genes, can be used. When an insert in such sites is made, the bacteria carrying the recombinant plasmid becomes susceptible to the antibiotic. By such alteration in sensitivity to the antibiotic, the appropriate colonies can be selected in agar plates.

Genetic engineering may be useful in the case of plant viruses for cloning viral genomes in prokaryotic systems and the development of vector systems for eukaryotes. Genetic engineering primarily aims to study the structure and function of plant virus genomes and to modify the plants using their viruses as gene vectors. The function of genes can be studied by deletions or insertions in the genome and such modifications in essential genes may result in the inactivation of the virus, because the mutation is lethal. But insertions into the genome in place on nonessential genes may render the foreign genes unstable or inactive. They may be either rejected or not expressed. The expression of a foreign gene in a vector derived from a plant virus is yet to be achieved. However, the basic requirement of understanding the mechanism of viral replication, transcription and translation of virus genes is being fulfilled by several investigations (Hull and Davies, 1983). Genetic engineering can be also used for the development of virus resistance in crop plants.

2. Properties of plant viruses and plant viruses used as vectors

The plant viruses offer a wide choice for consideration of plant viruses as vectors since there are many viruses capable of infecting large number of plant species. The different types of nucleic acids present in plant viruses as complied by Hull and Davies (1983).

Nucleic acid type	Number of viruses	Percentage
RNA		
Plus single stranded	262	78
Minus single stranded	42	13
Double stranded	11	3
DNA		
Double stranded	12	3
Single stranded	13	3

The DNA plant viruses have attracted most attention as the possible vectors. The caulimo viruses containing ds DNA have been studied most intensively among the plant viruses as possible vectors.

The double stranded DNA viruses used as vectors

The type member is the cauliflower mosaic virus (CaMV) will give local infections to cotton, soybean, peanut, cucumber, lettuce, tomato and spinach. The virus particles are isometric and about 50 nm in diameter. They are found associated with proteinaceous inclusion bodies in the cytoplasm of most mesophyll cells of infected plants.

The Caulimovirus group

Virus	Abbreviation
Cauliflower mosaic	CaMV
Carnation etched ring	CERV
Dahlia mosaic	DaMV
Figwort mosaic	FMV
Mirabilis mosaic	MMV
Strawberry vein banding	SVBV
Blueberry red ringspot	BRRV
Cassava vein banding	CVBV
Cestrum virus	CV
Petunia vein banding	PVCV
Plantago virus 4	PV 4

DNAs is that a large proportion of the molecules appear to have a twisted structure. The product from the open reading region IV is the precursor for coat protein the open reading region II product is the aphid transmission factor. Upstream of the 5' end are sequences generally recognized as eukaryotic promoter sequences (19 s RNA and 35 s RNA). There is a strong signal for polyadenylation just upstream of the 3'

end of the 1.9 kb mRNA. CaMV RNA is transcribed apparently by RNA polymerase II. The inclusion bodies were the site of CaMV replication.

Single stranded DNA viruses used as vectors

Only one group of viruses is recognized namely the geminiviruses. These are called so since they have double (twin) or geminate particles. The DNA genome is small of molecular weight about 0.75X 10^6 (2500 – 2700 nucleotides).

The geminivirus Group

Virus	Abbreviation
Abutilon mosaic	AbMV
Bean golden mosaic	BGMV
Cassava latent	CLV
Euphorbia mosaic	EuMV
Mung bean yellow mosaic	MYMV
Tobacco leaf curl	TLCV
Tomato golden mosaic	TGMV
Bean summer death	BSDV
(Beet) curly top	(B) CTV
Chloris striate mosaic	CSMV
Maize streak	MSV
Tobacco yellow dwarf	TYDV
Wheat dwarf	WDV

Single stranded RNA viruses

The majority, more than 75% of plant viruses, contain single stranded (+ strand) RNA as their genetic material. There are at present 21 groups of single stranded plant RNA viruses recognized. These are RNA viruses with broad host ranges (more than 500 copis) and others with narrow host range (one or two host species). Many plant RNA viruses replicate readily in plant cells and reach copy numbers of more than 10 particles per cell. Three RNA viruses which are potential vector systems.

Tobacco rattle virus and the pea early browning virus have wide ranges covering many crop plants. Particles of the two components of these viruses are rod shaped, the longer containing the infectious RNA 1 and RNA 2. RNA 2 codes for the coat protein and is not needed for infection; it is replicated in association with RNA1.

Barley stripe mosaic virus infects a range of monocotyledonous species. It is a multicomponent virus with rod shaped particles needing three RNA species for full infection. Alfalfa mosaic virus also has a wide host range. It is a multicomponent virus with bacilliform particles. These three virus systems are examples of the potential contribution that RNA viruses for vector development.

3. Application of genetic engineering in plant viruses

i. Cloning of viral nucleic acids

Plant viral nucleic acids can be cloned in bacterial vectors such as plasmids and bacteriophage DNA. For cloning, single molecules of nucleic acid have to be selected. Such a selection removes the variation in populations of virus particles and also the contamination of one component of a multicomponent virus with another component. There are other advantages such as ease, rapidity and the large scale production of viral nucleic acid when the viral nucleic acid is cloned in prokaryotic system. Moreover, manipulation of nucleic acids is also facilitated by cloning.

Cloned cDNA of plant RNA viruses have been used for sequencing studies because sequencing of DNA is easier than that of RNA. The full sequencing of TMV have been obtained by cloning in bacteriophage M13. The CLV- DNA, when isolated from the virus is single stranded and hence part of the sequence could be determined by endlabelling techniques. Cloned DNA has sequences complementary to both plus and minus RNA strands and it may be easy to label DNA with radioactivity by nick-translation. Hence cloned DNAs, can be used in probing for the DNA and the RNA transcripts of DNA viruses and for the RNAs of the RNA viruses. Blue berry red ring spot virus (BRRV) a member of the family caulimoviride was cloned and sequenced.

The dot blot hybridization technique has been used for the detection of potato spindle tuber viroid (PSTV) in extracts from sprouting potato tubers. A cDNA clone of PSTV was used as a probe to detect potato spindle tuber viroid. The method was used to compare five biologically distinct isolates of CTV. With CaMV transcripts and CaMV encapsidated DNAs, Southern and Northern blots of DNA and RNA respectively were probed using cloned CaMV DNA and cloned fragment of CaMV DNA. By this technique, the transcripts could be mapped in relation to full sized CaMV-DNA. Potato virus Y was detected in potato by nucleic acid probe (Singh and Singh 1995).

ii. Protection of plant virus diseases by genetic engineering

Plant virus diseases cause severe constraints on the productivity of a wide range of economically important crops worldwide. There are two approaches for developing genetically resistance depending on the source of genes used. The genes can be either from pathogenic virus itself or from any other source. The former approach is based on the concept of pathogen-derived resistance. The prospects for pathogen- mediated intervention in virus disease development were first realized in 1929 when H.H. McKinney demonstrated that tobacco could be protected from infection by a severe strain of TMV by prior inoculation with a mild strain of TMV (Gadani *et al.*,1990). This type of protective measure, known as cross-protection, has since been employed throughout the world on several important crops.

Development of virus-resistant varieties using classical breeding has also been limited for several crops due to the lack of known resistance genes and/or genetic barriers or complexity of the target crops. Engineered protection offers a new approach to manage virus diseases.

Engineered protection is referred to as resistance or protection conferred in plants by viral-derived nucleic acid sequences that are introduced into the plant genome via genetic engineering. Transgenic plants developed by this approach express viral sequences and are likely to be protected against infections by the virus from which the resistance gene is derived and closely related viruses.

Integrating the viral genome into plants for protection against viruses was first suggested by Hamilton (1980). Powell-Abel *et al.* (1986) were the first to demonstrate that constitutive expression of the tobacco mosaic tobamovirus (TMV) viral coat protein (CP) gene in tobacco plants provided a substantial level of protection against infection by this virus. Since this pioneering work on TMV, viral CP genes have been used extensively to engineer protection against numerous plant viruses. Coat protein-mediated protection is an example of a broad strategy proposed by Sanford and Johnson (1985) to genetically engineer resistance to pathogens by using parasite-derived genes.

Viral sequences encoding structural and nonstructural proteins have been used; these include genes coding for the coat protein (CP), replicase, movement protein, and protease. Viral coding sequences have been used as sense, antisense, full length, truncated or untranslatable constructs. In addition, several viral noncoding sequences have been used including satellite RNA, defective interfering RNA, terminal untranslated sequences, and ribozymes. Viral CP genes have been the ones most frequently used to engineer protection against plant viruses. The level of protection conferred by CP genes in transgenic plants varies from immunity to delay and attenuation of symptoms, and for some cases the protection is broad and effective against several strains of the virus from which the CP gene is derived. A number of reviews have discussed the different approaches developed for engineered protection (Beachy, 1993; Beachy *et al.*, 1990; Grumet 1994; Tomonossoff, 1995).

iii. Engineered Protection of Tomato

Several major virus diseases affect tomato production throughout the world including tomato (ToMV) and tobacco (TMV) mosaic tobamoviruses, CMV, potato virus Y potyvirus (PVY), tomato spotted wilt tospovirus (TSWV), tomato yellow leaf curl geminivirus (TYLCV), and tobacco etch potyvirus (TEV).

Control of tomato and tobacco mosaic tobamoviruses

These viruses cause significant yield losses in tomato. Their impact is more significant for tomato production under greenhouse than under field conditions because these two viruses are highly infectious, extremely persistent, and easily transmitted by mechanical inoculations. High sanitation standards and the use of virus resistant-varieties have provided effective control of these two tobamoviruses. The host gene Tm-22 is widely used and has proven very effective against ToMV and TMV in commercial plantings worldwide (Watterson,1993). However, breeding for resistance against tobamoviruses has been laborious for some cultivars due to undesirable traits tightly linked to the single partially dominant Tm-22 gene. Engineered protection has been used to develop resistance to ToMV and TMV in order to improve elite tomato varieties without altering their desirable characteristics.

Nelson *et al.*(1988) In the first field trial ever of transgenic plants engineered for virus resistance. evaluated two tomato lines expressing the CP gene of the TMV U1 strain. Transgenic plants displayed nearly complete resistance to mechanical infections by TMV and only 5% were symptomatic by at the end of the trial compared with 99% of the control plants. Quantitative ELISA analysis corroborated visual observations indicating that very low amounts of virions accumulated in transgenic tomato plants. Fruit yield was identical for inoculated transgenic and uninoculated control plants, demonstrating that viral transgenes do not alter the horticultural performance of elite tomato varieties.

Sanders *et al.*(1992) extended the field characterization of these transgenic tomato plants and demonstrated that they exhibit excellent resistance not only to the homologous U1 strain but also to the more severe PV230 strain. Only 1% and 2.5% of the transgenic plants inoculated with TMV-U1 and TMV-PV230, respectively, became infected within 8 wk of inoculation. No yield loss due to TMV infections was recorded for the transgenic plants versus 20-69% for the control plants. However, TMV-resistant transgenic plants had limited ability to protect against ToMV since 56-89% of them were infected with ToMV causing 11-25% yield loss. Tomato plants expressing the CP gene of ToMV were developed to introduce ToMV resistance. Transgenic line 4174 was not infected by ToMV in the field, while 93% of the control plants were infected. Interestingly, transgenic line 4174 also showed substantial resistance to TMV and only 7% of the plants were infected by TMV-U1.

On the left are CMV infected nontransgenic tomato plants and on the right are CMV resistant transgenic tomato plants. Note the differences in growth and fruiting

iv. Control of cucumber mosaic cucumovirus

Due to high CMV incidence, tomato production has been completely abandoned in some traditional growing areas of Spain (Jorda *et al.*, 1992).Resistance factors to CMV have been identified in several wild tomato species (Watterson,1993) but resistant varieties have not been developed yet because of the polygenic nature of the resistance and plant interfaces problems.

Tomato plants expressing the CP gene of CMV strain WL were developed using a parent tomato cultivar containing the Tm-22 gene for resistance to TMV (Xue *et al.*, 1994). These transgenic plants showed excellent CMV resistance in greenhouse tests and were subsequently evaluated under field conditions in New York over two consecutive years (Fuchs *et al.*, 1996). Transgenic plants displayed high resistance to mechanical inoculations since none of them became infected, as opposed to 80% of the mechanically inoculated control plants. Transgenic plants grew vigorously whether or not they were inoculated, and had a 17-fold overall increase in fruit yield relative to CMV-infected control plants. However, conclusions could not be drawn on resistance to infection by aphid inoculations because only 9-14% of uninoculated controls became infected despite the high inoculum incidence from CMV-Fny, a strain that is efficiently aphid-transmitted in cucurbit fields.

To evaluate the spectrum of resistance conferred by the CP gene of CMV strain WL, transgenic tomato plants were challenge inoculated in the greenhouse with CMV isolates from different geographic regions, including 10 strains from CMV subgroup I (California, Florida, Hawaii, France, Australia, New Zealand, Egypt, China, Japan, Taiwan) and 3 strains from subgroup II (New York, Mexico), some of which carried satellite RNA. Transgenic plants were completely resistant to all CMV field isolates tested so far (Provvidenti and Gonsalves, 1995).

v. Engineered Protection of Cucurbits

Transgenic squash and control of zucchini yellow mosaic and watermelon mosaic 2 potyviruses. Transgenic squash engineered for virus resistance have been developed by the Asgrow Seed Company. Several transgenic lines which express single or combinations of CP gene constructs of CMV, ZYMV, and/or WMV 2 were evaluated at Cornell University.

In 1993, the resistance of three transgenic lines expressing CP gene constructs of ZYMV and/or WMV 2 was evaluated in the field under severe incidence of ZYMV and WMV 2. The transgenic lines tested were: ZW-20 expressing the CP genes of both ZYMV and WMV 2, Z-33 expressing the single CP gene of ZYMV, and W-164 expressing the single CP gene of WMV 2. Transgenic line ZW-20 showed excellent resistance to mixed infections by ZYMV and WMV 2 in that none of the plants developed severe symptoms, i.e. foliar mosaic, chlorosis, malformation or plant stunting. Only a few ZW-20 developed very mild leaf symptoms in the form of localized chlorotic dots or blotches. In contrast, all plants of the transgenic lines Z-33 and W-164 expressing single CP genes developed severe symptoms, as did the control plants. ELISA data on ZW-20 plants confirmed visual observations. ZYMV and WMV 2 were detected only in chlorotic dots (56% of plants), but were not detectable in asymptomatic leaves even 10 weeks after planting. Transgenic Z-33 were substantially infected with ZYMV (21%) after 10 weeks and heavily infected by WMV 2 (98%) after 7 weeks. Both transgenic W-164 and control plants were totally infected with ZYMV and WMV 2 after 5 weeks.

Differences between squash lines in yield and fruit quality were even more dramatic. ZW-20 fruits were symptomless whereas all fruits from transgenic squash Z-33 and W-164, as well as from control plants were unmarketable because of severe discoloration and distortion. The high resistance of transgenic squash ZW-20 to

infections by ZYMV and WMV 2 has been confirmed in several field tests at different locations (Arce-Ochoa *et al.*, 1995; Clough and Hamm, 1995).

Transgenic line Z-33 containing only the ZYMV CP gene showed excellent resistance to ZYMV, but not to WMV 2, while transgenic line W-164 did not show high resistance to either WMV 2 or ZYMV. The low level of resistance of W-164 is likely due to the WMV 2 isolate which was able to severely infect transgenic plants also under greenhouse conditions. The combination of the CP genes of WMV 2 and ZYMV apparently provided synergistic resistance because the transgenic line ZW-20, which contains both genes, was resistant to severe dual infections by ZYMV and WMV 2.

Development of the transgenic squash hybrid ZW-20 is a significant breakthrough for squash improvement considering the economic importance of ZYMV and WMV 2, and the difficulties in developing resistant cultivars by conventional breeding. Transgenic squash ZW-20, subsequently renamed Freedom II, was approved as the first virus-resistant genetically engineered crop to be deregulated by USDA-APHIS (Medley, 1994). Seeds of Freedom II were marketed by the Asgrow Seed Company in the spring of 1995.

Transgenic squash lines Z-33 and W-164 with single resistance to either ZYMV or WMV 2 may be valuable for regions where only one of these potyviruses is prevalent. For example, ZYMV is prevalent in Maui, Hawaii but WMV 2 is not a major problem. The opposite situation occurs in central Florida where WMV 2 causes serious problems while ZYMV is less prevalent.

It is important to highlight the value of transgenic lines containing multiple CP genes to control several aphid-borne potyviruses because mixed virus infections are common. Resistance of the transgenic squash and cucumber lines described above can still be broadened by incorporating CP genes of other cucurbit viruses. Transgenic squash lines expressing the CP genes of ZYMV, WMV 2, and CMV have been developed (Tricoli *et al.*, 1995) and field tests demonstrated the potential of such transgenic squash in controlling mixed infections by these three viruses. This is the second transgenic squash line to be deregulated in the United States.

Transgenic cantaloupe and control of zucchini yellow mosaic and watermelon mosaic 2 potyviruses, and/or cucumber mosaic cucumovirus. Cantaloupes are severely affected by CMV, ZYMV, WMV 2, and PRSV-w. Melon varieties with multiple resistance to these viruses would be valuable to growers. Cantaloupes containing multiple viral CP gene constructs have been developed by the Asgrow Seed Company.One of these transgenic lines (CZW-30) was evaluated under field conditions at Cornell University.

Cantaloupe line CZW-30 expressing the CP genes of CMV, ZYMV, and WMV 2 was tested against infections by CMV, CMV and ZYMV, and CMV, ZYMV and WMV 2. Across all trials, transgenic plants showed excellent resistance against single or mixed infections. Transgenic homozygous plants developed localized mild symptoms late in the growing season whereas all control plants showed severe systemic symptoms and had high virus titers 5-6 weeks after transplanting. Transgenic hemizygous plants exhibited a significant delay 2-3 weeks in the onset of disease compared to control

plants but showed systemic symptoms 9-10 weeks post-planting. Strikingly, 3-10% of the homozygous and 31-35% of the hemizygous plants had dual or triple infections as opposed to the control plants which had 66-99% mixed infections with two or three viruses. Transgenic plants grew vigorously whereas control plants were severely stunted with a 34-50% reduction in shoot length. Although hemizygous plants had a 17% reduction in shoot length compared to homozygous plants, they yielded 7 times more marketable fruits than control plants. This was the first report on the evaluation of a cantaloupe line of commercial quality with resistance to three of the four major viruses affecting melon production.

High levels of resistance to a wide range of CMV strains was expected in cantaloupe plants expressing the CMV-WL CP gene based on earlier studies on tobacco (Namba *et al*., 1992) and tomato (Provvidenti and Gonsalves, 1995; Xue *et al*., 1994) plants containing this CP gene. However, transgenic cantaloupe lines expressing the CMV-WL CP gene were not highly resistant to infection by CMV in the greenhouse (Gonsalves *et al*., 1994). One of these lines designated H7-21 was further evaluated under field conditions. It showed a 4-weeks delay in infection relative to the control plants, but ELISA performed 9 weeks after planting revealed that 88% of the transgenic plants accumulated CMV similar to non transformed controls (98%) .

vi. Engineered Protection of Potato

Potato production for fresh market and seed tuber is often severely affected by several viruses including potato leafroll luteovirus (PLRV), potato virus Y potyvirus (PVY), potato virus X potexvirus (PVX), potato virus S carlavirus (PVS), potato virus A potyvirus (PVA), and potato virus M carlavirus (PVM) occurring alone or in combination. PVY and PLRV are aphid-transmitted in a nonpersistent and persistent manner; PVX is mechanically transmitted. When PVX and PVY occur together, they produce a synergistic increase in disease severity. The frequency in which mixed virus infections occur in potato plants stresses the need for developing multiple resistance, especially since viruses are readily disseminated in tubers. Conventional breeding methods to develop virus-resistant varieties have proven difficult because cultivated potato is an autotetraploid and highly heterozygous plant species. Although host resistance genes have been identified and used extensively for PVX, PVY, PVS, PVM, and PVA (Fore,1992), major commercial potato cultivars generally lack virus resistance. Incorporation of resistance to PLRV has been more difficult because of the polygenic nature of the resistance.

Vii. Control of potato virus X potexvirus

PVX usually causes mild or barely detectable symptoms in most potato varieties. This virus has a worldwide distribution and is found in most potato growing areas. Besides being mechanically transmitted, it is also disseminated through infected seed tubers. PVX infections can result in loss of seed tuber certification.

The first field test of transgenic potato expressing the CP gene of PVX was reported by Hoekema *et al*. (1989) for the varieties Bintje and Escort. This field test was designed to evaluate whether horticultural and morphological characteristics were maintained in transgenic potato plants, but virus resistance was not evaluated.

Jongedijk *et al.* (1992) examined the frequency of PVX transmission from tubers of transgenic potato varieties Bintje and Escort that became infected with PVX in the field. A significant reduction in PVX incidence was observed among clonal progenies from tubers of mechanically infected transgenic plants in the field. Among the twelve transgenic lines tested, two (MGE-32 and MGE-44) performed best giving no infected tubers as did the classically bred PVX-resistant varieties Bildstar and Sant. The other transgenic lines showed a range in reaction from moderate resistance (6–43% infection of tubers) to full susceptibility. The results obtained with lines MGE-32 and MGE-44 indicate that potato lines expressing the CP gene of PVX offer potential to control PVX in commercial production of resistant seed tuber stocks.

viii. Control of potato virus Y potyvirus

In the first field test reported in Europe with transgenic plants engineered for resistance to an insect-transmitted virus, Malnoe *et al.* (1994) relied on natural spread of two PVY strains, N and O, to evaluate the resistance of potato variety Bintje expressing the CP gene of PVYN. The transgenic line Bt6 was immune to the homologous PVYN under high inoculum levels, while 98% of the control plants were infected 15 weeks post-planting. ELISA performed with monoclonal antibodies revealed that 23% of the transgenic plants became infected with PVYO, the most common PVY strain in potato fields. In a second-year field trial, the excellent resistance of line Bt6 was confirmed since PVY was detected in only 7-10% of the transgenic plants while 86% of the control plants were infected. Analysis of PVYO transmission in tubers from plants infected by aphids showed that although all the progenies of infected non transformed control tubers were infected as early as 6 wk, only 28–41% of the transgenic progenies were infected 12 weeks after planting. Potato plants expressing the CP gene of PVYN exhibited complete resistance to PVYN and some degree of protection to PVYO under natural aphid transmission in the field. In addition, the PVYN CP gene conferred some resistance in progenies from tubers of plants infected with PVYO by aphids. No yield data were presented in this study probably because of a significant contamination by PVS.

ix. Control of PVX and PVY

Mixed infections occur frequently in potato plants, development of cultivars with multiple resistance is a valuable objective for potato improvement. Lawson et al., (1990) and Kaniewski *et al* (1990) demonstrated the usefulness of multiple genes to control mixed virus infections. Kaniewski *et al* (1990) evaluated the resistance of potato plants expressing the CP genes of PVX and PVY following mechanical inoculations with both viruses. Transgenic line 303 was the only one that remained symptomless and was highly resistant to both PVX and PVY. At the end of the trial period, a very low percentage of plants in this transgenic line were infected with PVX (6%) or PVY (2%), and none was infected with both viruses. Although some spread of PVY occurred in the field via aphid vectors, no natural infection was detected in any of the plants of transgenic line 303. Moreover, the yield of transgenic line 303 was high and unaffected by virus inoculations, indicating its potential to control mixed infections by PVX and PVY while maintaining high yield. Virus incidence in tubers from the experimental plants was not reported in this study.

x. Control of potato leafroll luteovirus

Host resistance genes available to control PLRV are polygenic, breeding PLRV-resistant varieties is difficult. Engineered protection offers an alternative. Potato plants expressing the CP gene of PLRV have been developed (Presting *et al*., 1995).Several transgenic potato lines tested under greenhouse conditions showed high levels of resistance to aphid inoculations of PLRV. Although greenhouse data appear to be very promising, resistance to PLRV has to be assessed under field conditions where plants are subjected to multiple random probing by aphid vectors throughout the growing season. Engineered protection of potato offers great value for virus management not only in commercial production but also in seed tuber production to ensure the continued commercial propagation of healthy seed tubers.

xi. Engineered Protection of Papaya

Although several transgenic fruit crops have been developed and evaluated in the greenhouse for virus resistance, extensive field tests have only been conducted with transgenic papaya plants so far.

In Hawaii, PRSV has limited papaya production on the island of Oahu in Hawaii since the 1960's, and by the 1980's, the virus had moved within 40 kilometers of the Puna area on the island of Hawaii where 95% of the state of Hawaii's papaya is produced. Thus, efforts were made to develop transgenic papaya plants expressing the CP gene of PRSV. In 1992, Fitch *et al*. (1992) reported that transgenic papaya line 55-1 expressing the CP gene of PRSV strain HA 5-1 was highly resistant in the greenhouse to mechanical inoculations with this PRSV strain from Hawaii. The same year, the resistance of R0 clones of transgenic line 55-1 was tested against natural aphid infections in the field on the island of Oahu. The field trial data showed that transgenic line 55-1 was highly resistant to PRSV. None of the transgenic trees developed symptoms throughout the trial period (20 months), and virions could not be detected by ELISA whether the plants were mechanically inoculated or not.Also, transgenic plants grew vigorously and produced fruits of marketable quality .In contrast, all control plants became infected within 2-4 months.

Papaya plants 9 months after planting. The plant on the left is transgenic.

xii. Protection of tomato and tobacco by cRNA or antisense RNA strategies

The use of RNA complementary to part of the viral genome (antisense) is another potential pathogen- derived resistance strategy that may have some utility for protecting plants from systemic virus infection. Expression of an RNA transcript complementary to a replication – associated portion of the viral genome of tomato golden mosaic virus, a ss DNA virus that replicates in the nucleus, resulted in a positive correlation between the accumulation of antisense RNA and reductions in symptom development of virus inoculated plants (Day *et al*.,1991). Transgenic tobacco plants expressing an RNA complementary to the coat protein gene of PLRV, a phloem limited plussense ssRNA virus, provided protection from virus infection comparable to that of transgenic plants expressing PLRV coat protein (Kawchuk *et al*.,1991).

xiii. Engineered Protection of other Crops

Several other transgenic vegetable, ornamental, cereal, and fruit crops, have been genetically engineered for protection against viruses. Some of these transgenic plants exhibit resistance under greenhouse or growth chamber conditions, however, no field data are available yet. Some of these examples include potato plants resistant to PVX, PVY and PLRV (Tacke *et al*., 1996) and to the carlaviruses PVS and, to some extent, to PVM (MacKenzie *et al*., 1991);alfalfa plants resistant to alfalfa mosaic alfamovirus (Hill *et al*., 1991);rice plants resistant to rice stripe tenuivirus (Hayakawa *et al*.,1992);cantaloupe plants resistant to ZYMV (Fang and Grumet,1993; corn plants resistant to maize dwarf mosaic potyvirus and maize chlorotic mottle machlomovirus (Murry *et al*., 1993); tomato plants resistant to TSWV (Kim *et al*., 1994; Ultzen *et al*., 1995); petunia plants resistant to CMV(Kim *et al*., 1995); and plum plants resistant to plum pox potyvirus (Scorza and Ravelonandro,1996)

Other transgenic vegetable, fruit, cereal, ornamental and pulse crops have been engineered for virus resistance but no resistance data are available yet. Some examples include: apricot plants expressing the CP gene of plum pox potyvirus (Machado *et al*., 1992), citrus plants expressing the CP gene of CTV (Moore *et al*., 1993), rapeseed (Herve *et al*.,1993) and cauliflower (Passelegue and Kerlan,1996) plants expressing the CP gene of cauliflower mosaic caulimovirus; grape plants expressing the CP gene of the nepoviruses grapevine chrome mosaic (Le Gall *et al*., 1994), grapevine fanleaf (Krastanova *et al*., 1995; Mauro *et al*., 1995) and tomato ringspot (Scorza *et al*., 1996), chrysanthemum plants containing the nucleocapsid gene of TSWV (Urban *et al*., 1994; Yespes *et al*., 1995); chinese cabbage plants expressing the CP gene of TMV(Jun *et al*., 1995), wheat plants expressing the CP genes of barley yellow dwarf luteovirus or wheat streak mosaic potyvirus (Hansen *et al*., 1995); peanut plants expressing the nucleocapsid gene of TSWV (Brar *et al*., 1994), soybean and bean plants expressing the CP precursor gene of bean pod mottle comovirus (Di *et al*., 1996) and antisense constructs of the AC1-3 and BC1 genes of bean golden mosaic geminivirus (Aragao *et al*., 1996); lettuce plants expressing the CP gene of lettuce mosaic potyvirus (Zerbini *et al*., 1995) or the nucleocapsid gene of TSWV (Pang *et al*., 1996), and sweet pepper plants expressing the CP gene of CMV

(Zhu *et al*., 1996); Mung bean yellow mosaic control by coat protein (Malik *et al*., 2005).

xiv. Cucumber mosaic Cucumovirus-Satellite RNA cross protection

CMV infects numerous plant species and is one of the most important viruses affecting vegetables worldwide (Palukaitis *et al*., 1992). Many isolates of CMV contain satellite RNAs, some of which can cause severe necrosis on tomato plants whereas most actually attenuate symptoms of the infection. CMV is very difficult to control because of its wide host range and its transmission by numerous species of aphids in a nonpersistent manner. Vegetable crops infected include cucurbits, tomato, and pepper plants.

Cross protection to control CMV has been through the use of CMV strains carrying satellite RNAs that attenuate symptoms on vegetables. This approach has been used over several thousand acres in China, and much less extensively in fields in Italy and the United States.

xv. Mechanism of virus resistance

Virus-resistant transgenic plants offer the possibility of excluding viruses or reducing their spread by limiting the availability of virus inoculum, by restricting the amount of virus-infected tissue, and also by reducing virus titers. Given the substantial reduction of virus incidence, transgenic plants are also likely to significantly lower the probability of transmission of vector-borne viruses by reducing the acquisition efficiency of the vectors. This aspect is of epidemiological importance.

xvi. Genetic engineering of plant viruses for antibody production

The 3'end of the TMV CP was modified by insertion of a region coding for an antigenic epitope from poliovirus type 3 and this modification resulted in the production of hybrid TMVCP polio 3.Both TMVCP- polio 3, when injected into rats, produced poliovirus neutralizing antibodies (Haynes *et al*., 1986).

The HCV HVR1 epitome sequence cloned into the open reading frame of alfalfa mosaic virus (AlMV) coat protein (CP). Introduced to transgenic tobacco plants. Plant derived HVR1/ AlMV CP reacted with HVR1 specific monoclonal antibodies and immune sera from individuals infected with HCV (El-Attar *et al*.,2004)

Cowpea mosaic virus (CPMV) is a bipartite RNA plant virus which has proved to be useful for epitope presentation. Short antigenic sequences are expressed on the surface of the assembled virus acted as epitope. Chimeric virus particles produced in this way stimulated the protective immunity in experimental animals (Li *et al*.,2005)

4. Essential considerations for developing virus-resistant transgenics

i. Variability

Variability may result due to host component as new host genotypes are introduced, or by vector component as they adapt to new host system or by the virus itself by

mutation, complementation or recombination. A periodical assessment of population structure is mandatory if virus-derived transgenic resistance strategy is adopted for the control of the disease. It is especially true of India, where strain variability is observed and which would result in breakdown of resistance.

ii. Biological risks

The concept of using pathogen-derived genes to induce transgenic resistance has no doubt raised a number of ecological concerns. Risk perceptions boil down to two major items, (i) recombination between viral-derived transgene and non target virus111. (ii) transmission/vector host range changes brought about by heteroencapsidation, i.e. encapsidation of the genome of non-target virus with the transgenically expressed CP. Field trials conducted so far with transgenics17 have not indicated that expression of viral transgenes leads to the emergence of new super strain or change in transmission behaviour of common viral pathogens. However, sufficient care should be taken to avoid any risks due to heteroencapsidation while designing the constructs. The strong linkages shown by CP with insect transmission of viruses, have made possible heteroencapsidation, an important factor to be considered while designing CPbased transgenes. Coat protein genes have been designed from PPV, such that a 'DAG' motif in the CP, believed to play an important role in vector transmission, was deleted to prevent any further insect transmission of heteroencapsidated virions. The use of these constructs in producing transgenic plants has shown that heteroencapsidation of ZYMV was significantly reduced without compromising virus resistance of the plants. Similar results have also been reported recently in transgenic *N. benthamiana* expressing mutated PPV CP, which were not only resistant to PPV, but were also suppressed in heteroencapsidation, when infected with chilli vein mottle virus and PVY.

iii. Comparison of anti-viral strategies

The success of transgenic approach varies for any specific host/virus combination. A range of phenotypes is observed amongst the virus-resistant transgenic plants. While CPMR confers broad-spectrum, less complete resistance. Rep-mediated resistance produces immunity against the virus, but to a limited spectrum of strains. Similarly, in RNA-mediated resistance, antisense RNA targeting mRNA of DNA viruses has more potential than against positivestranded RNA virus. Any antisense RNA ribozyme strategy should bear in mind the association/dissociation parameters of the molecules. Pyramiding of different transgenes or combination of transgenes with natural resistance targeting different events in viral life cycle will increase the confidence level in the management of viral diseases and will ensure stability of resistance at the field level. Durability, broad-spectrum character of the transgene derived resistance coupled with enhanced crop yield of the transgenics *viz-a-viz* healthy, untransfomed plants, etc. are some of the essential parameters, which any important strategy must incorporate.

5. Economically important plant viruses in India and future outlook

For most viral diseases, resistant lines have been developed by conventional breeding and along with judicious insecticide sprays to control the vector population, help in management of the disease. Some of the examples include cultivar Sree Vishakam in

cassava against ICMV, LR5166 in cotton against CLCuV, K-134 in groundnut against bud necrosis virus, Kufri Chandramukhi in potato against PLRV and PVY and Vikramarya in rice against the tungro virus disease. However, when the source of resistance is not available, a biotechnological approach becomes necessary. For the whitefly-transmitted geminiviruses like ToLCV, CLCuV, ICMV and yellow mosaic virus in legumes, results obtained in many laboratories with transgenics containing replication initiation protein are encouraging and this approach could be adopted. CPMR for Ilarviruses, both CPMR and PTGS for potyviruses, have shown promising results, which could be adapted for viruses of India. The *NS* and *NM* genes, similarly, have been used for tospoviruses. Characterization of *R* genes associated with the well-established resistant lines, if achieved, will lead to a long-lasting solution. Following the availability of molecular information on viruses, initiatives have been taken in some leading institutions in India towards the development of transgenic virus resistance in important crops. At the Indian Institute of Science, Bangalore, success has already been reported in controlling physalis mottle virus using pathogen derived resistance in tobacco and tomato . A similar approach has been recently shown to result in resistance to PVY in tobacco in a collaborative research programme between the Central Potato Research Institute, Shimla and the Bhabha Atomic Research Centre, Mumbai. Tobacco and tomato transformation using TLCV CP and replicase genes is being attempted at the National Botanical Research Institute, Lucknow. Similar approaches are also being used to generate resistance against viruses of important crops like cotton, rice, tomato and mungbean at the Indian Institute of Science, Bangalore, University of Delhi South Campus, New Delhi, the Indian Agricultural Research Institute, New Delhi, Madurai Kamaraj University, Madurai and Maharshi Dayanand University, Rohtak. Incorporation of PVY CP gene into tobacco and potato has been achieved by the Indian Agricultural Research Institute, New Delhi.

6. Conclusion

It can be said that genetic engineering of crop plants for virus resistance is undoubtedly a key biotechnological tool which can be used to minimize the losses to crop production incurred due to viral diseases in our country. Most of the important viruses have already been identified and the cloning and molecular characterization of their genomic components is at advanced stages. However, to successfully develop and test a series of virus-resistant transgenic crops, the following bottlenecks need to be removed:

(i) Absence of transformation and regeneration systems for all the major crops of the country

(ii) Insufficient variability studies of important viruses

(iii) Lack of basic research on the functional genomics of pathogenesis. Of all the major crop plants in our country, transformation systems are available for only a few cereals,vegetables, fibre crops and oilseed varieties. The dominant and virulent strains of each important virus in the country need to be identified for obtaining genes for resistance engineering. Studies should also focus on the degree of variability and the recombination of

viral genomes. This will help in the design of suitable constructs that will ensure durable resistance across the country. Emerging techniques of functional genomics need to be harnessed to understand the molecular interactions between the viral pathogen and the resistant and susceptible plants leading to resistance or pathogenesis. This is bound to result in novel insights at disease control. Insect-proof glasshouses and insectaries require to be modernized with facilities to provide ambient conditions for plant growth in our country. This needs to be looked into by funding agencies. It is also clear that the effort for producing viral-resistant transgenic crop plants needs to be multidisciplinary, with a close cooperation among virologists, molecular biologists, tissue-culture specialists, agronomists and the government. Their combined effort is sure to deliver to the Indian farmers, a range of virus-resistant crops in the near future, which will help mitigate the losses in crop yields due to viruses in India.

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Discussion

Which plant virus is used as a vector?

Cauliflower mosaic virus

Which region of the cauliflower mosaic virus is used as a promoter?

35 s region

Why the 35 s region is called so?

The poison of the promoter is on 35th region of the virus

What is the percentage success of the coat protein mediated transfer in transgenic tobacco?

About 75 percent age

Whether the 16 s and 35 s promoters as a same function?

No they have different function. But the efficiency is more in 35 s promoter.

Which is the transgenic work carried out in KAU against the virus management?

Tomato leaf curl virus

Whether the transgenic papaya released in Hawaii against papaya ring spot virus was a success?

No

What are all the limitations in the virus disease management through coat protein mediated transfer?

The success rate is much less

What is the mechanism of control in Gemini virus disease?

The coat protein of the virus encapsidates the nucleic acid of the virus

What is the plus strand of the virus?

The minus strand of the virus multiplies using the plus strand

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Admission number 2006-21-110

Venue Audio visual Lab
Dept of Pomology and Floriculture
Date and Time 04/05/07 9.15 AM

SEMINAR : PLPath.751

TITLE: PLANT VIRUSES AS TOOL FOR GENETIC ENGINEERING

Abstract

Genetic engineering is useful in the case of plant viruses for cloning viral genomes in prokaryotic systems and the development of vector systems for eukaryotes. It also useful to study the structure and function of plant virus genomes. Cloned cDNA of plant RNA viruses and cloned DNAs of DNA viruses were also used in the sequencing of plant viral nucleic acids. Cloned cDNA has sequences complementary to both plus and minus RNA strands used as probe. Plant viral sequences found to be of importance in the development of virus resistant plants. The introduction of coat protein gene of plant viruses in plants was based on the mechanism of cross protection (Powell *et al*., 1986).The protection developed in such transgenic plants were referred as genetically engineered cross protection. The stable integration of genes encoding viral coat proteins were reported for many viruses viz., tobacco mosaic virus in tobacco and tomato (Nelson *et al*., 1987); alfalfa mosaic virus in transgenic tobacco (Loesch-Fries *et al* ., 1987); papaya ring spot virus in papaya (Souza, M.T and Gonsalves, 1999). The coat protein of alfalfa mosaic virus was used to produce vaccine against hepatitis-C (Elattar *et al*., 2004). Mung bean yellow mosaic gemini virus coat protein controls the viral replication (Malik *et al*..2005).

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MOLECULAR METHODS OF DETECTION OF PHYTOPATHOGENIC BACTERIA

By

S.M.Purushothaman

(2006-21-110)

SEMINAR REPORT

Submitted in partial fulfillment for the requirement of the Course No. Pl. Path.752, Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

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Contents

CERTIFICATE

This is to certify that the seminar report titled "**MOLECULAR METHODS OF DETECTION OF PHYTOPATHOGENIC BACTERIA**" has been solely prepared by S.M.Purushothaman, (2006-21-110) under my guidance, and has not been copied from any seniors, juniors or fellow student's seminar reports.

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DECLARATION

I, S.M.Purushothaman (2006-21-110) hereby declare that the seminar entitled "MOLECULAR METHODS OF DETECTION OF PHYTOPATHOGENIC BACTERIA" has been prepared by me, after going through the various references cited here and has not been copied from my fellow students.

Vellanikkara

S.M. Purushothaman
(2006-21-10)

Molecular methods of detection of phytopathogenic bacteria

Introduction

The early detection of plant borne pathogens is an integral part of successful disease management and this is especially important in relation to the importation of foreign plant material. The rapid identification of a plant pathogen allows for the appropriate control measures to be applied prior to the further spread of the disease or its introduction. Since most plant based foodstuffs have a finite shelf life, it is imperative that any potentially infected material is diagnosed as rapidly and as reliably as possible, in order to avoid delays and expensive losses to the cash import.

Today, diagnosticians have an array of methodologies to help in this respect. Traditionally, cultural methods have been employed to isolate and identify potential pathogens. This is a relatively slow process, often requiring skilled taxonomists to reliably identify the pathogen. This practice is made more difficult due to a number of factors, such as ambiguities in morphological characters or the specific nutrient requirements and growth conditions of certain pathogens grown *in vitro*, or time constraints imposed by slow growing pathogens *in vitro*. However over the last 30 years, several techniques have been developed which have found application in plant pathogen diagnosis, these include the use of monoclonal antibodies and enzyme linked immunosorbant assay (ELISA), which drastically increased the speed in which pathogen antigens could be detected *in vivo*, and DNA based technologies such as the polymerase chain reaction (PCR) which enable regions of the pathogen's genome to be amplified several million fold, thus increasing the sensitivity of pathogen detection. Despite such advances, cultural diagnostics still predominates largely due to the technical experience and costs associated with the more recent techniques

The methods of detection of pytopathogenic bacteria are

1. Conventional techniques

a) Examination of dry seed

b) Use of indicator plants

c) Growing on test plants

d) Use of bacteriological media

2. Chemodiagnostic methods

a) Use of selective media

b) Biochemical tests

3. Electron microscopy

4. Serodiagnostic methods

a) Agglutination test

b) Gel diffusion test

c) Enzyme-Linked Immunosorbent Assay (ELISA)

5. Nucleic acid based techniques

a) Polymerase Chain Reaction (PCR)

b) Southern hybridization

Serodiagnostic methods

Antigen is a foreign protein and antibody is produced in response to antigen. Antiserum is a blood serum containing antibody. Polyclonal antibody (PABs) is obtained from serum of animal following injection with an antigen contains many antigenic sites. It can react with more than one epitope.

Slide agglutination test

Equal quantities of antiserum and antigen placed in the cavity slide and incubate the same at 25°C for 90 minutes. Precipitation indicate the presence of the bacterium. Used to detect *Pseudomonas syringae* pv. *phaseolicola*

Gel diffusion test

Used to distinguish and to identify the pathovars. Double diffusion test was used to differentiate and identify the *X. campestris* pathogen.

ELISA-(Enzymed Linked Immuno Sorbent Assay)

Direct antigen coating (DAC)-ELISA and Double antibody sandwich (DAS)- ELISA were used to detect the phytopathogenic bacteria by using polyclonal antibodies and monoclonal antibodies. Monoclonal antibodies used to detect the *X. oryzae*.pv.*oryzae* in rice plants.

DAS-ELISA (Double antibody sandwich)– Direct ELISA

The steps involved in DAS- ELISA is as follows

Step 1 : Coat ELISA plates with antigen specific IgG

Add 200 µl to each well. Incubate 3 hrs at 37 ° C

↓

Wash plate three times with PBS – Twen after pouring off IgG

↓

Step 2 : Add infected plant extracts (antigen)in antigen buffer and dispense200µl to each well. Incubate at 37 ° C for three hrs

↓

Wash the plate three times in PBS-Tween after pouring off the antigen

↓

Step 3 : Add alkaline phosphatase - conjucated bacteria specific IgG @

200 µl well and incubate at 37 ° C for 3 hrs

Wash the plate three times in PBS-Tween after pouring off the antibody

↓

Step 4 : Add substrate mixture 200 µl and incubate at room temperature

↓

Stop the reaction : The production of color by p-NPP can be stopped by the addition of 50 µl of 3 M NaOH / well

↓

Color development : Read the absorbance of yellow colour at 405 nm using ELISA –Reader

Polymerase chain reaction (PCR)

Polymerase chain reaction technique, developed by Kary Mullis in 1985, is extremely powerful. It generates microgram quantities of DNA copies of the desired DNA (or RNA) segment, present even as a single copy in the initial preparation in a matter of few hours. The PCR process has been completely automated and compact thermal cyclers are available in the market.

The PCR utilizes the following: (1) a DNA preparation containing the desired segment to be amplified, (2) two nucleotide primers (about 20 bases long) specific, *i.e.*, complementary, to the two 3^1 borders (the sequences present at the 3^1 ends of the two strands) of the desired segment, (3) the four deoxynucleoside triphosphates, *viz.*, TTP (thymine triphosphate), dCTP(deoxycyctidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate) and (a) a heat stable DNA polymerase, e.g. *Taq* (isolated from the bacterium *Thermus acquaticus*), *Pfu* (from *Pyrococcus furiosus*) and *Vent* (from *Thermococcus litoralis*) polymerases. Pfu and Vent polymerases are more efficient than *Taq* polymerases.

Procedure of PCR

At the start of PCR, the DNA from which a segment is to be amplified, an excess of the two primer molecules, the four deoxyriboside triphosphates and the DNA polymerase are mixed together in the reaction mixture that has appropriate quantities of Mg^{2+}. The following operations are now performed sequentially

Denaturation

The reaction mixture is first heated to a temperature between 90-98 ° C (commonly 94° C) that ensures DNA denaturation. This is the denaturation step. The duration of this step in the first cycle of PCR is usually 2 min at 94 ° C.

Annealing

The mixture is now cooled to a temperature (generally between 40 – 60 ° C) that permits annealing of the primer to the complementary sequences in the DNA. As a rule, these sequences are located at the 3^1 ends of the two strands of the segment to be amplified. The step is called annealing. The duration of annealing step is usually 1 min during the first as well as the subsequent cycles of PCR. Since the primer concentration is kept very high relative to that of the template DNA, primer- template hybrid formation is greatly favoured over reannealing of the template strands.

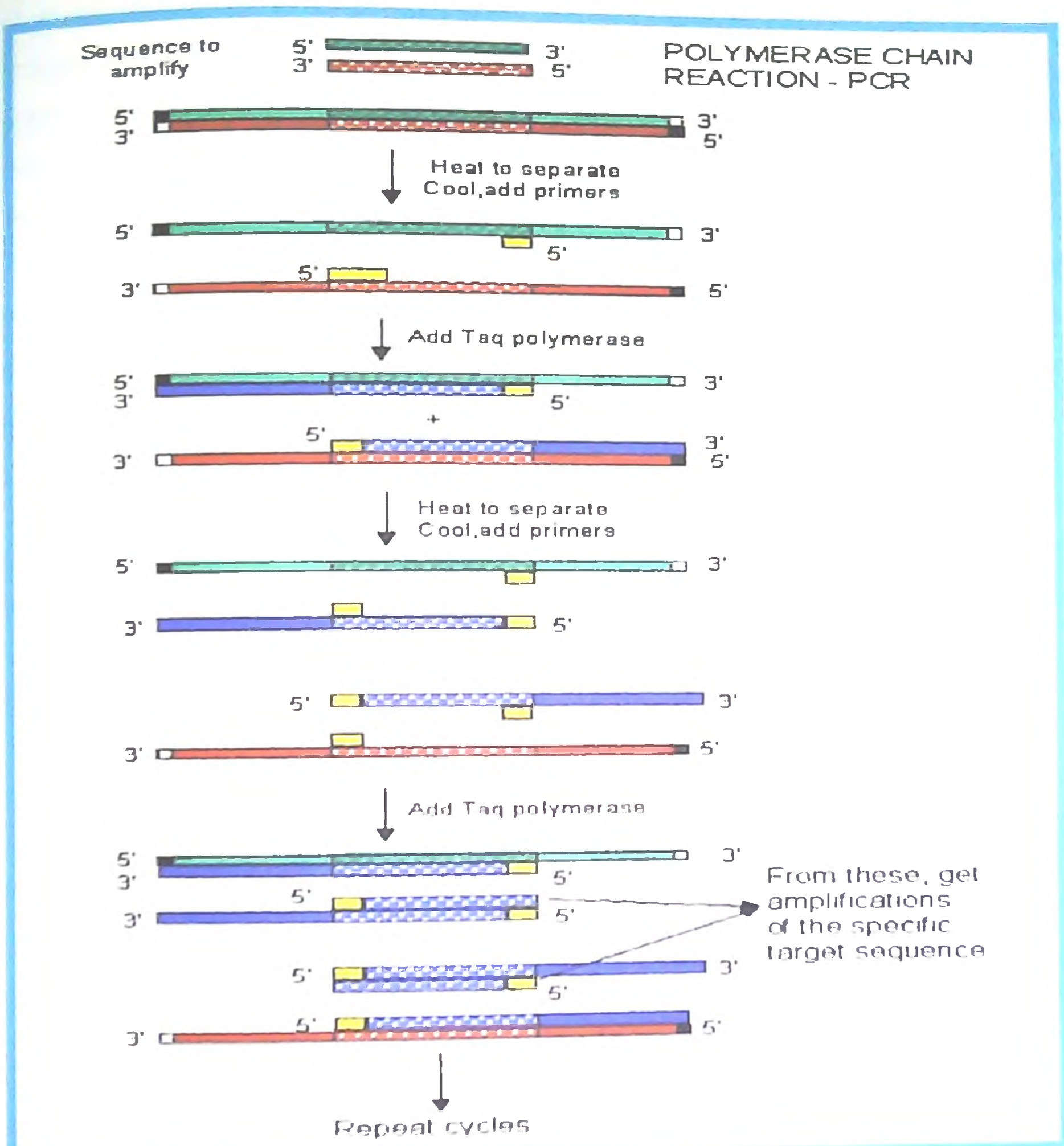
Primer Extension

The temperature is now so adjusted that the DNA polymerase synthesizes the complementary strands by utilizing the 3^1 – OH of the primers; this reaction is the same as that occurs *in vivo* during replication of the leading strand of a DNA duplex. The primers are extended towards each other so that the DNA segment lying between the two primers is copied; this is ensured by employing primers complementary to the 31 ends of the segment to be amplified. The duration of primer extension is usually 2 min at 72 ° C. Taq polymerase usually amplifies DNA fragments of up to 2 kb; special reaction conditions are necessary for the amplification of longer segments.

Applications of the PCR technique are as follows

- Sequencing reaction
- Genotyping of organisms
- Generating and identifying site specific mutations
- Detection of pathogens in the samples
- Cloning and subcloning of target DNAs
- For cDNA library construction
- Generation of specific DNA probes

Steps in the PCR reaction



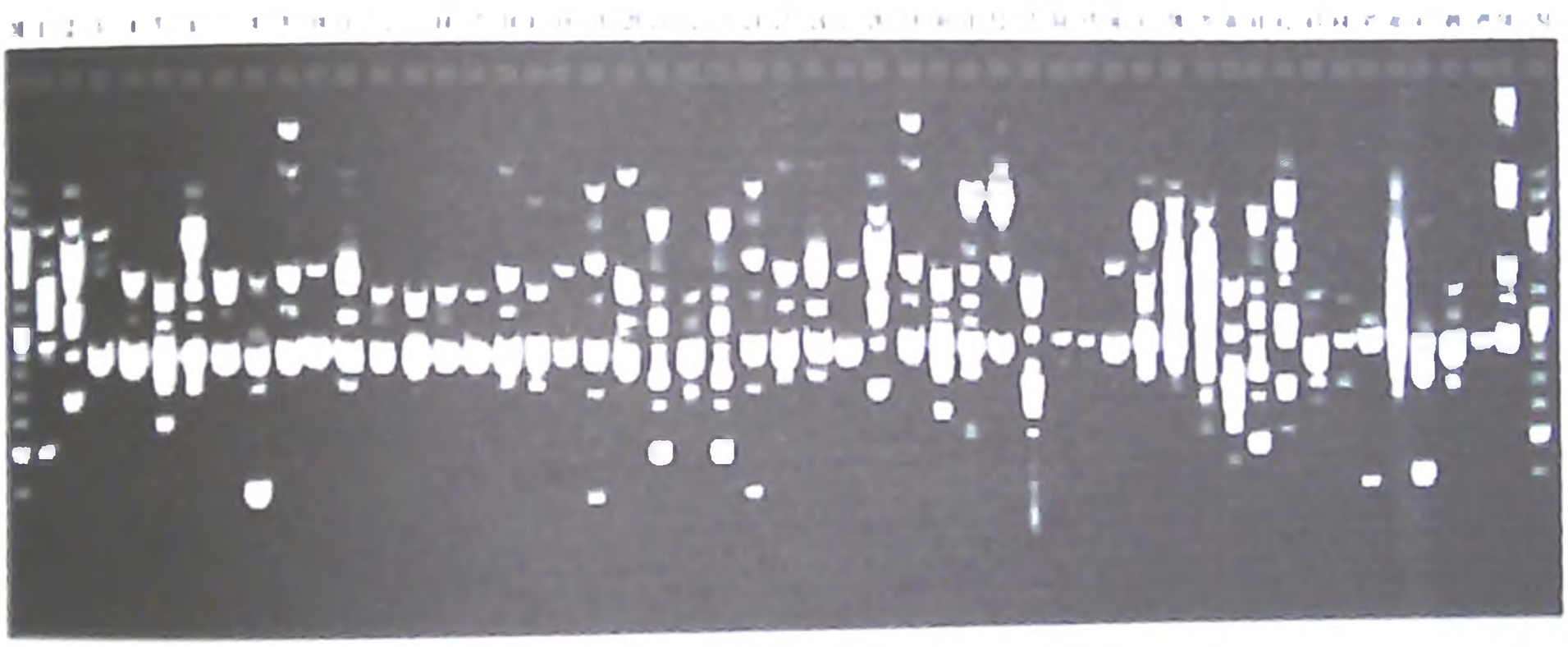
Use of genomic fingerprints to identify pathogens

DNA homology experiments and 16 S rDNA sequence analysis have proven to be highly effective for classification of microorganisms. Every bacterial isolate has a polymorphic genome sequence due to recombination, mutation and other genetic events. Polymorphisms characterized by DNA typing and diversity will facilitate in the identification

RAPD based genomic fingerprinting

Short oligonucleotides of an arbitrary DNA sequence, used in pairs or singly under low stringency conditions to target DNA sites that are partially or fully complementary to the primer sequence. An array of variable size PCR products are generated corresponding to the number of annealing site pairs proximal enough (~200 to 2000 bp) for PCR mediated DNA amplification. RAPD requires no prior knowledge of target DNA sequences but may require empirical screening of a number of arbitrary primers to generate the genomic fingerprints desired. DNA amplification with different random primers showed diversity among the *Ralstonia solanacearum* isolated from brinjal, chilli and tomato in three different agroclimatic zones of Kerala (Deepa *et al.*, 2003)

Gel showing Random Amplified Polymorphic DNA (RAPD) profile

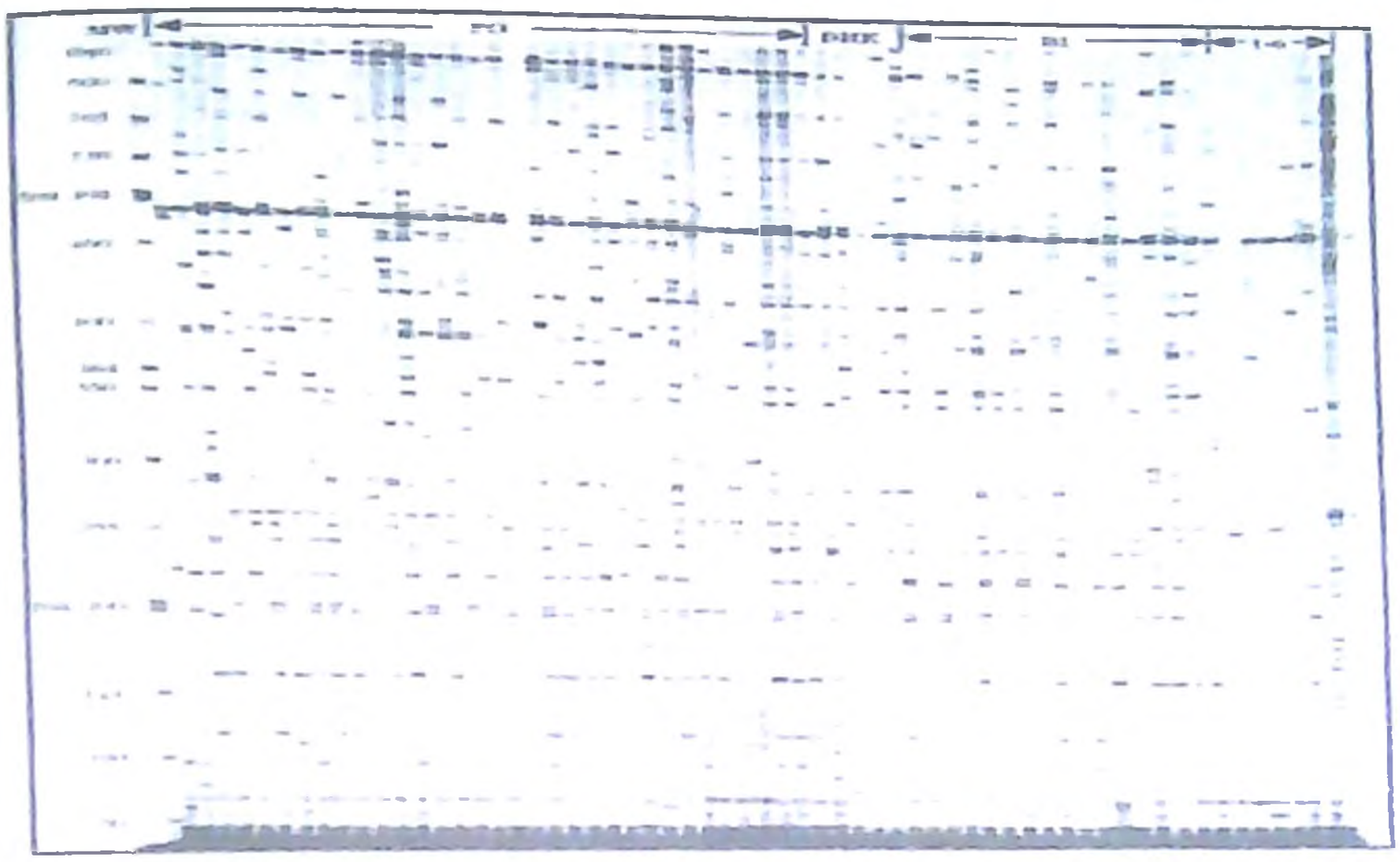


Amplified fragment length polymorphism genomic analysis

AFLP analysis also appears to be a universal protocol to fingerprint plant pathogenic bacteria. AFLP involves three sites. Restriction of genomic DNA using two restriction endonucleases, ligation of specific double stranded DNA adapters to the respective restriction fragments to function as priming sites, amplification of fragments using two primers complementary to the ligated adapters. The primers include one or two additional nucleotides at the 3' end designed to selectively match genomic DNA sequences flanked by the adapters in order to generate specific fragment sets that perfectly match the adapters and adjacent

bacterial nucleotides. AFLP analysis showed that *Ralstonia solanacearum* strains from ginger is genetically distinct from tomato (race 1) and Heliconia (race 2) (Yu *et al*., 2003).

AFLP GEL IMAGE

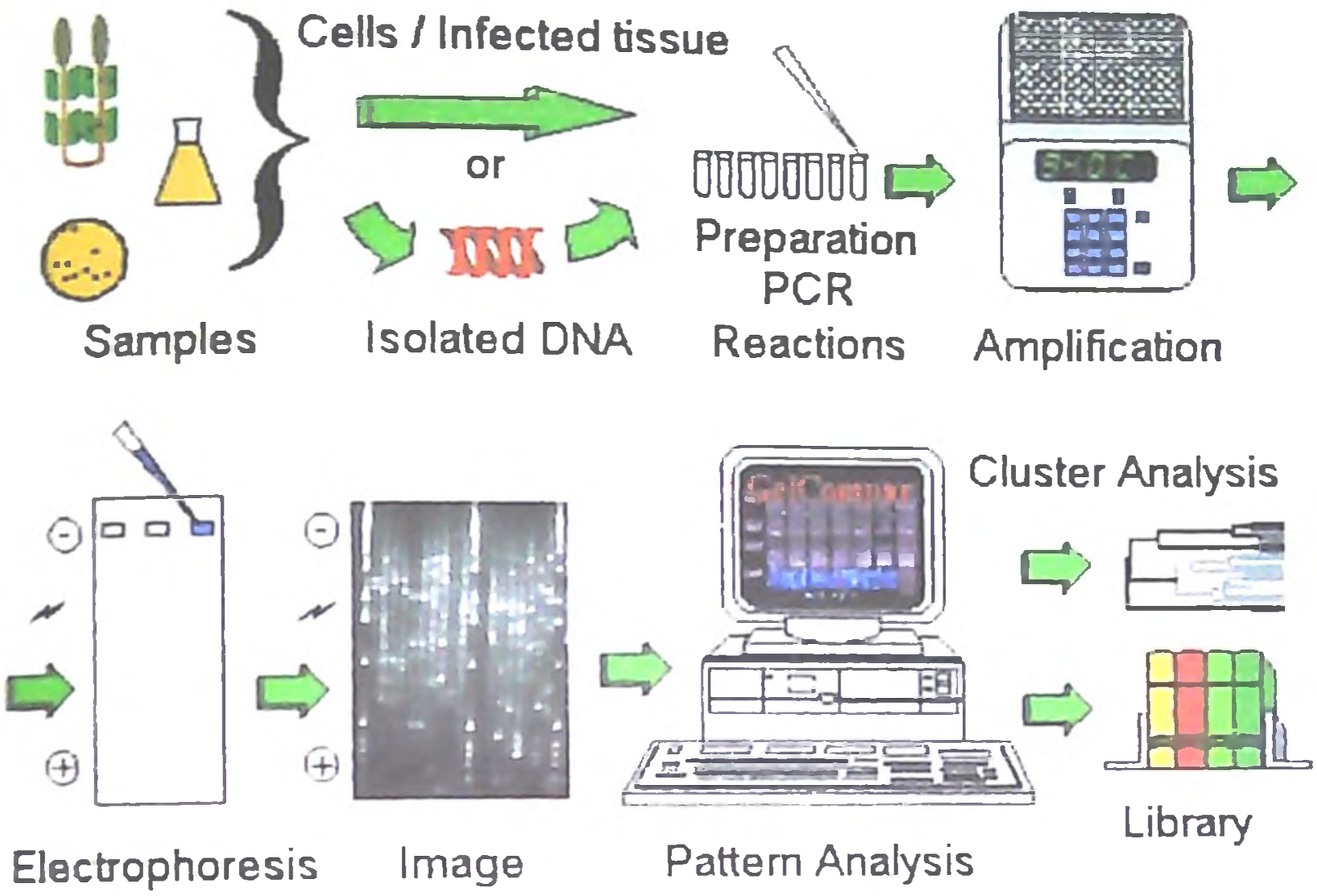


Different strains of *R. solanacearum* showing the variability

Repetitive DNA PCR based genomic fingerprinting

Rep- PCR analysis was developed based on the observed occurrence of specific conserved repetitive sequences (repetitive extragenic palindromic (REP) sequences, enterobacterial repetitive intergenic consensus (ERIC) sequences and BOX elements distributed in the genomes of diverse bacteria. Three primer sets are commonly used for rep – PCR genomic fingerprinting analysis, corresponding to REP, ERIC and BOX sequences. The protocols are reffered to as REP-PCR, ERIC-PCR and BOX-PCR. Rep-PCR has been extensively used to identify pathogens to differentiate strains and to assess the genetic diversity of plant pathogens. A more specialized form of rep-PCR employs primers designed to generate PCR amplification from the repetitive element IS 1112, commonly found in *Xanthomonas oryzae* pv.*oryzae*.

Steps in rep PCR-fingerprinting



Four types (A,B,C and D) within *C.michiganensis* subsp. *michiganensis* causing bacterial canker in tomato were grouped based on limited DNA polymorphisms (Louws *et al.*, 1998). *Xanthomonas* and *Pseudomonas* pathovar and strains generated with repetitive sequences (Louws *et al* , 1994).

Use of specific genes and DNA sequences to detect and identify pathogens

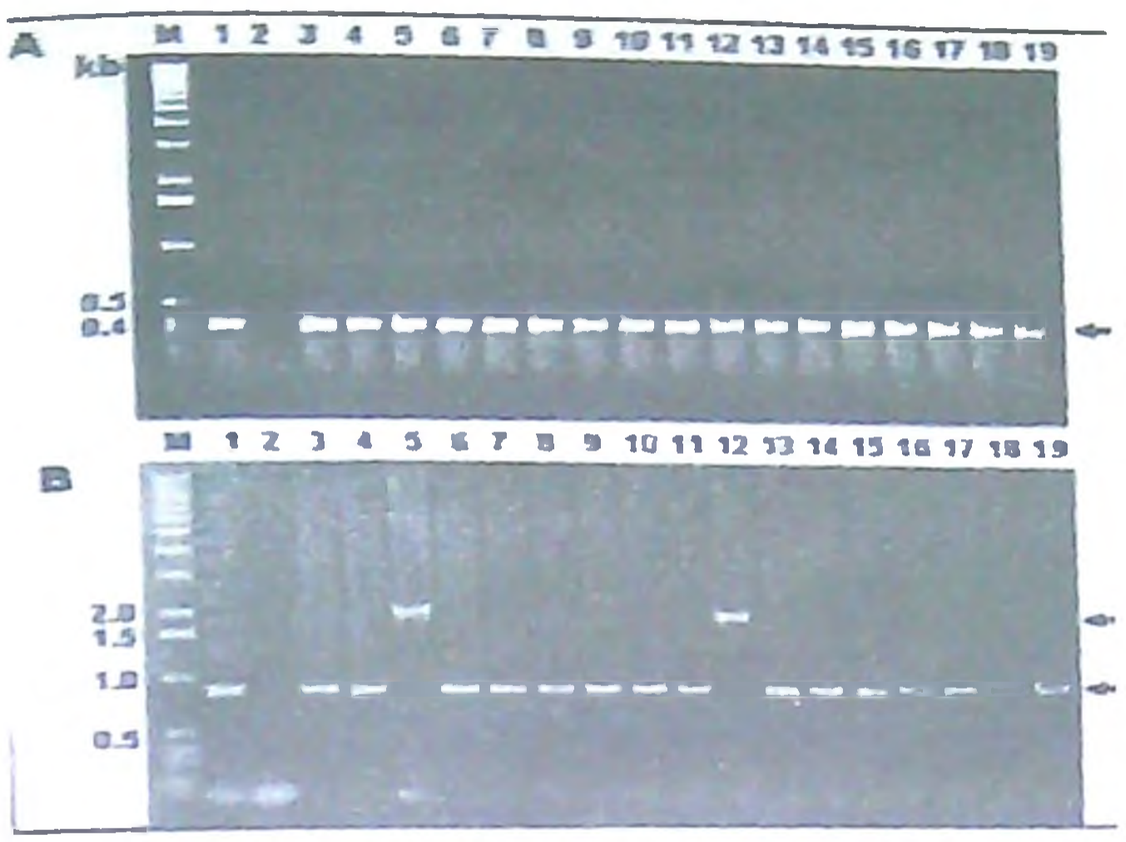
The utility of PCR protocols that employ specific primers that target known pathogenicity genes is employed to identify phytopathogenic bacteria

Genus/Sp./ subsp./pv.	Sequence(5^{I}.....3^{I})	Primer name	Target DN
Agrobacterium	ATCATTTGTAGCGACT AGCTCAAACCTGCTTC	VCF VCR	vir C
Agrobacterium tumefaciens	AAGTCGTCGAGATACTGTTT TATGATACCTTATGCTGATG AATCCTTCGCCTAGTCGTTA TCAATCGCCTTAACTTGAAC	Wide1 Wide2 Narrow1 Narrow2	T-DNA

Xathomonas	GGAGAGTTAGATCTTGGCTCAG AAGGAGGGGATCCAGCCGCA	FGPS6 FGPS1509	16 S rRNA gene
Ralstonia	TGGCTCAGAACGAACGCGGCGGC CCCACTGCTGCCTCCCGTAGGAGT	Y1 Y2	16 S rRNA gene
E. amylovora	ATTTCCGAATGGGGIAACCC GTGAGCTTTACGC TGTCTCACGACGTTTAAACCCAGCTC	C1 C2 C3	23 S r RNA gene
Pseudomonas	GAGTTTGATCATGGCTCAG TTGCGGGACTTAACCCAACAT GGTGGATGCCTTGGCAGTCA AGATGCTTTCAGCGGTTATC AGCCGTAGGGGAACCTGCGG TGACTGCCAAGGCATCCACC	A1 B6 N1 N2 D21 D22	16S rRNA gene 23S r RNA gene ITS region
Xanthomonas oryzae pv. *oryzae*	CTCAGGTCAGGTCGCC GCTCTACAATCGTCCGC	JEL 1 JEL2	*IS1112* insertion element
Xylella fastidiosa	AGATGAAAACAATCATGCAAA GCCGCTTCGGAGAGCATTCCT	CVC1 272-2	-
Clavibacter michiganensis	GTGATGTCAGAGCTTGCTCTGGCGGATC GTACGGCTACCTTCTTACGACTTAGT CCCCGACTCTGGGATAACTGCTA CGGTTAGGCCACTGGCTTCGGGTGTTAC CGA	CMR16F1 CMR16R1 CMR16F2 CMR16R2	16S rRNA gene Nested primers within F1 and R1
Clavibacter michiganensis Subsp. *sepedonicus*	TGTACTCGGCCATGACGTTGG TACTGGGTCATGACGTTGGT TCCCACGGTAATGCTCGTCTG GATGAAGGGGTCAAGCTGGTC	CMSIF1 CMSIR1 CMSIF2 CMSIR2	Insertion element Nested primers Within F1 and R1
Streptomyces scabies	ATGAGCGCGAACGGAAGCCCCGGA GCAGGTCGTCACGAAGGATCG	Nf Nr	Nec1

Carrot leaf blight caused by *Xanthomonas campestris* pv. *carotae* was detected by using PCR based assay. The cloned RAPD fragments 3 S and 9B used as primers for bacterial leaf blight pathogen detection in seed and carrot tissues. The 3 S primer amplifies the 350 base pair of the pathogen. The 9 B primer amplifies the 900 base pair of the pathogen. The PCR based assay can detect 2×10^2 to 2.3 to 10^8 CFU per gram of seed (Meng *et al*., 2004).

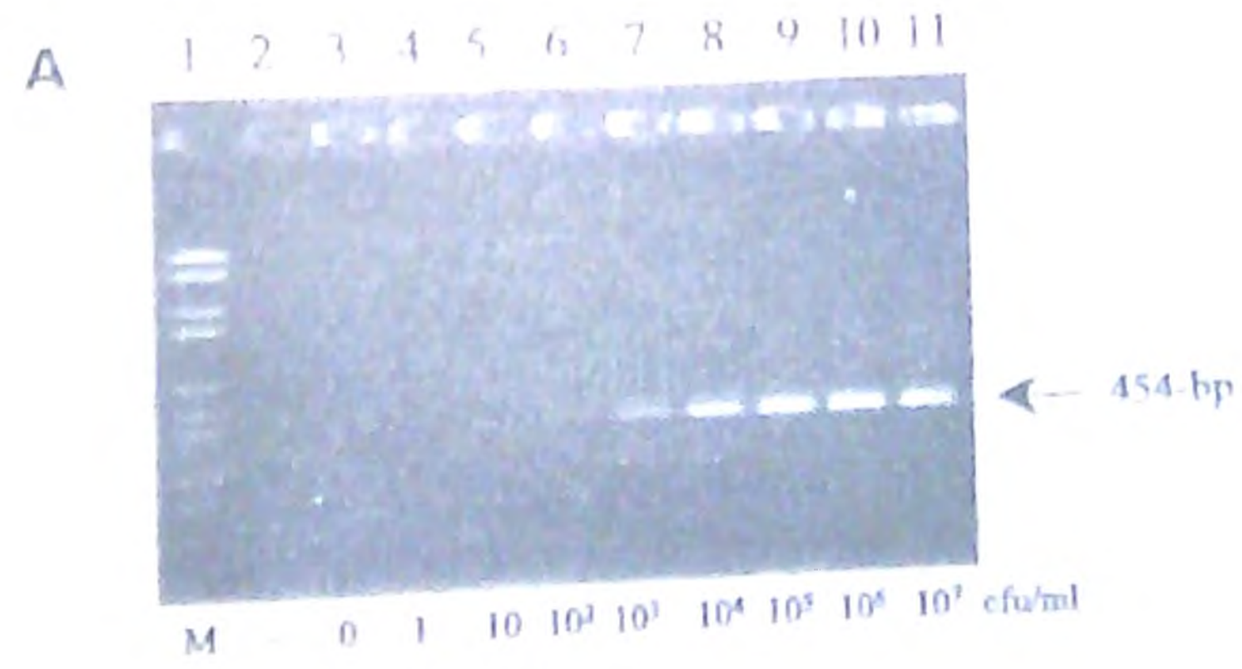
Detection of *Xanthomonas campestris* pv.*carotae*



Agarose gel polymerase chain reaction using the 3 S (A) and 9 B primers (B)

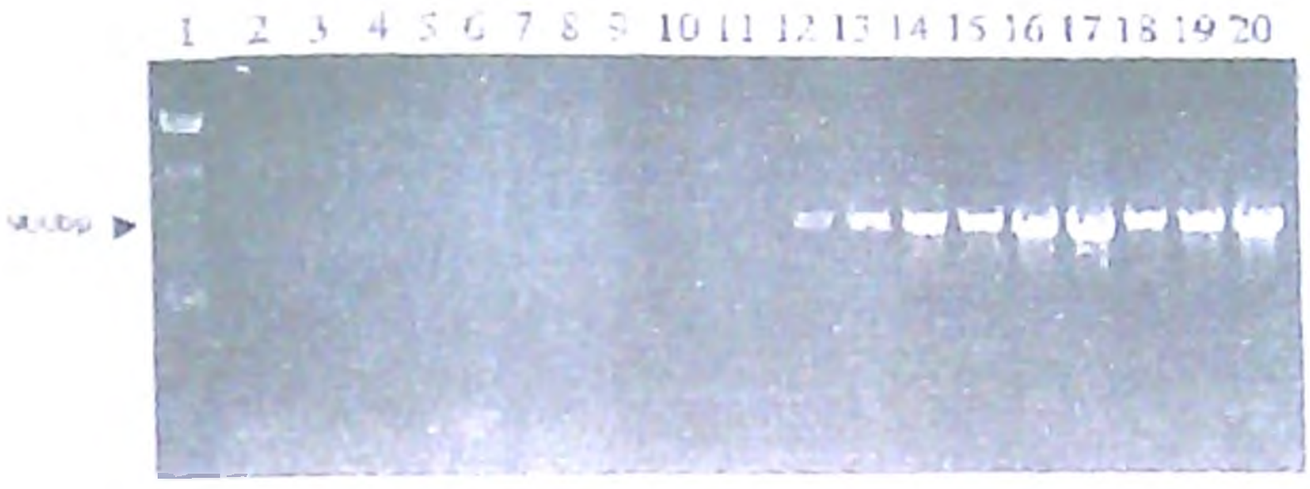
Pseudomonas savastanoi pv. *savastanoi* in olive plants was detected by enrichment PCR. iaaL derived primer can amplify the 454 base pair of *Pseudomonas savastanoi* pv. *savastanoi*. Bacteria enriched in the semi selective medium PVF-1 before carrying out PCR. The primer sequences used for iaaL amplification were IAALF 5[1] GGCACCAGCGGCAACATCAA 3[1] and IAALR 5[1] CGCCTCGGAACTGCCATAC 3[1](Ramon *et al*.,2000)

Gel showing the amplification of 454 base pair of the pathogen



Detection of Cassava bacterial blight pathogen *Xanthomonas axonopodis* pv. *manihotis* was detected by PCR Primer sequences used for the detection were 5^{1}TCGGCAACGGCAGTGACCACC 3^{1} (XV) and 5^{1} TCAATCGGAGATTACCTGAGCG 3^{1} (XK). These primer sequences can amplify 898 bp of *X. axonopodis* pv. *manihotis*. This method can detect 3x 10^{2} to 10^{4} CFU/ml in leaves and stem (Verdier *et al.*, 1998).

Detection of *Xanthomonas* strains by PCR



Lane 12 to 20 shows the presence of *X. axonopodis* pv. *manihotis* in the samples

Plasmid DNA as target

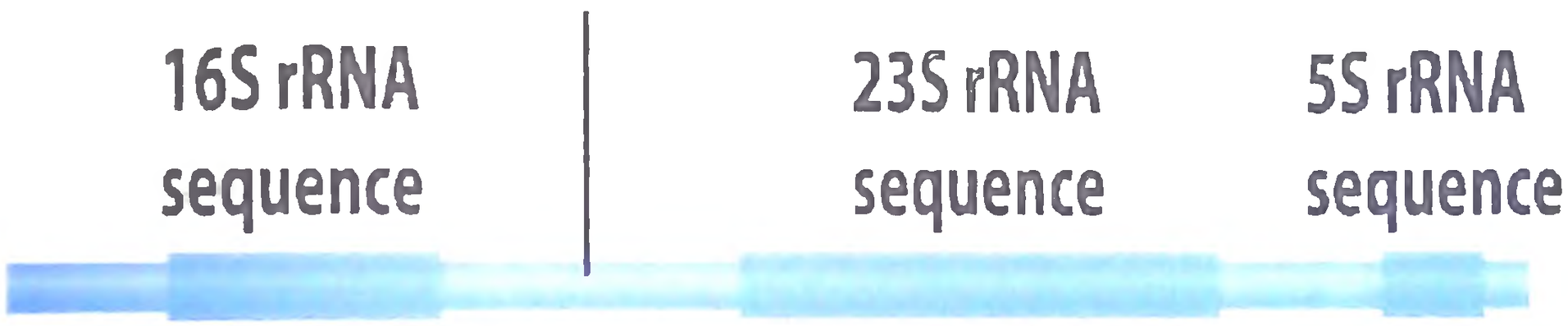
Plasmid DNA has served as template for PCR diagnosis of several pathogens. Plasmid encoded genes could also be associated with pathogenicity or function as an anonymous DNA target. A Pst I fragment from a plasmid named pEA 29 was partially sequenced and primers were developed for specific and sensitive detection of *Erwinia amylovora* (Bereswill *et al.*, 1992). Plasmid derived specific primers have also been developed to detect citrus canker causing bacteria (Hartung *et al.*, 1993). *Clavibacter michiganensis subsp. michiganensis* causal agent of bacterial wilt and canker in tomato was detected by DNA – probes derived from plasmid borne genes(*cel* A encoding an endocellulase and *pat*-1involved for pathogenicity) *cel* - A probe differentiated *C. m.* subsp. *michiganensis*, *C.m. insidious* and *C. m. sepedonicus* and *pat* -1 probe distinguished virulent from avirulent strains of *C. m. michiganensis* (Dreier *et al.*, 1994)

Ribosomal DNA based PCR analysis

rDNA- PCR

The ribosomal DNA operon in bacteria comprises three functionally and evolutionary conserved genes, the small subunit 16 S (rRNA) gene (rrr), the large subunit 23 S r RNA gene, and 5S r RNA gene interspersed with variable spacer regions (intergenic transcribed spacer,ITS). Multiple strategies have been adopted to characterize bacteria based on features of their ribosomal DNA. Genus specific rDNA sequences among phytobacteria are well documented and primers have been developed for *Pseudomonads*, *Xanthomonas* (Widmer *et al.*, 1998).

ITS



Maes (1993) deduced a primer set able to amplify a 480 bp fragment specific to the genus *Xanthomonas* by combining a universal 16 S DNA primer with a reverse primer specific to *Xanthomonas* permitting the detection of *Xanthomonas* DNA in wheat seed extracts.

ITS - PCR

ITS – PCR analysis employs conserved primers to the 16 S and 23 S ribosomal genes to amplify the internally transcribed spacer (ITS) region. *Xylella fastidiosa* causes Pierce's disease in grape vine. The pathogen was detected with ITS forward primer XfF1: 5^1 AAA AAT CGC CAA CAT AAA CCCA 3^1.ITS reverse primer XfR1 : 5^1 CCA GGC GTC CTC ACA CCC A 3^1,16 S forward primer XfR1: 5^1 CCA GGC GTC CTC ACA AGT TAC 3^1,16 S reverse primer XfR2 : 5^1 CTG GCG GCA GGC CTA AC3^1. The probes used for the detection were ITS probe XfP1: 5^1 6- carboxy – fluorescein (FAM) ACC TAT GCC AAC ATC AAA CCC TGA ATG CA 6 – tetramethylrhodamine (TAMRA) 3^1, 16 S probe XfP2 : $5^1$6 FAMATG TGC TGC CGT ACT TGCATG TAMRA 3^1 (Sahaad *et al.*,2002).

Amplified ribosomal DNA restriction analysis (ARDRA)

Amplified ribosomal DNA restriction analysis (ARDRA) exploits the use of universal primers to amplify bacterial rDNA sequences followed by digestion with frequently cutting restriction endonucleases to determine diversity and to identify/ classify bacterial isolates to the genus and sometimes species level.

The steps involved are as follows

16s rRNA gene (rDNA) / ITS region

↓

PCR amplified product

↓

Restricted with different RE

↓

Resolved banding pattern

↓

Similarity co-efficient

↓

Clustering

↓

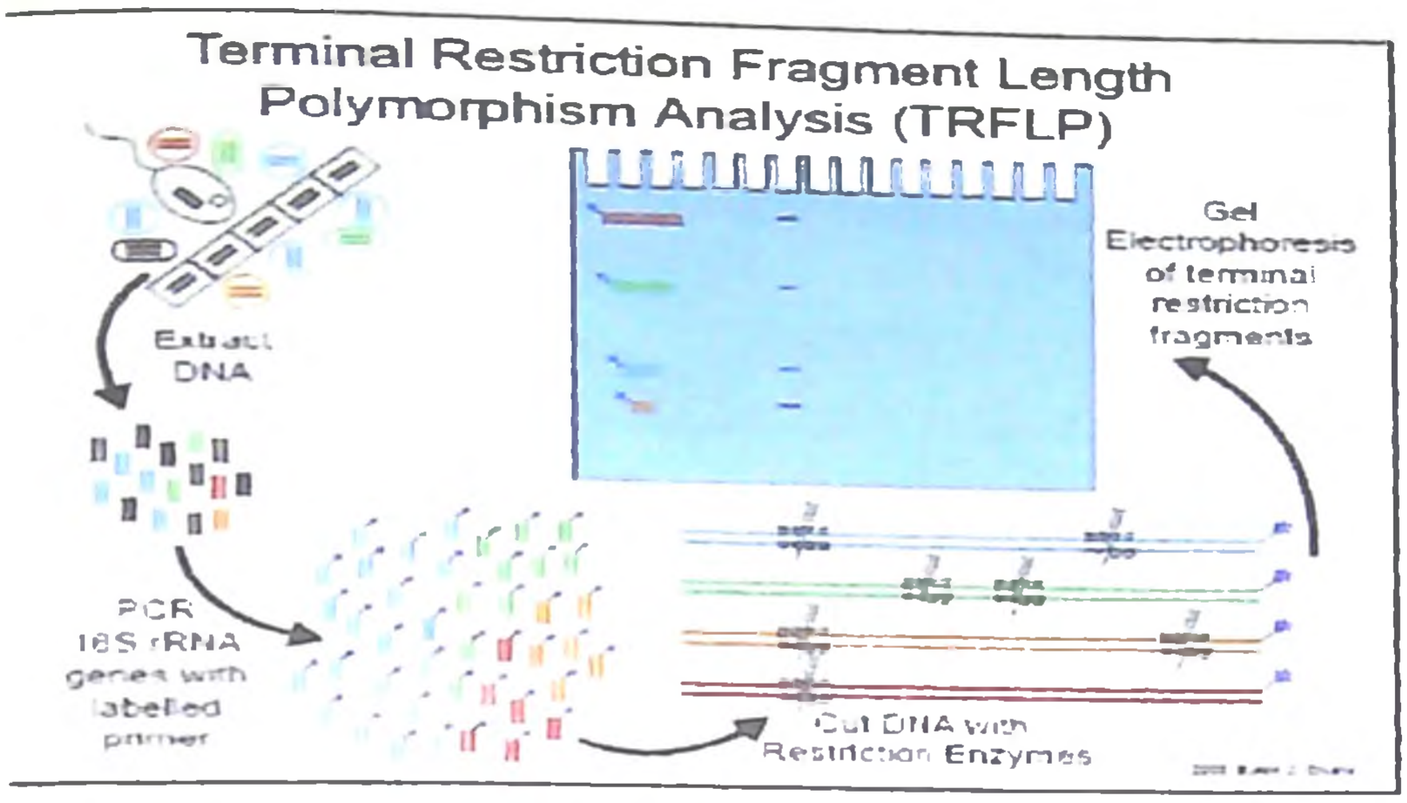
Dendrogram

16s r DNA reverse primer used for the detection of *Xanthomonas* (Maes 1993).

Terminal Restriction Fragment Length Polymorphism (TRFLP)

The Terminal Restriction Fragment Length Polymorphism exploits the amplification of 16 S rRNA gene to differentiate phylogenetic groups. The 5^{1} primer is labeled with a fluorescent dye and after restriction digestion of amplified 16 S fragments, the labeled terminal fragment can be specifically detected and its size determined plus or minus a few bases by using semiautomated electrophoresis and detection protocols. A primer set, fD2 and rP1 with conserved sequence for most eubacteria has been used to generate a 1.5 kb 16 S rDNA fragment of *Erwinia amylovora* which when digested with Hae III, generated a diagnostic RFLP pattern. A nested 16 S rDNA-PCR approach has been found to be useful to specifically identify strains of *C. michiganensis* and to differentiate the various subspecies after restriction endonucleases (Lee *et al.*, 1997). This method was used to differentiate *Agrobacterium* biovars 1, 2 and 3.

Diagram showing the steps in TRFLP



Molecular methods of detection using the PCR have following advantages

- Faster and highly accurate in detection capability
- Greater specificity and reliability
- Exact identification of strains and species
- Helpful in quarantine purpose
- Useful for disease indexing

Practical considerations using the PCR technique

False positives

False positives can result from cross amplification of nontarget DNA, exogenous DNA from cells/ cultures or aerosols or from contaminating DNA originating from carry-over of previous experiments. Genomic fingerprinting protocols like rep-PCR, AFLP and RAPDs always yield a fingerprint, and a negative control sample in each experiment is therefore essential to check if contamination has occurred

False negatives

False negatives can arise for many reasons, including the presence of compounds derived from extracted substrates that inhibit *Taq* polymerase, degradation of the DNA target sequence, or reagent problems. Genomic fingerprinting protocols like rep-PCR, AFLP and RAPDs a positive control sample in each experiment is useful to check the quality of the fingerprints.

Insufficient and excess sensitivity for pathogen detection

Sensitivity can be enhanced by nested PCR detecting amplified products with a probe, immuno-capture PCR and targeting multicopy genes compared with single copy genes. sensitivity and speed can be enhanced by immuno-enzymatic detection of amplified products (PCR- ELISA).

PCR reaction inhibitors

Plant derived compounds can inhibit the PCR reaction. The presence of spray chemicals on leaf surfaces can also inhibit PCR applications. Such problems can be circumvented by physical sample preparation methods include dilution, separation and concentration of cells by centrifugation.

Conclusions

PCR – mediated methods comprise a DNA based approach to assess the diversity of pathogen populations, detect pathogens, and diagnose disease. Combining different PCR protocols such as 16 S r DNA amplification and DNA sequence analysis, with total genome fingerprinting methods such as rep- PCR genomic fingerprinting could produce robust genetic diversity maps of plant pathogens from the family to the strain- specific taxon levels, complementary to other classification methods currently in use. Construction of precise genetic diversity maps will provide a better framework for addressing important plant disease problems related to detection of pathogens, diagnosis of disease, and ultimately management of disease risk.

Discussion

1. What do you mean by epitope?

Antigen reacting site is called as epitope.

2. What do you mean by Bio-PCR?

Samples are first placed on selective media to propagate viable cells and then subject to PCR analysis.

3. How PCR methods are useful in pathogen detection?

PCR methods are useful to detect the pathogen present in seed samples imported from some other countries and also helpful for indexing of the disease.

4. Which method of detection is more reliable?

PCR methods of detection are more reliable.

5. Why 16 S RNA is called as signature sequences?

Because of more conserved in nature

6. Which PCR method is more sensitive in detection of pathogen?

Real time PCR

7. How the pathogen presence in seed samples will be identified using ELISA method?

The antigen from the seed sample will be mixed with antiserum in slide. The formation of precipitation indicates the presence of the pathogen.

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Name of the student : S.M.Purushothaman **Venue: Conference Hall**
Admission number : 2006-21-110 **Date and Time: 07/07/07, 9.15 AM**

SEMINAR : PL.PATH.752

TITLE: MOLECULAR METHODS OF DETECTION OF PHYTOPATHOGENIC BACTERIA

Abstract

The polymerase chain reaction have facilitated the genomic analyses of microorganisms; provide enhanced capacity to characterize and classify strains and to assess the genetic diversity of populations. The diversity of large populations of *Clavibacter michiganensis* were assessed in a relatively efficient manner using rep-PCR (Louws *et al.*, 1998). Amplified fragment length polymorphism analysis showed genetic diversity of *Ralstonia solanacearum* strains isolated from ginger (Yu *et al.*, 2003).

Random amplified polymorphic DNA (RAPD) used for the detection of *Ralstonia solanacearum* race 3 causing bacterial wilt of solanaceous vegetables (Deepa *et al.*, 2003). Southern hybridization with DNA probes derived from plasmid borne genes used to distinguish *Clavibacter michiganensis* subsp. *michiganensis* causing bacterial wilt and canker in tomato (Dreier *et al.*, 1994). Detection of *Pseudomonas savastanoi* in olive plants by enrichment PCR (Ramon *et al.*, 2000).

A variety of PCR based finger printing protocols such as rDNA based PCR, ITS-PCR, ARDRA and T-RFLP were devised and innovative approaches using specific primers have been adopted to enhance both detection and identification of phytobacteria. PCR based protocols combined with computer based analysis have provided novel fundamental knowledge of the ecology and population dynamics of bacterial pathogens.

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SOIL HEALTH IN VIEW OF SUSTAINABLE FERTILITY

BY

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SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

Ag. Chem. 751 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

I hereby declare the seminar report on "**Soil health in view of sustainable fertility**" is a record of the seminar presented by me during the course and that this report has been prepared by me independently after going through the references cited herein

Vellanikkara,
31.05.2007

K. Sajnanath

CERTIFICATE

Certified that this seminar report, entitled "**Soil health in view of sustainable fertility**" is a record of seminar presented by **K. Sajnanath** under my guidance and that this has been prepared by him independently.

Vellanikkara,
31.05.2007

Dr. M. A. Hassan
Major Advisor

CONTENTS

SOIL HEALTH IN VIEW OF SUSTAINABLE FERTILITY

1. Introduction

Soil, Water and air are the three basic natural resources upon which most life forms depend. Like water, soil is a vital natural resource essential to civilisations but unlike water soil is non - renewable on a human time scale. Soil is a dynamic and natural living entity used to produce goods and services of value to humans but not necessarily with perpetual ability to withstand the degradative processes unless appropriately managed. Mismanagement and neglect can ruin this fragile resource and can become a threat to human survival.

As soon as land is newly put into production, the soil degradative processes are set into motion, triggering deterioration of soil structure and disruption of cycles of carbon, depletion of nutrient reserves and weakening of nutreint recycling mechanisms. The soils have an inherent capacity to restore the life support processes; provided that the disturbance created especially by human activities is not too drastic and sufficient time is allowed for the life support processes to restore themselves. It is for humans to decide, act and see that enough time is given for the soil to restore its capacity before it affects the capacity of soils to produce – *i.e.* soil quality or soil health.

Sustainable agriculture is one that, over the long-term, enhances environmental quality and the resource base on which agriculture depends, provides for basic human food and fibre needs, is economically viable, and enhances the quality of life of farmers and society as a whole (Schaller, 1990). The quality and health of soils determine agricultural sustainability, environmental quality and as a consequence of both, plant, animal and human health as well.

Sustainability of soil resources must be addressed in terms of the trends in per capita productivity in relation to changes in soil properties, water characteristics and climatic factors. But the heavy dependence of modern agriculture on non-renewable fossil fuels for synthesis of fertilizers and pesticides, and energy needs for cultivation, harvest, intensive animal production and grain processing raise questions about the long-term sustainability of agriculture. Moreover, the global climate change, depletion of the protective ozone layer, serious declines in species biodiversity and degradation and loss of productive agricultural land are among the most pressing concerns associated with our technological search for a higher standard of living for ever-growing human populations. Advancement in agricultural technologies to meet the needs of increasing populations has

taxed the resilience of soil and natural processes to maintain global balances of energy and matter.

The quality of a country's soil is that country's future. This topic covers concept of soil health, characteristics of healthy soil, factors affecting soil health, methods of assessment, management of soil health, policy decision to be taken and future line of research needed with special reference to our nation's situation.

2. Relevance of soil health

At the global level, out of the total ice free land area of 13.4 billion ha, 3.2 billion ha (about 22%) are potentially arable, of which 1.3 billion ha are moderately to highly productive and 1.9 billion ha are low productive. About one half of the potentially arable land is actually cultivated; other part is under permanent pastures, forests and woodland (Scherr, 1999). How much more potentially arable land can be brought under cultivation, will depend upon a host of factors including technical advances, socio-economic scenario, environmental concerns, political policies and world trade priorities. The global population, which increased from 1 billion in 1800 AD to 3 billion in 1960 and over 6 billion in 2001, is estimated to reach 12 billion by 2100 (Yadav, 2003). Considering the future population growth, very limited cultivated land is available in the world.

India is predominantly agrarian in nature and about 70% of the population depends on agriculture. At present, net sown area (143 m ha) and gross cropped area (187 m ha) in the country account for 43% and 57% respectively of its total geographical area. The per capita arable land decreased from 0.34 ha in 1950-51 to 0.15 ha in 2000-01 and is expected to shrink to 0.08 ha in 2025. No possibility of further horizontal expansion in the cultivated area seems to exist. In view of the galloping population from 361 million in 1951 to over 1 billion in 2001, with further projections of 1.4 billion in 2025 and 1.8 billion in 2050, the scarce soil resources are under heavy pressure. Agricultural statistics would indicate that India has made spectacular advances in improving food grain production because the total food grain production increased from nearly 51 million tones in 1950-51 to 177.6 mt in 1990-91 and to 206 mt in 2000-01. But we should have increased the production to a level of 240 mt when the population crossed 1 billion. Geometrically multiplying population has forced the agricultural managers to go in for high-input-high-output production model of the west.

From this, we can see that the use of chemical inputs, especially fertilizer, has played a pivotal role in increasing the agricultural production in the country. But in the modern agricultural scenario, the chemical component has been blamed for many of the

ill-effects facing presently. One of those is the plateuing trend in the productivity of many crops.

In recent years, many crops fail to increase yield in different regions, in spite of increased use of inputs, particularly nutritional ones. Several studies have been conducted to investigate the reasons for this phenomenon. The results of such attempts indicate that it is related to a decline in soil health. The above figures also clearly show fast diminishing availability of arable land and hence, emphasize the urgent need of conserving and sustaining good health of the finite soil resource through judicious management. Any damage to the soil resource ecosystem is bound to hamper the crop productivity and overall progress of the country. Therefore, the grave consequences of the abuse of the soil base have to receive priority attention.

3. Concept of soil health

Soil health is not a new concept. Greek and Roman philosophers were aware of the importance of soil health to agricultural prosperity over 2000 years ago, and reflected this awareness in their treatises on farm management. Since the land (earth) was considered as 'goddess' (*Bhoomi devi*) or as 'mother' who nourishes the human being, any activity that is harmful to soil was discouraged at that time. As the science of agriculture developed, plant nutrients were identified as essential components of soil health, at least with respect to sustaining biological productivity. This resulted in a paradigm of plant nutrition and soil management that relied heavily on the use of artificial fertilizers and intensive tillage. Increasing concern over agriculture's impact on the environment has created renewed interest in soil health.

In McGraw Hill Dictionary of scientific and technical terms, the term 'health' is defined as a 'state of dynamic equilibrium between organism an its environment in which all the functions of mind and body are normal'. Drawing similar analogy, soil health would imply as being 'a state of dynamic equilibrium between flora and fauna and their surrounding soil environment in which all the metabolic activities of the former proceed optimally without any hindrance, stress or impedance from the latter'.

Efforts to define soil health in the context of multiple soil functions began in 1977 (Warkentin and Fletcher, 1977), and were followed by more formalized definitions (Larson and Pierce, 1991; Karlen *et al.*, 1997), selection of indicators (Doran and Parkin, 1994), and specific strategies to enhance soil health (Doran *et al.*, 1996).

The presently accepted definition was given by Doran and Parkin (1994). They defined soil health as 'the capacity of soil to function within ecosystem boundaries to

sustain biological productivity, maintain environment quality and promote plant and animal health'. The terms soil quality and soil health are used as synonyms. Generally the scientific community uses 'quality' whereas farmers use 'health'. It mainly depends on intrinsic properties such as depth, texture, calcareousness, nutrient status, etc., external features like rainfall, wind velocity, topography, drainage, earthquake etc. and human interventions like tillage, cultivation, land use, constructions etc.

4. Characteristics of a healthy soil

Important soil functions related to crop production include: adsorption and infiltration of water, retention and cycling of nutrients, pest and weed suppression, detoxification of harmful chemicals, sequestering of carbon and production of food and fiber. When the soil is not functioning to its full capacity as a result of soil constraints then more inputs in the form of fuel are necessary for increased tillage to counteract compaction or more herbicides to manage problematic weeds, etc. the important 10 characteristics that describe a healthy soil are:

(i) Good soil tilth: Good soil tilth refers to the overall physical characters of the soil making it suitable for crop production

(ii) Sufficient depth: The extent of the soil profile to which roots are able to grow and function. Shallow soils are more vulnerable to extreme weather fluctuation like drought and flooding stress

(iii) Sufficient but not excess supply of nutrients: Adequate and accessible supply of nutrients is necessary for optimal plant growth and maintaining balanced nutrient cycling. Excess nutrients lead to leaching loss, ground water pollution, nutrient run off, green house gas losses, toxicity to plant and microbial population

(iv) Small population of plant pathogens and insect pests: Pest and disease population in healthy soil is low and/or inactive. This could result from direct competition from other micro-organism for nutrients, hyper parasitism, etc. Also, healthy plants defend themselves better against pest and diseases.

(v) Good soil drainage: Due to good soil structure and adequate distribution of different sized pore spaces, healthy soils drain rapidly but also retain adequate water for plant uptake.

(vi) Large population of beneficial organisms: Healthy soil will have a high and diverse population of beneficial micro organisms which help nutrient cycling,

organic matter decomposition, maintenance of soil structure, biological suppression of plant pests etc. thus maintaining a healthy status.

(vii) Low weed pressure: Weed pressure is a major constraint in crop production. Weeds compete with crops of nutrients and water, interfere with crop establishment, block sunlight, interfere with cultivation and harvesting and harbour diseases and pests. Low will be the weed population in a healthy soil.

(viii) Free of chemicals and toxins that may harm the crop: Healthy soils are either free from toxic chemicals or the diverse microbial population and richness in organic matter help detoxify their effect rapidly.

(ix) Resistant to degradation: A healthy soil system is more resistant to adverse events like erosion by wind and rain, excess rainfall, extreme drought, compaction etc. due to well aggregation.

(x) Resilience when unfavourable conditions occur: A healthy soil will rebound more quickly to normal situation after a negative event such as harvesting under wet soil conditions or if land constraints restrict or modify some planned crop rotations

5. Factors affecting soil health

The good health of a soil is a function of all the external and internal factors. These factors affect or maintain various parameters in the soil environment in which the growth of fauna proceeds without influencing the floral part. It is a balanced or equilibrium state of all the factors in optimum level. The important parameters considered here are (i) intensive cultivation, (ii) excessive use of chemical fertilizers, (iii) depletion of humus from soil and (iv) soil pollutants.

5.1 Intensive cultivation

Intensive agriculture, which implies harnessing of soil and water resources, genetic potential of plants and other inputs in a large measure, that has taken firm roots in the irrigated areas of India has no doubt succeeded in getting the country out of food shortage. This practice without conservation of soil fertility and soil structure would lead ultimately to the springing up of deserts. Vigorous exploitation of natural resources such as land and water will pose serious threat to the sustainability of environment. However, organic farming reduces degradation of natural resources and minimizes health and environmental hazards. It is good concept but in reality it may be difficult to practice and also it may not be the solution to the present problems.

Intensive agriculture practiced with the advent of new high analysis fertilizers, management responsive varieties, irrigation, coupled with indiscriminate use of chemical fertilizers, pesticides and other plant protection chemicals have caused irreparable damage to soil, water and air (Goswami and Rattan, 1992).

5.2 Excessive use of chemical fertilizers

Global fertilizer consumption has increased substantially since 1950 (Fig. 1). Between 1960 and 1995, global use of N fertilizer increased seven fold and it is expected to increase another 3 fold by 2050 unless there is substantial improvement in use efficiency. While the N, P and K fertilizer use has declined in developed countries since 1985, it has continued to increase in developing world at linear rates. In 1960, developed countries accounted for 88% of the world fertilizer consumption. By 2001 their share had fallen to 37%, developing countries accounted for 63% (IFA, 2002).

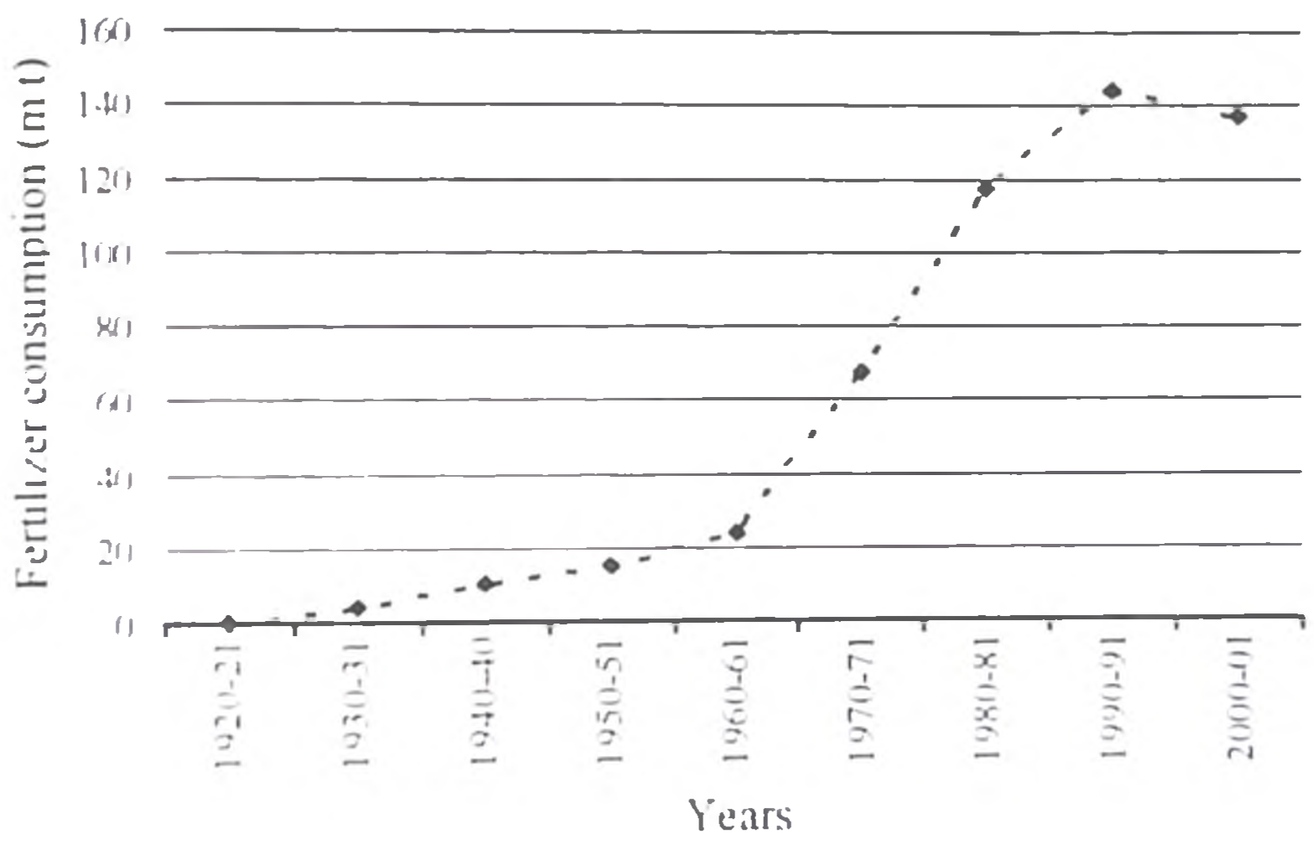

Figure 1 World fertilizer consumption over the years

Developing countries in Asia alone accounts for 49%. Moreover, there has been shift in the ratio of N, P and K use. Since 1960, world N consumption has increased much faster than that of P and K (Fig. 2). The ratio between N, P and K has changed from 1:0.91:0.86 in 1930 to 1:0.18:0.22 in 2000. This suggests that in recent decades, farmers have tended to rely primarily on nitrogen fertilizers to maximize crop yields, rather than targeting optimal achievable yields determined by local agronomic, economic and environmental conditions. The preference of N fertilizers by farmers is because of relatively low cost per unit of nutrient, easy availability, and the quick yield response of

new varieties to N. Farmers also view N inputs as a risk reducing factor because of its influence on growth and yield, in particular when their financial resources are limited (Tandon, 1996).

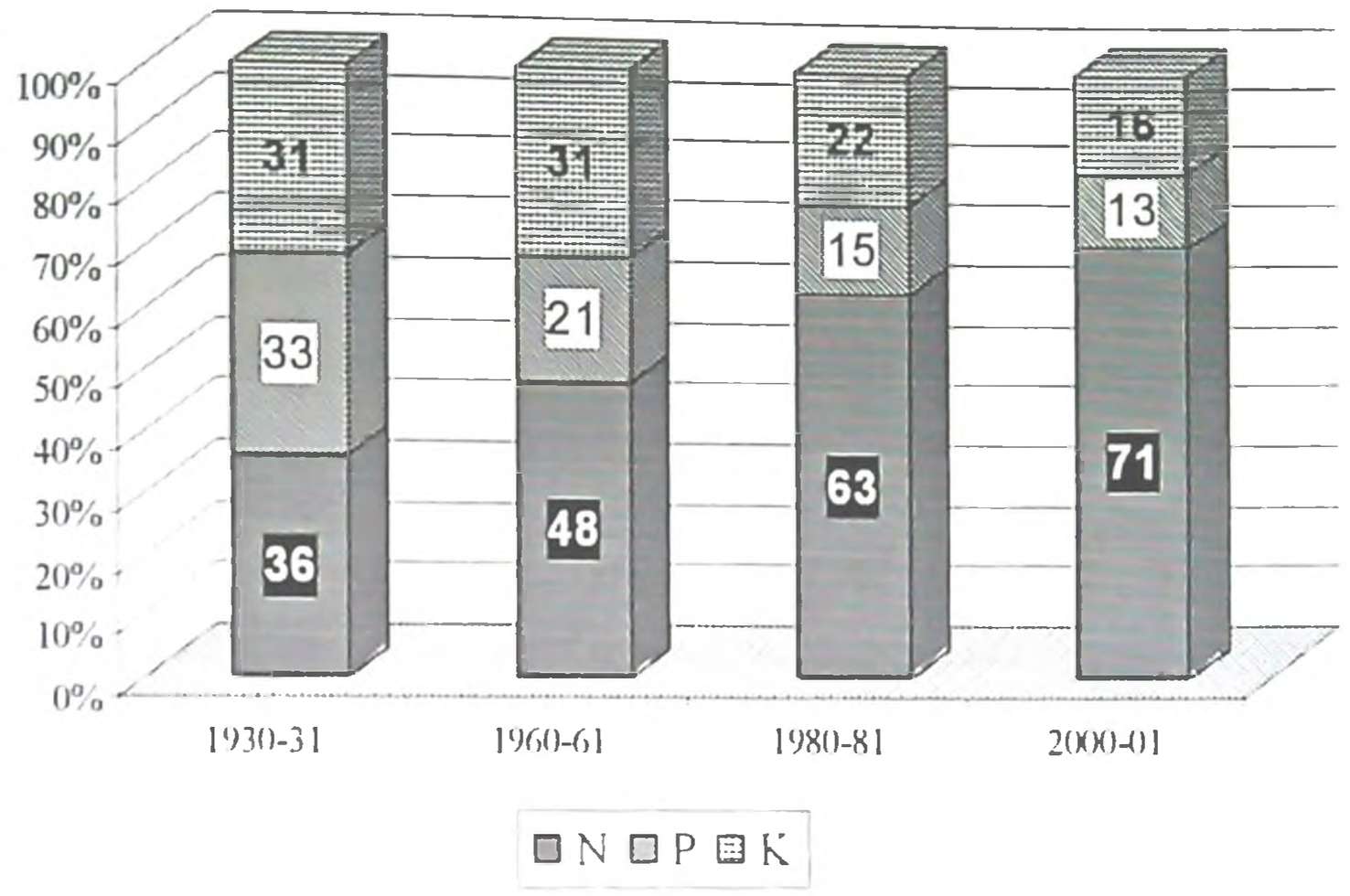

Figure 2 Relative consumption of N, P and K in the world over the years

Fertilizer consumption in India

In India, fertilizer consumption increased from less than 50,000 tonnes in 1950 to 15 million tones in 2000 and the food grain production increased from 50 mt to 200 mt in the same period, indicating a direct relationship between the fertilizer use and yield increase. The green revolution or spectacular increase in production would not have been possible without many fold increase in use of fertilizers. The high yielding varieties became a catalyst for the conversion of chemical energy into biological productivity.

There is a strong relationship between fertilizer consumption and food grain production in the country over years (Pathak *et al*., 2004). The consumption of fertilizer nutrients in the country increased drastically with corresponding increase in food grain production till early nineties. Nevertheless, here exists a wide gap between demand for fertilizers and their production that has necessitated their import leading to increase of fertilizer prices. Thus, fertilizers constitute one of the most effective and costlier inputs in increasing crop production and their rationalized use needs no emphasis.

There are vast differences in consumption of fertilizers per ha of cropped area in different regions. The fertilizer consumption varies from 114, 103, 58, 47 kg (NPK) per ha

cropped area in north, south, east and west respectively. Some states like Punjab are using more than 167 kg nutrients per ha as against some using less than 10 kg nutrients per ha. About 70 – 80 per cent fertilizer is used for growing rice and wheat. Besides these the major recipients of the remaining fertilizer use are sugarcane, cotton, potato, plantation and horticulture crops. The lowest fertilizer use is in rainfed farming, which covers nearly 66 per cent of the total cropped area in the country.

There are also wide differences in the consumption ratio of three major nutrients N : P_2O_5 : K_2O in different regions, crops and cropping systems. These differences also got magnified and showed aberrations due to adhoc changes in pricing policy of fertilizers during the recent years. The NPK ratio for India has changed from 6:2.4:1 in 1990-91 to 7:2.7:1 in 2000-01 against favourable ratio of 4:2:1 adopted (Ghosh *et al.*, 2004). There is also divergence in ratios in different regions. In 1990-91, the ratio was 8.5:3.1:1 in northern states and 4.2:1.8:1.0 in southern states. Such divergence in new ratio is also due to the differences in the quality of land, inherent soil fertility, cropping systems and degree of exploitive agriculture

Use of high analysis fertilizers for high yielding varieties in attaining maximum production resulted in the deficient conditions of organic matter, secondary and micronutrients in the soil (Yadav, 2003). Excessive use of macronutrients, especially nitrogen, leads to imbalance of nutrients in the soil ecosystem, which also causes unfavourable microclimate for microorganisms.

5.3 Depletion of humus from soil

The organic matter in the soil is important as it provides everything to the soil good health, character, relation with crop, microclimate for micro-organism etc. It is the live, fertile and dynamic system of soil.

Influence of organic matter on soil properties

- It influences soil colour. Soils with high organic matter content may be darker in colour
- It is a storehouse of several essential nutrients like N, P, S etc.
- It serves as a source of food and energy for soil micro- organisms.
- It absorbs heavy metal pollutants in soil, thus preventing the contamination of water, to some extent.
- It increases the cation exchange capacity of the soil.
- It improves soil physical conditions especially soil structure.
- It increases water-holding capacity of the soil.

- It enhances rate of infiltration and percolation of water.
- It binds soil particles, forming stable aggregates, thus reducing soil loss by erosion.
- It helps to maintain the proper soil temperature and soil aeration.
- It acts as a buffering agent, stabilizing the soil pH.
- It forms water soluble chelates with metals like Fe, Zn, Cu, Mn etc., thereby increasing their availability to plants

The total soil carbon content includes organic carbon and inorganic carbon, out of which the former has greater positive influence on the soil quality. The highest total carbon stock is found in the soils of hot arid, semi-arid and hot sub-humid regions, but the bulk is of inorganic nature mainly as calcium carbonate and hence, the soil quality in these regions is poorer. In general, the content of organic carbon decreases and that of inorganic carbon increases with soil depth.

Among the various factors, the amount and quality of organic carbon are crucial to influence the physical, chemical and biological properties of soil and its productive capacity. Owing to wide variations in climatic condition, topographic situation, vegetative cover, land use and management practices, the quantity and nature of organic carbon in the diverse soils vary widely. The cooler and humid to per-humid climate is most conducive for soils to be enriched with soil organic carbon as in the case of Mollisols of the sub Himalayan region (Bhattacharya and Pal, 2003).

The soil organic carbon status in India has declined owing to heavy losses arising from erosion, deforestation, vegetation removal, excessive grazing, burning of crop residues, use of cow dung a fuel, over ploughing, leaching, other soil disturbances and less addition of organic carbon sources.

The concept of organic farming and organic products is gaining popularity in India. Many farmers are using vermi-compost prepared from the farm wastes as a source of manure. However, the available data reveal that the increasing plant nutrient requirement for producing the targeted quantities of farm produce cannot be met from utilization of all available organic sources alone. Therefore, an alternative feasible option is to use predominantly all locally available organic sources supplemented with the minimum chemical fertilizers to serve dual purpose of (a) maximizing farm produce of high competitive quality with no adverse health hazard in order to harness the export potential on the one hand, and (b) raising the SOC content for better soil quality on the other.

5.4 Soil pollutants

The fast increasing trends of urbanization and industrialization in India are having a pronounced detrimental impact on soil health. Mismanagement of huge quantities of city garbage, sewage sludge, sewage water, industrial effluents, agricultural chemicals etc. is leading to soil contamination. Most of the medium and large industries are located in and around the cities. The highly polluting industries are related to primary metallurgy, paper, pulp and newsprint, pesticides, refineries, fertilizers, paints, dyes, leather tanning, rubber, plastics, cement etc. Very few industries consistently produce treated effluent as per the statutory standards. It s reported that the effluents from these industries are contaminated with Cd, Cr, Cu, Fe, Mn, Pb and Zn. Accumulation of heavy metals beyond the permissible limit adversely affects soil health and in turn, human and animal health (Rattan *et al*., 2002)

High nitrate content, a problem particularly found in leafy green vegetables such as lettuce and spinach, has been linked additionally to reduce protein quality and lower vitamin content of food crops. An increase in the nitrate N content of water has been registered in the heavily fertilized areas and irrigated cropping systems. In Ludhiana, average nitrate N content of shallow wells increased from 0.42 to 2.29 mg l^{-1}. After the analysis of water samples of 144 tube wells serving intensive cropping systems, it was noticed that percentage of samples having more than 5 mg nitrate N per litre was the highest under vegetables cultivation, followed by potato-wheat and rice-wheat systems. There is a wide spread concern in the developed world that groundwater resources are deteriorating in long term, both in quality and quantity.

6. Assessment of soil health

Recent efforts to quantify soil health have resulted in the development of tools to evaluate the impact of management on the soil and environment. The soil quality test kit (Cramer, 1994) and soil health scorecard (USDA-NRCS-SQI, 1999) are two examples of tools that provide users with a means to quickly evaluate soil properties and processes with minimal equipment and expertise. These tools, along with numerous extension-oriented presentations by USDA and university personnel, have increased awareness among producers, conservationists, scientists, and policy makers regarding the importance of soil to agricultural and natural resource sustainability.

The health of a soil can be assessed by Wisconsin Soil Health score card as a function of soil, plant, animal and water properties identified by farmers. It was developed by the Wisconsin Soil Health Programme, Department of Soil Science, University of

Wisconsin, Madison. The card was structured from interviews with 28 farmers in conjunction with the Wisconsin Integrated Cropping System Trial. Farmers who were interviewed operated conventional and low input cash grain and dairy farms typical of southeast Wisconsin. Each parameter is having ratings as 0, 2 and 4. The superscript numbers with parameters indicate the relative importance ad rank of the property. After the collection of data, review the score card and tally the number of indicator properties that reside within the 3 categories of health as: Healthy (score 3-4), Impaired (score 1.5-2.5) and Unhealthy (score 0 -1.0). Divide the number in each health category by the total number of questions answered and multiply by 100% for the percentage within each category.

The score card is field tool to monitor and improve soil health based on field experience and a working knowledge of a soil.

Soil- questions refer primarily to the plow layer

Descriptive properties

1. Earth worms
2. Erosion
3. Tillage ease
4. Soil structure
5. Colour(moist)
6. Compaction
7. Infiltration
8. Drainage
9. Water retention
10. Decomposition
11. Soil fertility
12. Feel
13. Surface crust
14. Surface cover
15. Hardness
16. Smell
17. Soil texture
18. Aeration
19. Biological activity
20. Topsoil depth

Analytical properties

21. Organic matter
22. pH
23. Soil test- N, P and K
24. Micro nutrients

Plants- Questions concern typical years with adequate rainfall and temperature

Descriptive properties

25. Crop appearance
26. Nutrient deficiency
27. Seed germination
28. Growth rate
29. Roots
30. Stems
31. Leaves
32. Resists drought
33. Resists pest and disease
34. Mature crop

Analytical properties

35. Yield
36. Feed value
37. Test weight
38. Cost of production and profit

Animals- Question should not relate to improper housing, poor water or severe weather conditions

Descriptive properties

39. Human health
40. Animal health
41. Wildlife

Analytical properties

42. Chemicals in ground water
43. Surface water

So many other scientists also developed different health score cards by taking into account various parameters which are considered important to the area where they have conducted surveys (Table 1)

There are a number of physical, chemical and biological indicators of soil health. The important physical indicators are aggregate stability, bulk density and soil compaction, chemical indicators are total organic carbon, total N, pH, EC and CEC. The biological indicators are microbial biomass, enzyme activity and soil respiratory rate. The generally used ones are microbial biomass and enzyme activity.

The Palouse and Nezperce Prairies Soil Quality Indicator Card

	Indicator	Preferred ----------> 1 2 3 4 5 6 7 8 9 10	Observations	Rating the Indicator 1	5	10
1	Infiltration			Water ponds or runs off field following moist rains, long wait to get on the field following rain, soil surface crusted.	Water drains slowly with some ponding.	Soil drains well after rain; little or no ponding or runoff following rain; can get into field after rain.
2	Compaction			High resistance to penetration by soil probe, shovel, wire flag, tillage implement, etc., tillage pan present.	Some resistance to penetration by soil probe, wire flag, tillage implement, etc.	Little resistance to penetration by soil probe, shovel, wire flag, tillage implement, etc. No tillage pan present.
3	Tilth and Structure			Soil has a cloddy, powdery, massive or flaky structure with no visible crumbs.	Soil has some crumb structure. Crumbs break under only slight pressure and are fragile after wetting.	Soil is crumbly with a definite crumb structure. Aggregates maintain shape with pressure.
4	Organic Matter			Light colored surface soil, surface color similar to subsoil color. Visible organic material in soil, no smell, soil test shows low organic matter.	Soil surface closer to subsoil color, shows medium organic matter levels on soil test.	Dark colored soil surface; visible organic materials, earthy smell; soil test shows high organic matter. Topsoil defined, darker than subsoil.
5	Plant Residue			No visable residue Residue does not decompose.	Some plant residue slowly decomposing.	Noticeable residue in all stages of decomposit-ion; earthy, sweet smell.
6	Earthworms			0-1 worms in shovelful of top foot of soil. No worm casts or holes. Few insects or fungi.	2-10 worms in shovelful. Few casts, holes or worms. Some insects and fungi.	10+ worms in top foot of soil. Lots of casts and holes in tilled clods. Birds behind tillage. Many insects and fungi.
7	Erosion			Large gullies over 2" deep joined to others, thin or no topsoil, rapid runoff with dark colored water.	Few rills or gullies, gullies up to two inches deep. Some swift runoff, lighter colored water.	No gullies or rills, clear water or no runoff.
8	Seedling Emergence			Slow and uneven emergence.	Some variability in emergence.	Rapid and even emergence.
9	Plant Growth			Uneven color, variable height and population, poor growth, visible evidence of plant stress.	Some variation in color, height and population; moderate growth; mild stress.	Uniform deep-green color, rapid growth, even stand (height and population), no visible signs of stress.
10	Rooting Systems			Few or no roots present; roots short & coarse, not uniformly distributed; roots growing sideways; obvious restrictions.	Roots present in profile; some misshaped roots; some restriction to root growth.	Robust, large, deep, well-dispersed root system; no obvious restriction to root growth; many fine roots.

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7. Management of soil health

Since the initiation of green revolution in late sixties, India has made a remarkable progress in fertilizer nutrient use with the introduction of high yielding varieties of wheat and rice. Crop production under intensified agriculture over the years has resulted in large scale removal of nutrients from the soil, resulting in negative balance and declining soil fertility.

The important approaches to be adopted for improving soil health are balanced nutrition, Site Specific Nutrient Management (SSNM), Integrated Plant Nutrient Supply (IPNS) and Soil Test Crop Response Correlation studies (STCR) etc.

7.1 Balanced Nutrition

Balanced fertilization does not mean a certain definite proportion of nitrogen, phosphorus and potash or other nutrients to be added in the form of fertilizer, but it has to take into account the availability of nutrients already present in the soil, crop requirement and other factors. It should take into account the crop removal of nutrients, the economics of fertilizers, profitability, farmers' ability to invest, agro-techniques, soil moisture regime, weed control, plant protection, seed rate, sowing time, soil salinity, alkalinity, physical environment, microbiological condition of the soil, available nutrient status of soil, cropping sequence, etc. It is not a state but a dynamic concept.

This concept mainly aims to (a) increasing crop yield, (b) increasing crop quality, (c) increasing farm income, (d) correction of inherent soil nutrient deficiencies, (e) maintaining or improving lasting soil fertility, (f) avoiding damage to the environment, and (g) restoring fertility and productivity of the land that has been degraded by wrong and exploitative activities in the past

Balanced use of plant nutrients corrects nutrient deficiency, improves soil fertility, increases nutrient and water use efficiency, enhances crop yields and farmers' income, betters crop and environmental quality. To reap the benefits of balanced use of plant nutrients, it is important to have good quality seed, adequate moisture and better agronomic practices with greater emphasis on timeliness and precision in farm operations.

A generalised equation for balanced fertilization could be taken as:

Balanced fertilization = f (soil type, crop/cropping system, inputs, residual effects, available soil nutrients, yield target, economics of fertilizer use, time etc.)

No single source of plant nutrient, whether it is chemical fertilizer or organic manure or green manure or biofertilizer or crop residue is in a position to meet the growing crop nutrient need. Moreover, the right kind of nutrients required by the crops

may not be achieved from a single source. For example different chemical fertilizers can supply the nutrients like N, P, K, Zn and S; Green manuring can meet a part of nitrogen requirement, one tonne organic manure can add about 12 kg NPK and also some micronutrients; crop residue like rice straw is a good source of potassium and use of biofertilizers can supply nitrogen @ 20-25 kg/ha and mobilize soil phosphorus. This implies that integrated use of plant nutrients is essential mainly for two obvious reasons (i) to increase nutrient supply and (ii) practice balanced fertilization. In addition integrated use of different sources of plant nutrient helps to increase their efficiencies and also crop productivity.

Nitrogen no doubt is the most limiting factor for Indian agriculture, but nitrogen alone is not enough and fertilizer does not mean nitrogen fertilizer only. Lack of this appreciation has led to poor results in most cases. Improving N use efficiency is the major problem for improving economy of its use especially in rice growing areas. Several studies report that vegetables grown with biological sources of nitrogen showed significantly lower excess nitrate than those grown under mineral fertilizers regimes, but caution must be exercised when determining whether one source of fertility is superior to another.

Green manuring with legumes and other means of biological nitrogen fixations such as through Blue Green Algae, Azolla etc can contribute to some of the N needs of rice crop but there are numerous technological, economic and operational problems to their use At the best they can be relied upon for 30 – 60 kg supply under good management The efficiency of use of biofertilizers is more crop specific, location specific and management specific and unless there is a reliable system of quality control and a good system of storage, transportation and management in the field, the expected contribution will not be realized

Cereals remove 20-27 kg N, 8-18 kg P_2O_5 and 20-40 kg K_2O per tonne of grain harvested, amounts being larger for coarse grains (Prasad *et al* , 2004) Chickpea (*Cicer arietinum*), rapeseed-mustard and cotton, remove 39-50 kg N, 6-9 kg P_2O_5 and 30-50 kg K_2O per tonne of grain or seed cotton Thus, as compared to cereals more N and K was removed by pulses and oilseeds per tonne of grain produced For most crops, K was removed in the same amount as N or more than N. The average N:P_2O_5:K_2O removal ratio for the crops studied was approximately 1:0.4:1.

Long term fertility trials revealed the importance of conjunctive use of organic manure with inorganic source for sustaining productivity. For a number of years, the responses to NPK+FYM were not distinctly different from NPK alone, but after some

years, responses to NPK alone were lower than those to NPK+FYM. This indicates that with time, intensive agriculture relying mainly on NPK would accentuate deficiencies of secondary nutrients such as S and micronutrients, mainly Zn and B. The effect of NPK+FYM was comparable or often superior to that of NPK+lime in acid soils, indicating that to a certain extent the benefits of limiting were met by the FYM which also has buffering effect on soil acidity correction (Nambiar and Ghosh, 1984). The results of Long Term Fertilizer Experiments have shown that continuous cropping along with balanced fertilizer application results in increased status of soil organic carbon (Swarup, 2001).

Surekha *et al.* (2004) conducted field experiments on a sandy clay loam soil in Andhra Pradesh, to evaluate the influence of different crop residue management practices on rice yields, nutrient balance and soil health indicators. Treatments include incorporation of 100 and 50% paddy straw in both the seasons. Significant improvement in rice productivity was observed with recycling of 100% straw directly or its ash or straw + GM (*Sesbania aculeata*) over straw removal or 50% straw addition after two crop cycles. Recycling of crop residues substantially improved partial N balance in the system by 123, 33 and 24% with 100% straw + GM, 100 and 50% straw, respectively, over straw removal or burning indicating nutrient addition to soil reserves. Partial K balance was positive with crop residue treatments and negative when straw was removed (-107 kg ha^{-1}). A decrease in bulk density and increase in infiltration rate was observed by crop residue incorporation over control or straw burning. Available K increased significantly with incorporation of straw or its ash (440-519 kg ha^{-1}) over control (377 kg ha^{-1}) with no significant change in available N and P. Soil organic carbon content and soil respiration rate increased significantly in all the crop residue treatments over control with maximum values in straw + GM treatment. Thus, incorporation of paddy straw alone or in combination with green manure improved yields, nutrient balance and soil health under intensive rice mono-cropping conditions.

7.2 Site Specific Nutrient Management

Site-specific nutrient management (SSNM) is an approach to feed the crop with nutrients as and when required. The application and management of nutrients are dynamically adjusted to crop needs of the location and season.

Dobermann and co-workers conducted on-farm experiments at 179 sites in eight key irrigated rice domains of Asia were conducted from 1997 to 1999 to evaluate site-specific nutrient management (SSNM) approach. The eight experimental sites were Philippines, Thailand, Mekong Delta (Vietnam), Indonesia, Old Cauvery Delta (Tamil

Nadu, India), New Cauvery Delta (Tamil Nadu, India), River Red Delta (Vietnam) and Zhejiang, China. Large variation in the initial soil fertility characteristics and indigenous supply of N, P and K was observed among the eight intensive rice domains as well as among farms within each domain. Field- and season-specific NPK applications were calculated by accounting for the indigenous nutrient supply, yield targets and nutrient demand as a function of the interactions between N, P and K. Nitrogen applications were fine-tuned based on season-specific rules and field-specific monitoring of crop N status. The performance of SSNM was tested for four successive rice crops. Average grain yield in the SSNM increased by 0.36 Mg ha^{-1} (7%) compared to the current farmers' fertilizer practice (FFP) measured in the same cropping seasons or 0.54 Mg ha^{-1} (11%) compared to the baseline FFP yield before intervention. Average nutrient uptake under SSNM increased by about 10% in the same seasons or by 13% (N) and 21% (P, K) compared to the baseline data. Yield increases were associated with a 4% decrease in the average N rate, but larger amounts of K fertilizer at sites where the previous K use was low. Average N use efficiency was increased by 30-40%, mainly through the use of improved seasonal N management schemes.

This concept aims to increase farmers' profit through: i) increased yield of rice per unit of applied fertilizer, ii) higher rice yields; and iii) reduced disease and insect damage.

The main features of this method are:

i. Optimal use of existing indigenous nutrient sources such as crop residues and manures.
ii. Application of nitrogen (N), phosphorus (P), and potassium (K) fertilizer is adjusted to the location- and season-specific needs of the crop. It ensures that nutrient is applied at the right time and in amounts needed by the crop. This prevents wastage of fertilizer.
iii. Local recommendation for application of zinc, sulfur, and micronutrients are followed.
iv. Selection of the most economic combinations of available fertilizer sources.
v. Integration with other integrated crop management (ICM) practices such as the use of quality seeds, optimum plant density, integrated pest management, and good water management.

Features

Increased nutrient use efficiency

It provides an approach for "feeding" rice with nutrients as and when they are needed. Current fertilizer recommendations often advise fixed rates and timings for large rice-growing areas. Such recommendations assume the crop need for nutrients is constant from one place to another, one year to the next. But crop growth and crop demand for nutrients are strongly influenced by climate and other growing conditions, which can vary greatly according to location, season and year.

Increased profitability

The major benefit for farmers from improved nutrient management strategies is an increase in the profitability of rice cropping (Dobermann *et al.*, 2003). SSNM eliminates wastage of fertilizer by preventing excessive rates of fertilization and by avoiding fertilization when the crop does not require nutrient inputs. It also ensures that N, P and K are applied in the ratio required by the rice crop.

The principles of SSNM can accommodate a wide range of socio-economic conditions, including situations of labor shortage. Small amounts of additional labor may be required, but labor costs for nutrient management are relatively small compared to those for land preparation, transplanting or harvesting. Efficient N management may also result in off-farm environmental benefits through a reduction of fertilizer N use without a reduction in yield especially in situations where N inputs are very large (e.g., China and Java Island in Indonesia). This may increase profitability, particularly in cases of very high fertilizer N inputs (China, Indonesia). Researchers have compared SSNM with current farmers' fertilizer practices in more than 200 farmers' fields in China, India, Indonesia, Philippines, Thailand, and Vietnam. In most cases, using SSNM increased grain yields and farmers' profit.

The SSNM, as developed for rice across Asia through partnerships within the Irrigated Rice Research Consortium (IRRC), is a plant need-based approach for optimally applying N, P, and K. It provides two equally effective options for dynamic field-specific application of fertilizer N during the growing season, based on leaf colour as determined with a leaf colour chart (LCC). Fertilizer P_2O_5 and K_2O requirements are determined through a plant-based nutrient omission approach, which enables the selection of P_2O_5 and K_2O rates sufficient to overcome deficiencies of these nutrients and also maintain P and K fertility of soil. It is compatible with integrated management of organic and fertilizer nutrient sources. The SSNM approach has been evaluated and promoted through the IRRC

in 8 Asian countries, including India. Activities in India have focused on the rice-rice cropping system in the Cauvery Delta of Tamil Nadu and the rice-wheat cropping systems in Uttaranchal. The use of SSNM by rice farmers at both locations increased yield and profit through improved management of fertilizer N and increased use of fertilizer K to better supply the plant's need for nutrients (Buresh, *et al.*, 2005).

Gill and Rathore (2004) conducted a field experiment in rabi season in Rajasthan, to evaluate nutrient management for maximizing crop yield of wheat. Twelve treatment combinations of N, P, K, Zn, B and S (kg ha^{-1}) were made, with a common control of state recommended dose of fertilizer for wheat in a randomized block design, and with three replication of each treatment. The results of the experiment revealed that application of all nutrients, viz., N, P, K, Zn, B and S increased the grain and straw yield of wheat. Application of treatment T5 ($N_{150}P_{80}K_{40}Zn_{20}B_5S_{40}$) significantly increased parameters such as number of tillers per plant, plant height at harvest, grain yield, straw yield and harvest index. Results of the present investigation reveal significance of site-specific nutrient management in wheat crop for maximizing of yield.

7.3 Integrated Plant Nutrient Supply (IPNS)

Soil fertility decline occurs mainly through intensive cultivation and the inadequate application of replacement nutrients and through deforestation and clearance of vegetation on sandy soils. Large amounts of soil nutrients are also lost to the terrestrial ecosystems through wind and water erosion. In order to maintain the soil condition suitable for crop production, we have to modify the nutritional method in such a manner to better utilize the natural resources along with balanced use of chemical fertilizers and other inputs. We are aware that for increasing the food production to fulfill the food requirements of the burgeoning population of the country, sustainability of agriculture and environmental safety are the priority issues. To avoid wastage of precious national resource and to minimize the environmental damage there is need to develop and demonstrate balanced use of chemical fertilizer. This will not only improve the crop production in sustainable way but also economize the crop production. Higher food production needs higher amount of plant nutrients. As no single source is capable of supplying the required amount of nutrients, integrated use of all sources is a must to supply balanced nutrition to plants.

Integrated plant nutrient supply (IPNS) as well as balanced fertilization is conceptually the same. The IPNS aims at maintenance or adjustment of soil fertility and of plant nutrient supply to an optimum level for sustaining the desired crop productivity through optimization of benefit from all possible sources of plant nutrients in an integrated

manner. Balanced fertilization must be based on the concept of integrated nutrient management for a cropping system as this is the only viable strategy advocating accelerated and enhanced use of fertilizers with matching adoptions of organic manures and bio-fertilizers so that productivity is maintained for a sustainable agriculture.

China has successfully sustained the higher productivity level of wheat-rice for over 100 years by meeting more than 50% of the N requirement through organic sources as against our experience with same cropping system by using mineral fertilizers within 3 decades, which convincingly shows the importance of integrated nutrient management (Ghosh *et al.*, 2004).

The long term fertilizer experiments in different agro-ecological zones of India clearly demonstrate that even the full application of recommended doses of NPK fails to sustain the soil health and crop productivity, but combined use of chemical fertilizers and FYM could produce higher crop yields beside improvement of soil fertility (Swarup, 1998). In dry land areas, application of 2.5t ha of compost can give a significant increase in crop yield.

Jat *et al.* (2004) conducted an investigation on a sandy loam soil to evolve efficient management practices for rice residue recycling in the succeeding wheat crop. The treatments are as follows:

T1: Rice residue incorporated

T2: Residue burnt *in situ*

T3: Residue incorporated + *Trichoderma* + *Aspergillus*

T4: Residue incorporated + N enriched phospho-compost

T5: Residue burnt + N enriched phospho-compost

In the case of rice yield and soil fertility, T4 and T5 were on par (Table 2 and 3). There was increase in bacterial population through incorporation of rice residue, which may be due to increase in heterotrophic phototrophic N- fixing micro organisms. They observed that soil microbial count was higher under rice residue incorporated treatment than treatment with *in situ* burning in rice wheat cropping system (Table 4).

Table 2 - Effect of rice residue management practices on grain yield of wheat

Treatment	Grain yield(tonnes/ha)			
	1999-2000	Increase (%)	2000-01	Increase (%)
T1	2.95	-	3.05	-
T2	4.05	37.2	4.09	34.1
T3	3.96	34.2	4.00	31.1
T4	4.20	42.3	4.28	40.3
T5	4.39	48.8	4.41	44.6
CD(p=0.05)	0.80	-	0.95	-

Table 3 - Effect of rice residue management practices on soil fertility status

Treatments	1999-2000			2000-2001		
	Organic C (%)	Available P (kg/ha)	Available K (kg/ha)	Organic C (%)	Available P (kg/ha)	Available K (kg/ha)
T1	0.74	27.5	89.5	0.78	28.9	92.3
T2	0.76	26.6	81.0	0.79	27.0	83.8
T3	0.76	29.2	75.6	0.83	31.5	79.4
T4	0.81	34.8	92.5	0.82	35.9	93.4
T5	0.82	30.5	78.0	0.84	31.2	79.6
CD(p=0.05)	0.07	2.43	6.21	0.06	2.33	7.20

Table 4 - Effect of rice residue management practices on soil microflora count
(Days after sowing)

Treatments	Azotobacter x 10^2		Fungi x 10^4		Bacteria x 10^6		Actinomycetes x 10^5	
	30	60	30	60	30	60	30	60
T1	30.0	34.5	30.0	41.0	50.0	59.0	20.0	31.0
T2	27.0	32.0	25.5	33.5	40.5	54.0	18.5	28.0
T3	32.0	38.0	40.5	59.0	86.0	90.0	27.5	39.5
T4	34.0	48.5	55.0	64.5	80.5	87.5	42.5	70.0
T5	32.5	39.5	50.0	62.0	74.0	85.0	40.5	66.0
CD(p=0.05)	NS	12.3	13.1	13.8	18.6	16.3	8.9	11.0

A study was conducted during the first crop season to assess the effect of long term application of manures and fertilizers on soil properties, utilization efficiency of nutrients and quality of rice making use of the soil and plant samples taken from the existing permanent manurial trial (dwarf indica) at Regional Agricultural Research Station, Pattambi. The treatments consisted of application of entire quantity of N (90 kg ha^{-1}, as organic alone (cattle manure alone, green manure alone and cattle manure + green manure), inorganic alone (ammonium sulphate alone and NPK fertilizers) and combination of organics with inorganic (cattle manure + NPK fertilizers, green manure + NPK fertilizers and cattle manure + green manure + NPK fertilizers). At different stages of

crop growth significant variation was noticed only in the case of organic carbon content and availability of P in soil due to treatments. Maximum grain and straw yield were recorded by the treatment receiving application of cattle manure alone and the results clearly indicated that application of cattle manure along with inorganic fertilizers is essential to maintain high yields. Significant correlation existed between organic carbon content and available P content of soil with grain yield. The results obtained clearly indicated that application of cattle manure along with inorganic fertilizers or green leaves is essential for maximum utilization efficiency of N applied to the soil (Padmam, 1992).

In Maharashtra, a field experiment was conducted during kharif, to investigate the effect of incorporation of leafy foliage of *Gliricidia sepium* with different levels of NPK on the physico-chemical properties of soil and yield of rice. The different NPK levels include: T1-100:50:50; T2-100:50:00; T3-100:00:50; T4-00:50:50; T5-100:00:00; T6-00:50:00; T7-00:00:50; and T8-00:00:00. Incorporation of Gliricidia leafy foliage at same site for 5 years increased organic carbon, total N, available NPK, and water holding capacity, and reduced bulk density compared to the control. The treatment also recorded significantly higher grain (52.08 q ha^{-1}) and straw (83.28 q ha^{-1}) yield over control (Chaphale and Badole, 1999).

In another experiment in the permanent manurial trial, conducted at RARS Pattambi, Mathew (2003) revealed that application of straw has increased yield in *rabi* to an increase of 7 and 13.5 % continuously for 2 years. In *kharif* season, yield increase was upto 5%. Here they have harvested the straw by retaining two third portions in the field itself. It was also noticed that there is an increase in plant height, shoot dry matter production and spikelets per panicle.

Mahavishnan *et al.* (2004) conducted an experiment to investigate the effects of organic sources with fertilizers on the growth and yield of rice. The treatments were NPK at 7, 100, 125% of recommended dose of fertilizer (120:60:40 kg ha^{-1}), combined with FYM at 10 t ha^{-1}, poultry manure at 5 t ha^{-1}, and glyricidia at 10 t ha^{-1} along with control. The crop growth and yield obtained were higher with 125% RDF + poultry manure and 100 % RDF + poultry manure compared to the other treatments.

The soil application of FYM with 1:1 integration improved soil health as depicted by increase in soil nutrients (organic C and available P and K). The highest leaf N content was also recorded with the treatment with FYM (Sharma *et al.*, 2005).

7.4 Soil Test Crop Response (STCR) approach

The fertilizer recommendations of crops, generalised state level, are based on fertilizer trials conducted in research stations and in farmers' fields. Adoption of this fertilizer recommendation uniformly throughout a region does not ensure economy and efficiency in fertilizer use since variations in soil fertility are not taken into account. It leads to discrepancy in fertilizer use. Scientific and economic fertilizer use must take into account the soil fertility status as well as the crop needs. This has necessitated the formulation of fertilizer dose for crops based on soil tests.

After soil testing, in order to interpret soil test values, peculiarities of both the soil and crop have to be taken into consideration. Different soils with given soil test values for nutrients differ in their capacity to supply nutrients to crops. Crops vary in their nutrient requirements and in their response to added nutrients in different soils. Soil test values should be closely correlated with nutrient uptake by crops and hopefully with the yield for making fertilizer recommendations. There comes the relevance of soil test crop response correlation studies.

Soil test crop response correlation studies based on fertility gradient approach provides a basis for soil test calibration for site specific and situation specific formulation of fertilizer dose. In this approach, soil fertility variations are created in the same field. Actual soil test values for soil available nutrients are then determined in the laboratories and correlated with crop responses to applied nutrients as observed in the field. Accordingly, fertilizer prescription equations can be derived for recommending fertilizer doses for maximum yield, economic yield and specific yield targets of crops. Such soil test based recommendations ensure balanced use of soil and fertilizer nutrients for sustained crop production. The fertilizer prescription equations have to be test verified in farmers' fields before they are recommended for large scale adoption.

The environmental hazards caused by prolonged or heavy rates of mineral fertilization can be easily mitigated by optimizing the fertilizers with judicious application of organics. The complementary use of organics and inorganic helps not only in increasing nutrient use efficiency but also in sustaining high yields of crops. Hence soil test crop response studies are being conducted under integrated plant nutrition system.

This project was started in 1967-68 with 8 centres. Presently, STCR project is functioning with sixteen cooperating centres. The present location of the coordinating cell of the project is at Indian Institute of Soil Science, Bhopal. The project mainly conducts applied and some basic research work on the development of soil test based nutrient

recommendations for crops and cropping systems in different states. The recommendations generated are then tested in the follow up trials at research farms or in a nearby village and also in frontline demonstrations in .villages. The state soil testing laboratories are involved in the conduct of frontline demonstrations on farmers' fields.

Targeted yield concept strikes a balance between fertilizing the crop and the soil. The procedure provides a scientific basis for balanced fertilization and balance between applied nutrients and soil available nutrients. In the targeted yield approach, it is assumed that there is linear relationship between grain yield and nutrient uptake by the crop, as for obtaining a particular yield, a definite amount of nutrients are taken by the plant. Once this requirement is known for a given yield level, the needed fertilizer can be estimated taking into consideration the contribution from soil available nutrients.

Prediction equations for the cropping sequence was prepared by following the methodology outlined by Ramamoorthy and co-workers. It was done by developing post harvest soil test value prediction equations making use of the initial soil test values, applied fertilizer doses and the yields obtained or uptake of nutrients. The post harvest test values were taken as dependent variable and a function of the pre-sowing soil test values and the related parameters like yield/uptake and fertilizer nutrient doses. Prediction equations for post harvest soil test values were developed from initial soil test values, fertilizer doses applied and the yield of crops/uptake of nutrients in order to obtain a basis for prescribing the fertilizer amounts for the crops succeeding the first crop in the sequence

Targeted Yield Equations

Nutrient Requirement in kg per q of grain, NR = $\frac{\text{Total uptake of nutrient (kg)}}{\text{grain yield (q)}}$

Contribution from soil (available)

$$\%CS = \frac{\text{Total uptake in control plots (kg/ha)} \times 100}{\text{soil test values of nutrient in control plots (kg/ha)}}$$

Contribution from fertilizer

CF = Total uptake in treated plots - (soil test values of nutrient in treated plots x CS)

% contribution from fertilizer, CF

$$\%\,CF = \frac{CF \times 100}{\text{(Fertilizer dose (kg/ha)}}$$

Calculation of fertilizer dose

$$= \frac{NR}{\%\,CF} \times 100 \times T - \frac{\%\,CS}{\%\,CF} \times \text{soil test value}$$

= a constant x Yield target (q/ha) – b constant x soil test value (kg/ha)

An example is

FN = 7.8 T – 0.37 SN
FP_2O_5 = 2.8 T – 0.64 SP
FK_2O = 10.6 T – 0.835 SK

For implementing this research methodology, there are three steps:

1. Experiment with gradient crop: for developing a gradient in nutrient status of the soil with an exhaustive crop like maize
2. Test crop experiment – with crop under consideration
3. Test crop verification experiment – to verify, conducting field trials with formulated prescription equations. The results will be verified with the recommendation of SAUs and farmers' practices

A lot of experiments were done in this aspect and researchers developed equations for targeted yields for various crops raised in the locality. Santhi and Natesan (2004) conducted correlation studies on soil test crop response using okra (*Abelmoschus esculentus*) in mixed black calcareous soils (Vertic Ustropept) of Coimbatore, Tamil Nadu, India, and fertilizer prescription under integrated plant nutrition system. In both locations, the highest green fruit yield was recorded with 17 tonnes ha^{-1} IPNS treatment, followed by 17 tonnes ha^{-1} NPK alone, 15 tonnes ha^{-1} IPNS and 15 tonnes ha^{-1} NPK alone. The mean response ratio (RR) for various treatments ranged from 30.7 kg kg^{-1} in farmers' practice to 66.2 kg kg^{-1} in 15 tonnes ha^{-1} IPNS treatment. The mean benefit cost ratio ranged from 19.6 in farmers' practice to 30.8 in 15 tonnes ha^{-1} IPNS and the trend of the results was the same as that of RR.

The results of soil test crop response field experiments conducted by Srinivas *et al* (2001) in Warangal, Andhra Pradesh, during kharif and rabi, indicated that there existed a significant correlation between mean variables of yield and nutrient uptake of rice with soil and fertilizer variables in Vertisols. A fertilizer ready reckoner indicating fertilizer doses at varying soil test values for attaining maximum yield, economic yield and desired rate of return for rice grown in that soil also worked out.

A study was conducted by Swadija *et al.* (1998) in the Instructional Farm at the College of Agriculture, Vellayani, Kerala, to establish soil test-crop response correlation for soil test based fertilizer recommendation for cassava cv. M4 in a laterite soil. Calibrated multiple regression models indicated yield predictability of >70% using soil test values and applied nutrients as independent variables. But optimization of fertilizer

dose for maximum and economic tuber yield at varying soil test values could be done for the nutrient nitrogen only. In Andhra Pradesh, Reddy *et al.* (1999) formulated soil test based fertilizer prescription for summer groundnut grown in alluvial soils of Nellore. Meena *et al.* (2001) conducted soil test crop response calibration studies on onion (*Allium cepa*) in Alfisols.

8. Policy decisions to be taken

Some policy decisions should be taken in such a way that farmers and researchers are supposed to move together hand in hand to improve soil health. The researchers have to standardize the approaches for maintaining the healthy conditions of the soil on a long term basis.

The concept of balanced fertilization must go beyond N, P and K. The soil testing service should include the component of secondary and micronutrients status of soils. The required amount of these nutrients may be recommended with suitable fertilizers.

The maximum efforts should be made to educate farmers to practice balanced use of fertilizers for higher crop yields in a sustainable way. The campaign programme may be organized for popularizing the relevance of this approach as well as the ill-effects of present day practices.

It is necessary to improve the quality of soil testing service with respect to reliability of analysis report, maintenance of laboratories, availability of trained staff and timely availability of results etc. The faith among the farmers about the soil testing service should be regained. The necessary steps have to be taken so that the results should be given in time. For this, trained technical staffs should be appointed with sufficient updated equipment support. The soil testing should be a service with continuous support and monitoring.

The soil testing laboratory persons can maintain soil health cards for each sample or farmer. In this the results of the same sample need to be recorded year after year. With this we can assess the degree of variation in the soil properties through the years.

Important indicators of soil quality in relation to soil organic carbon content are mean weight diameter of aggregates, available water capacity, CEC, bulk density, aeration porosity, and biodiversity. The relative importance of these indicators varies among different soils and therefore, site specific information is needed for quantitative assessment of soil quality. To collect and develop a record of information on this regard, steps should be made from the side of administrative authority.

The general $N:P_2O_5:K_2O$ ratios of 4:2:1 frequently used in India needs re-examination. A more desirable approach is the supply of adequate amount of primary, secondary and main nutrients on the basis of soil test crop response data. Since fertilizer is a costly input and also involves drain on foreign exchange, it is suggested that all efforts be made to meet part plant nutrient needs through green manuring, incorporation of crop residues and bio-fertilizers

9. Future line of research

In view of the emerging trends in modern agriculture there is a need for optimum utilization of fertilizer for the quality of environment and economic use of fertilizers. Future research should include generation of data base on nutrient status of soil, their available pools and the extent of deficiencies in major soil types and agro-ecosystems.

Research efforts should be make to develop a soil quality index based on easily measurable soil properties, which can be utilized as an indicator of soil health because presently adopted methods are very cumbersome and also not suitable to farmers. The novel technology should be easy and based on visual observation than the analytical results. We have to compare the health of a closed ecosystem (eg: forest system), where there only a small portion is lost as output, with that of a barren land. The health condition of such closed system can be taken as healthy and we can estimate the status of other land as percentage of this healthy condition.

Research on the effect of changing the cropping system and associated management practices on soil health needs to be monitored regularly.

Role of integrated plant nutrient supply system on soil health needs to be assessed. The impact of IPNS should be monitored properly and the positive research results should be exposed at the earliest to the farming community. The extent of influence of various organic sources on neutralizing the degraded condition should be identified and the better sources need to be popularized.

Soil fertility and soil health map of the country should be developed with high precision using GIS technology and the recommendation to the farmers on input management should be made based on this information

Crop residues remove a large chunk of nutrients from soil. Efforts need to be made to develop techniques for crop residue recycling in different cropping systems including tillage options, nutrient supplementation and moisture availability.

Conclusion

The actual time has come; the farmers, researchers and other related communities should come forward and act in the process of managing and maintaining soil health. Chemical fertilizers should be used judiciously and manures have to be used in conjunction with chemical fertilizers for improving the crop yield and soil productivity in a sustainable way. Many more activities are being planned to promote the balanced use of fertilizers. It is hoped that all these efforts would lead to desired awareness and as a result, 'healthy soil' would become a reality in the near future.

The task of conserving soil health for sustained agricultural production is two fold: (i) to manage healthy soils without any further degradation through adoption of sound agricultural practices including optimization in the use of water, fertilizers and pesticides, and (ii) to improve the problem soils by alleviating soil limitations for crop production by adoption of suitable technologies like integrated plant nutrient supply and balanced fertilizer application. Any method suggested for maintaining good soil health with sustained fertility and productivity should be cost effective, eco-friendly and scientifically sound for food security, environmental safety and human welfare. The vigorous and faithful accomplishment of this primary goal by the agriculturists especially soil scientists will be the best service to the country.

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DISCUSSION SESSION

1. Whether organic farming practices can improve soil health? What will be its impact on crop production?

✓ Organic farming practices will improve the soil health definitely, but it will take much time. The productivity of the crop will be reduced to a drastic range when wee adopt organic farming at present system. Since the soil is deteriorated to that extent, this will affect the food grain production. So it is better to adopt IPNS and then slowly to organic farming when soil attains enough production capacity.

2. What may be reason for the excessive use of the nitrogenous fertilizers by the farmers?

✓ The high response nature of the nitrogenous fertilizers attracted the farmers. Since vegetative growth is increased to notable range, they started applying in bulk quantity. Moreover low price of the fertilizers compared to others also encouraged them.

3. The data on fertilizer consumption in India shows that the nitrogenous fertilizers are used in excessive quantity for the last 20 years. But Indian soils are deficient in N. Why?

✓ The use efficiency of N in Indian soils is less. Moreover, the applied quantity is either used by the crop and or lost form the soil by leaching, nitrification, volatilization etc.

4. How much time will be taken to assess the health of a soil?

✓ A period of 4-5 days is necessary to assess the soil quality. First we have to do survey in the field. Visual parameters will be obtained on that day itself. Then analytical parameters are to be assessed. The interpretation of the rating should be done

5. How does excessive use of nitrogenous fertilizers result in accelerated depletion of other nutrients?

✓ When nitrogenous fertilizers are applied in excessive amount, the vegetative growth of the plant will be higher. Hence the nutrient absorbing rate by the plants will also be higher.

6. What is the role of soil testing laboratories for improving soil health?

✓ The soil testing laboratories can do a campaign for the soil health improvement programme. By improving the efficiency of the labs, the relevance of soil testing

can be popularized. Then only people will come forward for seeking help from the STL.

7. Whether the assessment procedure is possible in the farmers' field?

✓ The present system of assessment is a little bit laborious process. It needs technical skill. An easy and more convenient method has to be developed.

8. How soil health is affected by the excessive N contamination in the water?

✓ If the water is contaminated with excessive N, the algal growth will be higher. That will result in the nutrient loss and leads to disease incidence.

9. What are the methods to be adopted for the assessment of soil nutrient status in the field?

✓ Leaf Colour Chart (LCC) and SPAD meter can be used for the assessment of soil nutrient status.

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KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara

Ag. Chem. 751 **Seminar**

Topic: SOIL HEALTH IN VIEW OF SUSTAINABLE FERTILITY

Student: K. Sajnanath (2006-21-111)
Time: 10.15 a.m., 04-05-2007

Venue: Audio Visual Laboratory,
Dept. of Pomology & Floriculture

Abstract

Soil health is defined as capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environment quality and promote plant and animal health (Doran and Parkin, 1994). It mainly depends on intrinsic properties, external features and human interventions. Intensive cultivation with the use of high analysis fertilizers for high yielding varieties in attaining maximum production resulted in the deficient conditions of organic matter, secondary and micronutrients in the soil (Yadav, 2003). Excessive use of macronutrients, especially nitrogen, leads to imbalance of nutrients in the soil ecosystem, which also causes unfavourable microclimate for microorganisms. The NPK ratios were 6:2.4:1 in 1990-91 and 7:2.7:1 in 2000-01 against favourable ratio of 4:2:1 adopted in India (Ghosh *et al.* 2004).

The results of long term fertilizer experiments have shown that continuous cropping along with balanced fertilizer application leads to an increased status of soil organic carbon (Swarup, 2001). Padmam (1992) observed that application of cattle manure along with inorganic fertilizers is essential to maintain high yields. Jat *et al.* (2004) reported that soil microbial count was higher under rice residue incorporated treatment than treatment with *in situ* burning in rice-wheat cropping system.

The health of a soil can be assessed by Wisconsin soil health score card. There are a number of physical, chemical and biological indicators of soil health. The task of conserving soil health for sustained agricultural production is two fold: (i) to manage healthy soils without any further degradation through adoption of sound agricultural practices, and (ii) to improve the problem soils by alleviating soil limitations for crop production by adoption of suitable technologies like integrated plant nutrient supply and balanced fertilizer application.

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ENVIRONMENTAL IMPACTS OF CHEMICAL FERTILIZERS

BY

K. SAJNANATH
(2006-21-111)
Ph. D Scholar
Dept. of Soil Science and Agricultural Chemistry

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

Ag. Chem. 752 - Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala
2007

DECLARATION

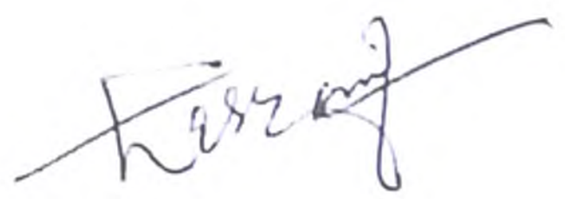
I hereby declare the seminar report on "**Environmental impacts of chemical fertilizers**" is a record of the seminar presented by me during the course and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara,
08.08.2007

K. Sajnanath

CERTIFICATE

Certified that this seminar report, entitled "**Environmental impacts of chemical fertilizers**" is a record of seminar presented by **K. Sajnanath** under my guidance and that this has been prepared by him independently.



Vellanikkara,
08.08.2007

Dr. M. A. Hassan
Major Advisor

CONTENTS

ENVIRONMENTAL IMPACTS OF CHEMICAL FERTILIZERS

1. Introduction

We are familiar with the two terms namely; manures and fertilizers since they are the suppliers of nutritional inputs to plants. Both these terms were using synonymously in earlier days. By usage we differentiated them into two classes of inputs i.e. one is of organic in nature and other is of chemical in nature. So the nutrient supplier which are of organic in nature are called as organic manures. Those inputs that are synthesised and chemical in nature are called as chemical (inorganic) fertilizers or mineral fertilizers.

The principles of nutrition is developed in 18^{th} century. Till that some of the mateiral in the nature were using as manure even without knowing the chemical theory behind the same. After the scientific developments, the relevance of the nutritional inputs were noticed by various workers. The scientific community started giving fertilizer recommendations to various crops after considering the physiological need as well as the increase in food production. The mineral input could led to a better results due to its high concentration of nutrients, which made them acceptable by the farming community within a short period.

In India, the use of mineral fertilizers was started during the late 18^{th} century. For the first 40 years, after the initiation of single super phosphate manufacturing, these were used mainly for plantation crops. Undoubtedly, India is proud of its 'Green revolution' since it enabled to attain a four fold increase in food production in the last 50 years. But this achievement has been followed by indiscriminative use of chemical fertilizers that led to a decline in crop production and detrimental impacts on environment.

To achieve leadership in agriculture sector, the emerging areas that need to be given urgent consideration are environmental engineering and pollution control. For this to happen, all the scientists working in the various fields of agriculture will have to work in tandem so that full potential of scientific advances is realized through better food productivity, better quality and high yield potential

With a view of giving an emphasis on the impacts of non-judicious use of fertilizers on our environment, an attempt has been made to compile the information. Thus the seminar was formulated and the information embodied are featured in different subtitles namely; (i) history and relevance, (ii) fate of fertilizer applied, (iii) impacts on soil, water and air, (iv) management aspects, (v) policy decision to be taken, (vi) future line of research needed and (vii) conclusion.

2. Historical perspective and recent trends

The art of agriculture in India dates back to pre-historic times. The use of dung as manure appears to have been practiced since *Rigvedic age* (2500-1500 B.C.). The value of green manure appears to have been known in periods as far as back as 1000 B.C. The practice of application of bones dates back to 300 B.C., while the value of excreta of goat as manure was recognized before 500 A.D. The use of oil cakes appears to have come in this country from 1000-1400 A.D.

Little was known to abut the principle of fertilization till Liebig in 1840 propounded hid theory of mineral nutrition. The first single super phosphate manufacturing unit was set up in the country in 1906 at Ranipet in Tamil Nadu. For the first 40 years or so the use fertilizers were confined mainly for plantation crops. After green revolution, usage of fertilizers were increased to a larger extend.

In India, fertilizer consumption increased from less than 50,000 tonnes in 1950 to 15 million tones in 2000 and the food grain production increased from 50 mt to 200 mt in the same period. indicating a direct relationship between the fertilizer use and yield increase. There is a strong relationship between fertilizer consumption and food grain production in the country over years (Pathak *et al.*, 2004).

The consumption of fertilizer nutrients in the country increased drastically with corresponding increase in food grain production till early nineties. Nevertheless, here exists a wide gap between demand for fertilizers and their production that has necessitated their import leading to increase of fertilizer prices. Thus, fertilizers constitute one of the most effective and costlier inputs in increasing crop production and their rationalized use needs no emphasis.

There are also wide differences in the consumption ratio of three major nutrients N : P_2O_5 : K_2O in different regions, crops and cropping systems. These differences also got magnified and showed aberrations due to adhoc changes in pricing policy of fertilizers during the recent years.

Even then, there was a decline in production and productivity of the crops by mid 1980s (Table 1). Then it is clearly evident that mere the application of mineral fertilizers will not increase the crop production but its use efficiency is also taken into consideration.

Table 1 – Compound growth rates (% per year) of production and productivity

Crop	Production		Productivity	
	1981- 1990	1991 - 2000	1981- 1990	1991 - 2000
Rice	3.55	1.74	3.47	0.92
Wheat	3.57	3.27	3.10	2.21
Total cereals	3.03	1.86	2.90	1.36
Total pulses	1.52	-0.04	1.61	0.55
Total food grains	2.85	1.66	2.74	1.28

3. Relevance

The increase in food grain production is mainly attributed to the higher fertilizer use. Over the years, the fertilizer consumption in the country has increased to about 16 m t in 2005 (Table 2). The steep hike in fertilizer consumption during the period 1965-84 was mainly attributed the introduction of high yielding varieties and bringing more area under cultivation (Table 3)

Table 2 - Consumption of fertilizers in India

Years	Consumption (M tonnes)	Consumption (kg/ha)
1970-71	2.18	13.13
1980-81	5.52	31.83
1990-91	12.54	67.49
2000-01	16.71	93.32
2001-02	17.54	101.02
2005-06	16.02	106.00

Table 3 - All India area under cultivation and production of food grains

Years	Area (M ha)	Production (M t)
1950-51	97.32	50.82
1960-61	115.58	82.02
1970-71	124.32	108.42
1980-81	126.67	129.59
1990-91	127.84	176.39
2000-01	121.05	196.81
2002-03	111.5	174.19
2004-05	-	204.6
2006-07	-	220.0

The per hectare consumption of NPK increased from the meager 0.6 kg in 1950 to more than 106 kg by 2006 (Tiwari, 2007). Indian agriculture has made significant progress

in the area of food production. It was about 50 m t in 1950 and now reached upto 220 mt in 2006. The projected data fro the year 2025 is about 300 Mt of food grain production to feed our population. Since the available cultivable land remains constant, we require raise productivity to a higher level. This might be possible by the application of modern technologies using good seeds, irrigation and suitable plant protection measures with sufficient application of fertilizers.

While the N, P and K fertilizer use has declined in developed countries since 1985, it has continued to increase in developing world at linear rates. In 1960, developed countries accounted for 88% of the world fertilizer consumption. By 2001 their share had fallen to 37% and developing countries accounted for 63% (IFA, 2002).

The fertilizers, which are the most valuable inputs used, affect the environments due to its indiscriminative usage. This also influences the cost benefit ratio of the cultivation. The use efficiency of the inputs has to be improved by adopting various management practices that are scientifically proved. This is highly essential to develop a sustainable production system.

To highlight the importance of the above matter, we should to know the ill-effects of the mineral fertilizers in the various ecosystems. The residual effects in the soil contribute a little to the total. The main loss is due to leaching to other systems namely aquatic and gaseous systems. The ground water gets polluted with nitrates and air with various gases. This will finally affect the human population in the form of diseases as well as other environmental impacts.

4. Fate of fertilizers

It is highly necessary to have knowledge on the fate of various fertilizers when they applied in the soil. With respect to N, the available forms of N are NO_3^- and NH_4^+. The nitrogenous fertilizers applied will be converted to either of the above forms finally. But the nitrate forms are more preferred by most of the crops. This form is susceptible to leaching more than that in the case of ammoniacal form because ammoniacal form can be adsorbed on the soil clay particles. The sources of N in the soil and their mode of losses are depicted in the figure 1.

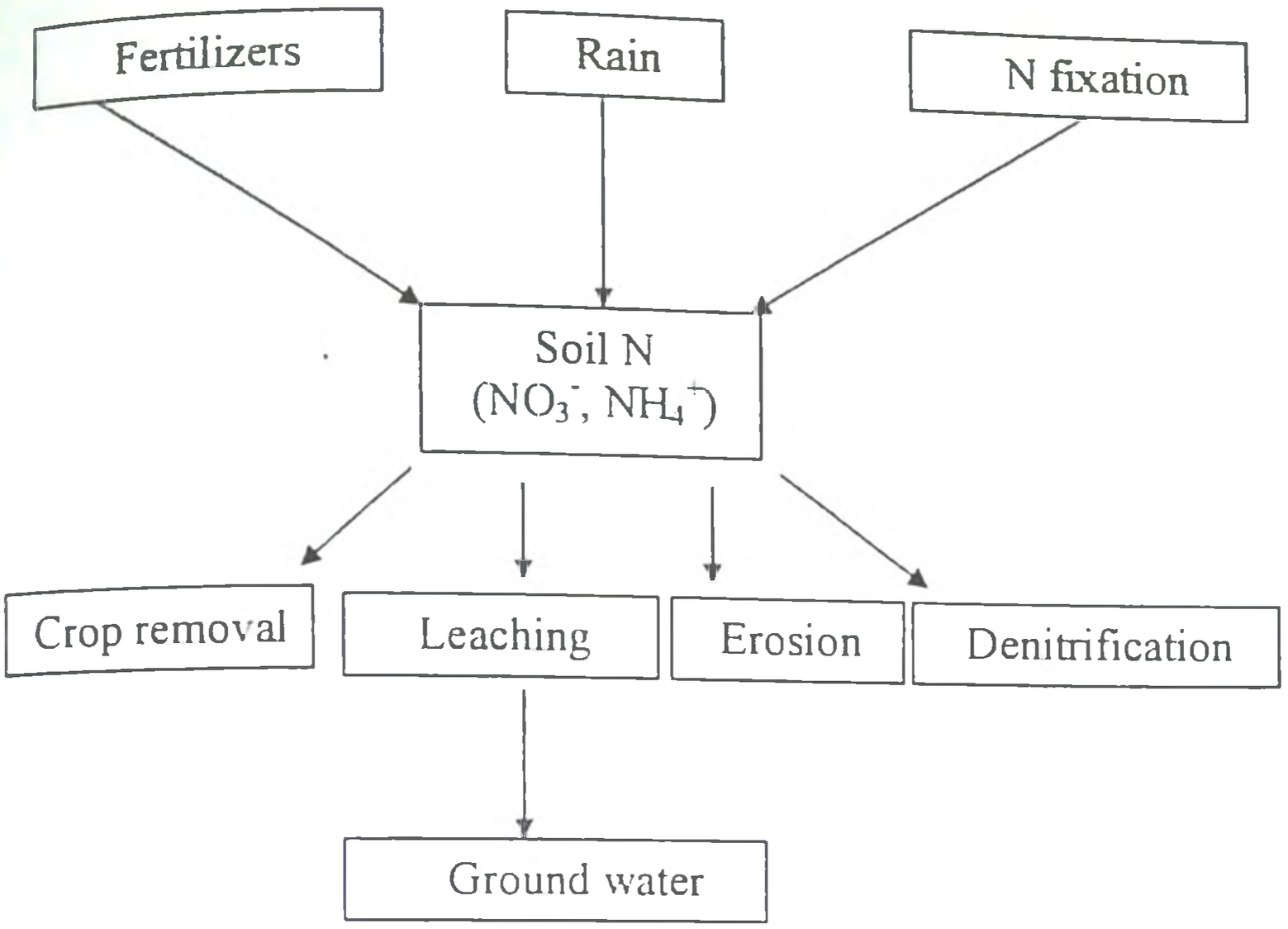


Fig. 1 – Sources and changes of N in the environment

With regard to phosphatic fertilizers, it is highly lost or become unavailable through erosion and fixing in soil. The available forms of P in the soil are $H_2PO_4^-$ and HPO_4^{2-}. Due to water erosion, soil has been removed and deposited in the banks or basins of the rivers, lakes or other water bodies. In acidic soil conditions, phosphorus will be fixed as iron and aluminium phosphates.

As far as K ions are considered, its available form is K^+. Since it is imported fertilizers, its usage is limited in the agricultural field.

5. Impact of fertilizers on environment

In recent years agricultural chemicals have become the focus of investigations into their contribution to non-point source pollution of surface and ground waters. In terms of plant mineral nutrients and the fertilizers used to supply them, efforts have focused on ntrogen and phosphorus as potential contaminants of surface and ground waters. The effect was also noticed in soil and atmospheric environments.

5.1 Impact on soil environment

The losses of the chemical fertilizers from the soil occurred mainly by leaching, run off, volatilization, fixing or erosion. The adverse effects of these chemical inputs on soil environments are nutrient mining, soil degradation, heavy metal contamination, etc.

The results of Long Term Fertilizer Experiments have shown that the continuous cropping with fertilizer application alone leads to a decrease in soil health (Nambiar, 2002). It also shows that the availability of nutrients and the uptake of the same were less in the treatments with chemical fertilizers alone. The contaminations of heavy metals and chloride to some extend are some of the problems other than those of ill-effects on soil organisms.

5.1.1 Nutrient mining

The use of high analysis fertilizers resulted in the increased food production. So the farming community started applying these inputs in large quantities especially those of macronutrients. The increased biomass production was also a byproduct of the absorption of secondary as well as micronutrients from the soil. This was not known to them. Thus a reduction in the secondary and micronutrients occurred in soil. It was finally reflected as un-proportional crop production with fertilizers applied. The exhausted condition of organic matter also reflected in the results.

The table given below shows the potential nutrient removal of various crops to produce one tonne of yield. It varies with crop husbandry and its growth characters.

Table 4 - Average nutrient removal per tonne yield production by diverse crop under field condition

Crop	Produce	N	P_2O_5	K_2O
		Kg per tonne of produce		
Rice	Paddy	20.1	11.2	30.0
Wheat	Grain	24.5	8.6	32.8
Groundnut	Nut	58.1	19.6	30.1
Sugarcane	Cane	1.7	0.2	2.0
Cassava	Tuber	7.8	1.2	5.1
Grapes	Fruit	4.9	1.5	5.9
Banana	Fruit	8.2	3.0	32.3
Coconut	1000 nuts	7.1	3.5	10.7

For the last 25 years, the Indian soils have been experiencing on an average s net negative balance @ 8-10 Mt of nutrient per annum (fig 2) (Tiwari, 2002.)

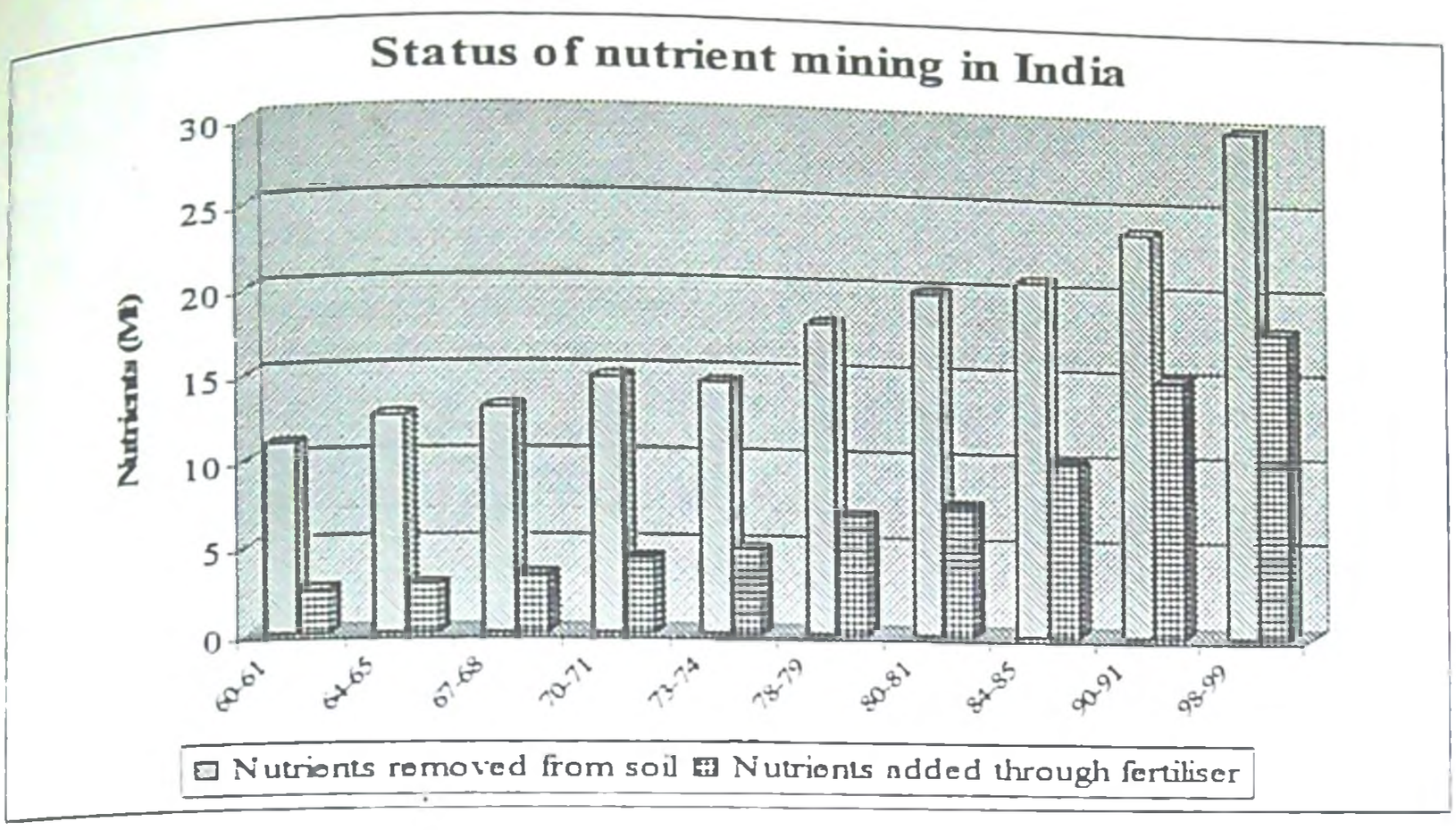


Fig. 2 – Status of nutrient mining in India

5.1.2 Soil degradation

Continuous cultivation of the same crop, combined with intensive use of chemical fertilizers, can cause significant reduction in soil nutrient status and organic content. In a study, rice and wheat in India, the growth in productivity had slowed considerably over time where fertilizer use was above recommended levels (Yadav *et al.*, 1998). This study, along with several other studies of long-term yield declines in agrochemical intensive cropping systems highlight how these systems can, over time, affect soil properties and the ability of crops to utilize applied nutrients.

5.1.2.1 Soil physical properties

The important physical properties of soil which are affected by the continuous application of chemical fertilizers for a long period of time are bulk density, aggregate stability, hydraulic conductivity, water retention characteristics etc. This was assessed by the AICRP on Long Term Fertilizer Experiments.

The history of long term manorial experiments in India dates back to 1885 with the establishment of first permanent manorial experiment at Kanpur (Uttar Pradesh). The results of PMT revealed the effect of fertilizers on soil physical properties. With the objectives to study the effect of continuous use of plant nutrients, single and in combination, in organic and inorganic forms including secondary and micronutrients on crop production and soil health under high input soil management technology, ICAR sponsored AICRP on LTFE in selected soil types in 1970 (Swarup, 2001).

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From the results of LTFE, it was seen that there is slight decrease in the bulk density of soils having treatment of mineral fertilizers with organic manure than those having treatment with chemical fertilizers alone (fig. 3).

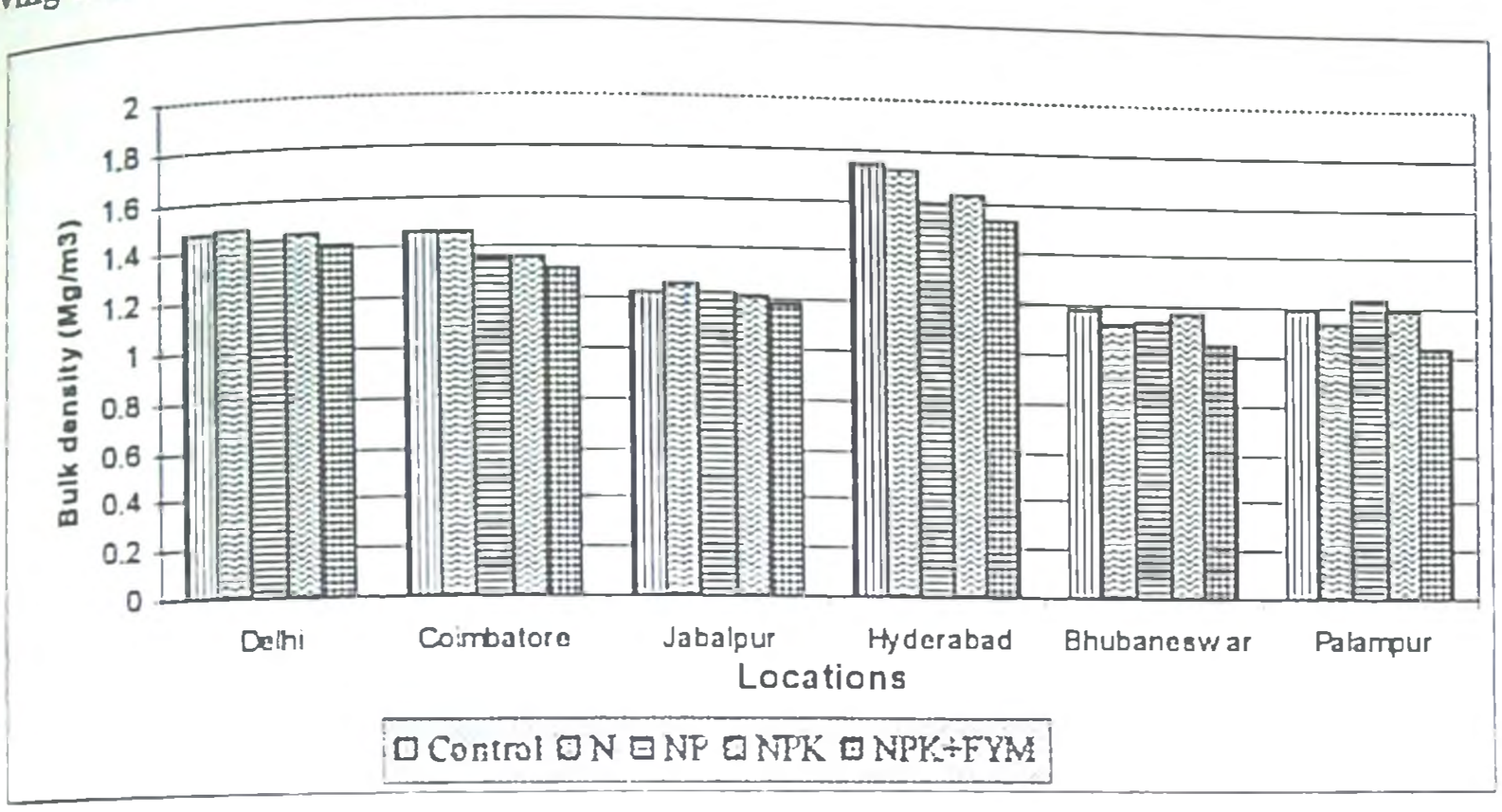


Fig. 3 - Effect of long term manuring and cropping on bulk density (g/cm3) in 15 years

It was also seen that there is an increase in the aggregate stability of soils having treatment of mineral fertilizers with organic manure than those having treatment with chemical fertilizers alone (fig. 4) in most of the locations.

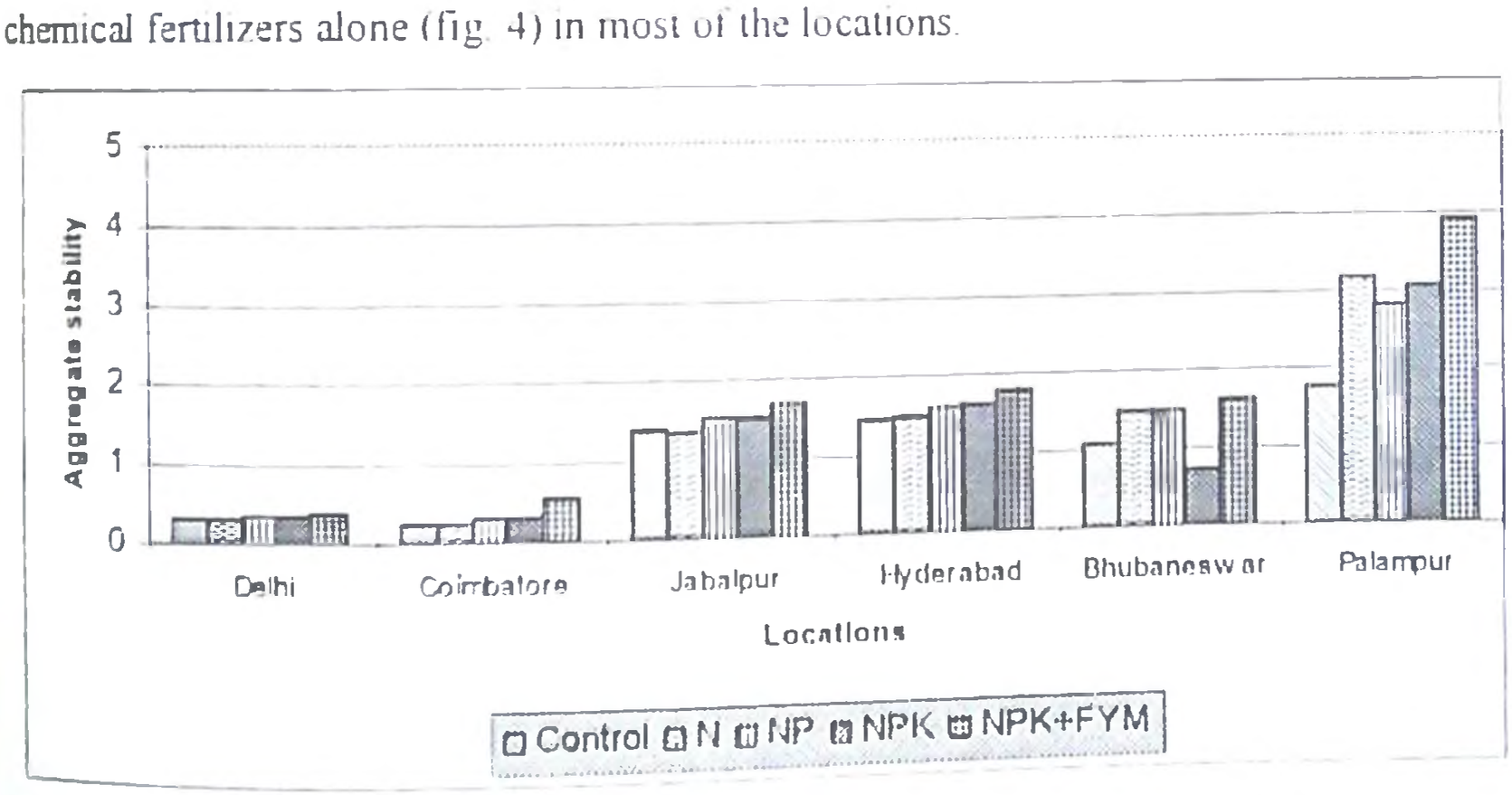


Fig. 4 - Effect of long term manuring and cropping on aggregate stability in 15 years

The results of LTFE also showed an increase in the water retention characteristics of soils having treatment of mineral fertilizers with organic manure than those having treatment with chemical fertilizers alone (Table 5) in most of the locations

Table 5 - Effect of long term manuring and cropping on water retention (%) characteristics in 10 years

Location	Treatments				
	Control	N	NP	NPK	NPK+FYM
Delhi [0.3]	19.9	20.1	20.5	20.6	21.8
Delhi [15.0]	7.7	7.8	7.8	8.0	8.2
Jabalpur [0.3]	34.9	35.3	35.7	36.0	37.3
Jabalpur [15.0]	20.8	20.8	20.9	20.8	21.1
Bhubaneswar [0.3]	9.1	9.9	1.2	10.2	12.0
Bhubaneswar [15]	2.7	3.0	3.0	3.1	3.2
Palampur [0.3]	25.3	24.6	25.0	26.9	27.8
Palampur [15.0]	15.5	15.3	15.6	15.9	15.3

It was also seen that there is an increase in the hydraulic conductivity of soils having treatment of mineral fertilizers with organic manure than those having treatment with chemical fertilizers alone (Table 6) in most of the locations

Table 6 - Effect of long term manuring and cropping on hydraulic conductivity (cm/hr) in 10 years

Location	Treatments				
	Control	N	NP	NPK	NPK+FYM
Delhi	0.66	0.67	0.68	0.69	0.70
Coimbatore	0.088	0.095	0.095	0.095	0.106
Jabalpur	0.126	0.116	0.125	0.124	0.133
Bhubaneswar	3.090	3.030	3.020	3.020	3.095

5.1.2.2 Soil chemical properties

The use of heavy dose of inorganic fertilizers has not played havoc with the soil health but reduced the fertilizer efficiency of crops. Narayanan (2005) reported that the availability of nutrients were decreased or not improved in the fields where the fertilizers alone were applied (Table 7)

Table 7 - Effect of long term manuring and cropping on nutrient availability

Years	NPK + OM		NPK alone	
	Org. C (%)	Available P (kg/ha)	Org. C (%)	Available P (kg/ha)
1993	0.38	12.1	0.38	12.1
1994	0.4	12.6	0.36	12
1995	0.46	14.5	0.35	12.9
1996	0.52	15	0.38	12

Mukherjee (2003) observed in rice field after harvest, the physical properties such as porosity have not improved in the treatments with chemical fertilizers alone (Table 8)

Table 8 - Effect of chemical fertilizers on soil characters

Treatment	Soil fertility status					
	Pore space (%)	Total N (%)	Org. C (%)	Avail. N (kg/ha)	Avail. P (kg/ha)	Avail. K (kg/ha)
Control	36	0.06	0.7	241	16	295
NPK alone	36.3	0.069	0.71	252	18.1	301
NPK + FYM	41	0.071	0.81	266	18.5	320

Another important effect is the depletion of humus from soil. Addition of organic manures is necessary to improves water movement in fine textured soils by formation of larger water stable aggregates and to reduces the water movement in coarse textured soil through reduction in non-capillary porosity

5.1.3 Heavy metal contamination

Heavy metals are those metals having density > 5g/cc or atomic number more than 20. There are two types of heavy metals; Elements essential to life (micronutrients; Fe, Cu, Zn, Ni) and elements non -essential to life (As, Cd, Cr, Hg, Pb)

Depending on the material, there are some inherent impurities in conventional fertilizer materials. The base material for making phosphorus and potassium fertilizers are naturally formed mineral materials, which typically are mined from either below ground or surface deposits. Of these two macronutrients, concerns of heavy metals are generally associated with phosphorus. Other plant nutrient or soil conditioning additions are derived from mined mineral deposits are also associated with agricultural lime being a familiar example. Additionally, several of the essential mineral elements often provided as either granular or liquid fertilizers are considered heavy metals- eg: copper, zinc, and iron. Historically the issue that has received the most attention is cadmium in phosphorus fertilizers (FAI, 2006). A recent study from Australia reports a wide variability in cadmium content of potato tubers grown on commercial farms, however, tuber cadmium could not be related to soil cadmium content

To address the issue of cadmium loading, nine countries have either mandatory or voluntary restrictions on the cadmium content allowed in phosphorus fertilizers. Canada has long had regulations on the amount of impurity loading allowed with fertilizers use. Developing these limits involves determining the amount of a heavy metal contaminant naturally occurring in soil and then restricting the amount that can be added as impurities to focus on preventing a build up over time (Table 9).

Table 9 - The content of heavy metals in fertilizers available in India

Source	Content of heavy metals in fertilizers in mg/kg				
	Cd	Cr	Cu	Pb	Zn
Ammonium nitrate	1.1	2.5	3.6	5.4	11.7
Super phosphate	16.6	15.0	22.6	20.6	244.0
NPK fertilizers	4.9	54.3	8.3	3.2	97.5

Palaniappan (1995) reported the micronutrient contents of as heavy metals in various rock phosphates generally used in India (Table 10).

Table 10 - The content of heavy metals in rockphosphates available in India

Source	Micronutrients in ppm			
	Fe	Mn	Zn	Cu
Mussoorie	4	0.2	100	115
Jhamarkota	2	5	200	125
Purulia	14	0.7	350	140
Udaipur	0.6	0.2	75	85
Kasipatanam	9	0.1	100	190

He also highlighted the heavy metal contaminations in the imported rock phosphates which are having low use in the country (Table 11).

Table 11 - The content of heavy metals in imported rock phosphates

Source	Heavy metals in ppm					
	Cd	Hg	Pb	Ni	Zn	As
Morocco	18	0.04	2	30	270	10
Florida	5	0.09	12	13	80	5
Tunisia	34	0.03	2	16	290	2

Mismanagement of huge quantities of city garbage, sewage sludge, sewage water, industrial effluents, agricultural chemicals etc is leading to soil contamination. Most of the medium and large industries are located in and around the cities. The highly polluting industries are related to primary metallurgy, paper, pulp and newsprint, pesticides, refineries, fertilizers, paints, dyes, leather tanning, rubber, plastics, cement etc. Very few industries consistently produce treated effluent as per the statutory standards. It s reported that the effluents from these industries are contaminated with Cd, Cr, Cu, Fe, Mn, Pb and Zn (Table 12). Accumulation of heavy metals beyond the permissible limit adversely affects soil health and in turn, human and animal health (Rattan *et al*., 2002).

Table 12 - The content of heavy metals in sewage – sludge

Source	Content of heavy metals in sewage – sludge (mg/kg)				
Sewage/sludge	Cd	Cr	Cu	Pb	Zn
	20.0	500.0	250.0	700.0	3000.0

5.1.4 Chloride contamination

Accumulation of Cl in the soil leads to increase in osmotic pressure and low water availability. It also increases pH and EC. There is a reduction in nitrification rate by reducing microbial activity. A level of NaCl of higher than 0.25mg/g is enough to reduce the microbial activity in soil. It competes with nitrate in nutrient uptake process

5.1.5 Effect on soil organisms

Excessive use of macronutrients, especially nitrogen, leads to imbalance of nutrients in the soil ecosystem, which also causes unfavourable microclimate for microorganisms.

The count of microorganism obtained in the various experiments was lowest when NPK alone was applied. The nodulation of soyabean in an experiment having fertilizers alone and with FYM, conducted in Ranchi, was at its peak with NPK+FYM. Treatment with N alone adversely affected the growth of azotobacters and other microbes.

5.2 Impact on aquatic environment

In discussing non-point impacts of agricultural practice, recognition must be given to water management in the system. Nutrients are taken up by plant roots only when they are dissolved in the soil water (soil solution). Water in an agricultural system (irrigation or rainfall) that results in movement of nutrients below the root zone or off of a management area as runoff plays a highly significant role in controlling environmental impacts of nutrients.

Historically, P has been understood to have very low solubility in soil systems. From a geochemical perspective, there is a very narrow range of soil pH where P is not tied up in low solubility complexes with iron and aluminum (pH 6.5). Due to this low solubility, a common practice is to add P fertilizers in amounts in excess of plant removal to increase the amount of plantavailable P.

The impact of phosphorus as a non-point source contaminant has been an issue in agriculture for nearly 30 years in all over the world. There has been a resurgence of attention to this issue due to a number of factors. The driving force has been the development of large confined-animal-feeding operations leading to land application of

manures that have resulted in excessive P application and resultant issues related to nutrient runoff and fresh water quality. From a crop production perspective, the resurgence in interest relates to new discoveries in terms of phosphorus soil chemistry, which have shifted our understanding of phosphorus mobility in soil.

5.2.1 Nitrate contamination

There has been shift in the ratio of N, P and K fertilizers in use. Since 1960, world N consumption has increased much faster than that of P and K. The ratio between N, P and K has changed from 1:0.91:0.86 in 1930 to 1:0.18:0.22 in 2000. This suggests that in recent decades, farmers have tended to rely primarily on nitrogen fertilizers to maximize crop yields, rather than targeting optimal achievable yields determined by local agronomic, economic and environmental conditions (Table 13). The preference of N fertilizers by farmers is because of relatively low cost per unit of nutrient, easy availability, and the quick yield response of new varieties to N. Farmers also view N inputs as a risk reducing factor because of its influence on growth and yield, in particular when their financial resources are limited (Tandon, 1996).

Table 13 - The pattern of N fertilizer consumption in last 50 years

Years	Consumption ('000 tonnes)
1951-56	408.10
1961-66	2088.90
1971-76	8301.30
1981-86	21417.90
1991-96	38733.70
2000-2001	41870.70

The NPK ratio for India has changed from 6:2.4:1 in 1990-91 to 7:2.7:1 in 2000-01 against favourable ratio of 4:2:1 adopted (Ghosh *et al.*, 2004). There is also divergence in ratios in different regions. In 1990-91, the ratio was 8.5:3.1:1 in northern states and 4.2:1.8:1.0 in southern states. Such divergence in new ratio is also due to the differences in the quality of land, inherent soil fertility, cropping systems and degree of exploitive agriculture.

Non-point groundwater contamination with nitrate is an issue in most agricultural production systems. The nitrate can come from soil organic matter, crop residues or added organic materials, nitrogen fertilizers, or microorganisms that convert N_2 gas into soluble nitrogen (e.g. Rhizobia sp. on legumes). Conversion of organic or ammonium nitrogen to

nitrate is a microbial process that is well documented and highly effective in all but highly acidic pH.

An increase in the nitrate N content of water has been registered in the heavily fertilized areas and irrigated cropping systems. In Ludhiana, average nitrate N content of shallow wells increased from 0.42 to 2.29 mg l^{-1}. After the analysis of water samples of 144 tube wells serving intensive cropping systems, it was noticed that percentage of samples having more than 5 mg nitrate N per litre was the highest under vegetables cultivation followed by potato-wheat and rice-wheat systems. There is a wide spread concern in the developed world that groundwater resources are deteriorating in long term, both in quality and quantity.

Nitrate pollution in groundwater is mainly occurred in areas with light textured soil, high rainfall or intensive irrigation. It was reported that a few areas (Table 14) in Maharashtra, Karnataka and Rajasthan were having nitrate contaminated ground water (Palaniappan, 1995). The maximum permissible limit for nitrate (mg/l) in groundwater as per FAO is 10, but in India we have taken it as 45.

Table 14 - The percentage of samples having NO_3 > 45mg/l in statewise

State	Percentage of samples having NO_3 > 45mg/l	State	Percentage of samples having NO_3 > 45mg/l
Andra Pradesh	18	MP	20
Bihar	26	Maharashtra	47
Gujarat	18	Orissa	10
Haryana	22	Rajasthan	42
Karnataka	49	Tamil Nadu	11

The content of nitrate in the samples of Tamil Nadu as per a survey conducted by Ramachandran *et al.* (1993) is comparatively low in most of the cases (Table 15).

Table 15 - The content NO_3 of samples in location wise

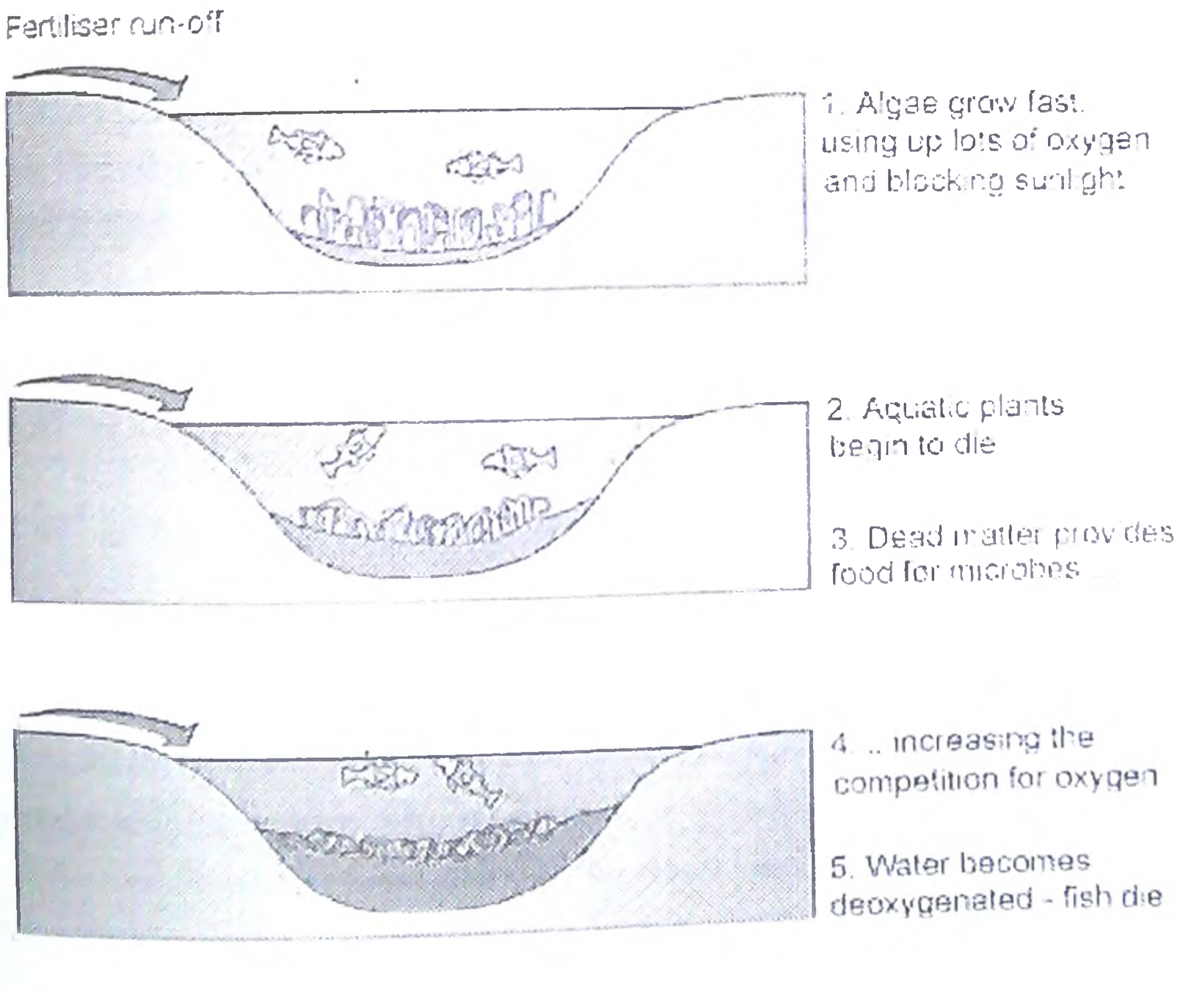
Location	NO3 level (mg/l)	Location	NO3 level (mg/l)
Bengrampel	0.577	Peruduvakkam	0.440
Komakambedu (DW)	0.487	Kanniputhur	0.385
Komakambedu (BW)	0.577	Periyapalayam	0.450
Thamarapakkam	0.239	Puduvayal	0.128
Karanur	0.538		

5.2.2 Methaemoglobininemia

The excess content of nitrate in drinking water causes Metheamoglobineamia (blue baby syndrome) in infants and ruminant livestocks. It is mainly due to the delay in absorption of nitrate in the body that results. in conversion of of NO_3 to NO_2 by the action of *Coliform* and *Clostridia sp.* The NO_2 prevents heamoglobin from carrying O_2.

5.2.3 Eutrophication

Another adverse effect of excessive nitrate level in water is the eutrophication. Lakes with 0.3 ppm NO_3 may support algal bloom. this results in recreational uses will be diminished, phytoplankton and fishes succumb and offensive smell and taste. The content of P is the limiting factor here. By loss of applied P fertilizer due to soil erosion, the water bodies will be getting enough P. If there is excessive nitrate, P will have an additive effect in algal growth. Excessive growth of algae can be controlled by use of less soluble P fertilizers.



Phosphogypsum is a by-product of production of phosphoric acid from rock phosphate. It is having CaSO4 with small amount of P, F, Si, Fe, Al. In the leachate from phosphogypsum, F content is higher than the EPA limit of 1.5 mg/l of F in drinking water. In Indian phosphogypsum, content is 5-40g F/kg, so harmful only when it is applied in higher rate.

5.3 Impact on atmospheric environment

It is mainly due to emission of methane gas and nitrous oxide contamination in the air by various activities.

5.3.1 Nitrous oxide emission

Nitrous oxide constitutes only 350 ppb of the atmosphere. The major source of this gas is combustion of fossil fuels. The content is 1.5 Tg of N_2O. It was reported that the concentration of N_2O has been rising at an increasing rate since about 1960. In 1990, increasing rate was 0.5% per year. The nitrous oxide is harmful to ozone layer in the atmosphere that protects the gaseous system from infrared rays.

Fertilizer constitutes 18% of the total emission. The fraction of fertilizer lost to the atmosphere as N_2O for various types of N fertilizers are: 0.01-0.5% for nitrates, 0.04-1.71% for NH_4NO_3, 0.02 – 1.5% for other NH_4 fertilizers, 0.86-6.84% for anhydrous NH_3 and 0.01-6.84% for other N fertilizers (Chanda, 2006).

5.3.2 Methane emission

Methane is the second important green house gas which is contributing 5-20% of global warming. It can trap about 32 times more heat than a molecule of CO_2 can. Natural sources are wetlands, lakes, etc. It is reported that Asian countries are contributing a good amount from their rice fields. The emission from India on an average is 2.7-6.4 Tg/yr (Roy, 2004).

Addition of NO_3 to water logged soil might suppress the methane emission by acting as terminal e- acceptor in absence of O2 for anaerobic respiration (Ponnamperuma, 1972). Addition of SO_4 reduces the methane emission because sulphate get reduced first (Jakobsen *et al*., 1981).

In an experiment conducted at KAU, showed that application of urea resulted in higher methane emission than factamphos (Mathew, 2003).

6. Management strategies

The issues of both nitrate and phosphorus as potential non-point pollutants are related to the movement of these elements to areas where the plant is no longer able to use

them. There are a number of alternative crop and fertilizer management strategies that offer approaches to reduce potential non-point pollution.

To meet crop production needs while reducing potential negative environmental impacts, nitrogen applications timing can be manipulated. Rather than a single bulk pre-plant application, applications can also be made in season to coincide with periods of high plant nutrient demand. In a combination rainfed/irrigated potato production system, split applications have been shown to increase N use efficiency, reduce nitrate leaching, and maintain yield. The effectiveness of this technique to increase crop nutrient-use efficacy has been demonstrated and enabled growers to reduce total nitrogen inputs.

A new approach for nutrient application strategies is site specific crop management (SSCM)-more commonly referred to as precision agriculture. With this crop production technique, inputs are applied where they are needed in the amount needed and when they are needed. As the producer's control of water decreases, so decreases the effectiveness of SSCM as a tool for reducing non-point movement of plant nutrients. Systems have been developed to control water, and hence liquid fertilizer, on a site specific basis. Thus, using a site specific approach of pre-plant and in-season N applications provides a system to reduce application rates in areas (either within or between fields) and phenological periods associated with higher environmental risk.

A developing country like India is concerned, in agriculture the role of fertilizer becomes inevitable. Since the status of Indian soil are low in most of the nutrients. In a survey and soil testing conducted followed in India, clearly shows the above fact. In that survey, samples from 228 districts fall under low, 118 in mediun and 18 are in high ranges as far as N is concerned. For P, it was 190, 184 and 17 respectively. Regarding K, it was 47, 192 and 122 respectively. In the case of S, deficiencies are scattered in 100-120 districts.

6.1 Nitrogen fertilizers

The loss of n can be reduced to an extent by adjusting agronomic management practices. The N loss depends on type of fertilizer used, soil conditions and management practices adopted. The ammoniacal fertilizers are less susceptible to leaching than nitrate since it can be adsorbed on clay particles. But in acidic conditions, the loss of ammoniacal fertilizers by volatilization is more.

The potential approach for managing nitrogen movement beyond the root zone is the use of slow-release materials. Slow-release fertilizer technology is not new. However, recent developments with polymer-coated fertilizers offer slow-release materials that are

primarily temperature dependent in their release rates and are more predictable than conventional slow release materials such as sulfur-coated urea. By altering the type of polymer coating, nitrogen release rate can be theoretically adjusted to plant demand based on soil temperature. If historical temperatures and nitrogen uptake characteristics of the crop are known, then the correct mixture of materials can be designed and applied for optimum nitrogen release.

Polymer-coated urea fertilizer seems to offer some promise in reducing nitrate losses for potato production. Recent work in Minnesota on coarse-textured irrigated soils has shown improvements in potato yield and quality with polymer-coated urea compared to the same rates applied as conventional urea. In these studies optimum N rate was 30% to 40% lower with coated urea compared to soluble urea. Implicitly, this indicates a higher uptake efficiency of nitrogen with the coated urea and a concomitant decrease in nutrient loss from the root zone. The primary disadvantage of slow release materials is the cost, which can be four to eight times the cost of urea. In addition, in years where leaching of nitrogen is not significant, response to slow-release fertilizers is often negligible or not observed. Unless some incentives are provided or nitrogen use becomes regulated, adoption of slow release fertilizers by potato producers will depend on whether the price can be reduced to be competitive with conventional fertilizers. However, despite the price, these materials do offer an appealing alternative to areas where high risk of environmental contamination might otherwise prevent production.

Use of nitrification inhibitors can be practiced, eg. N- serve (nitrapyrine) 2-chloro-6(trichloromethyl) pyridine and AM (2-amino--4-chloro-6-methylpyrimidine). It acts only when conditions suitable for unwanted conversion to NO_3 coincide with the effective period of the inhibitor

6.2 Phosphatic fertilizers

Phosphate solubilizing microorganisms (PSMs) are considered to play a significant role in making available soil phosphates for the growth of plants. PSMs solubilize mineral phosphates by bringing about a drop in the soil pH of the surrounding either by proton extrusion or by the secretion of mono, di and tricarboxylic acid. Eg. *Citrobactor koseri*, *Bacillus coagulans* etc. (Panda and Hota, 2007). Phosphate loss from the soil can be controlled by reducing soil erosion by establishing a vegetative cover.

7. Policy decisions to be taken

Since the dawn of the civilization, governments of all kinds have enforced standards of quality, environment, safety and health. Such standards have been formulated

by both political governments as well as non-political bodies i.e.; professional societies, industry associations, independent laboratories, organizations etc (Shukla *et al.*, 1999). These programmes have enabled human society to mankind some kind of regulation for the well-being of mankind.

Some policy decisions should be taken in such a way that farmers and researchers are supposed to move together hand in hand to improve environmental quality. The researchers have to standardize the approaches for maintaining the healthy conditions of the soil, water bodies and air on a long term basis.

The maximum efforts should be made to educate farmers to practice balanced use of fertilizers for higher crop yields in a sustainable way. The concept of balanced fertilization must go beyond N, P and K. The soil testing service should include the component of secondary and micronutrients status of soils. The required amount of these nutrients may be recommended with suitable fertilizers. The campaign programme may be organized for popularizing the relevance of this approach as well as the ill-effects of present day practices.

The general $N:P_2O_5:K_2O$ ratios of 4:2:1 frequently used in India needs re-examination. A more desirable approach is the supply of adequate amount of primary, secondary and main nutrients on the basis of soil test crop response data. Since fertilizer is a costly input and also involves drain on foreign exchange, it is suggested that all efforts be made to meet part plant nutrient needs through green manuring, incorporation of crop residues and bio-fertilizers by adopting integrated plant nutrient supply system.

8. Future line of research

In view of the emerging trends in modern agriculture there is a need for optimum utilization of fertilizer for the quality of environment and economic use of fertilizers. Future research should include generation of data base on nutrient status of soil, their available pools and the extent of deficiencies in major soil types and agro-ecosystems.

For the application of chemical fertilizers to be environmentally sustainable, there must be no deleterious effects on the ecosystem. Environmental sustainability can be equated with long-term and large-scale economic sustainability – for example, the application of agrochemicals should not affect future productivity of downstream fisheries, jeopardize drinking water sources, or cause degradation of lands that will impact regional climate.

Role of integrated plant nutrient supply system on environmental health needs to be assessed. The impact of IPNS should be monitored properly and the positive research

results should be exposed at the earliest to the farming community. The extent of influence of various organic sources on neutralizing the degraded condition should be identified and the better sources need to be popularized.

Conclusion

The practices of application of mineral fertilizers can't be avoided by the farming community since its pivotal role is essential. We can say that the actual time has come; the farmers, researchers and other related communities should come forward and act in the process of managing agricultural practices and maintaining environmental quality. Chemical fertilizers should be used judiciously and manures have to be used in conjunction with chemical fertilizers for improving the crop yield and soil productivity in a sustainable way. Many more activities are being planned to promote the balanced use of fertilizers and adopt integrated nutrient supply system. It is hoped that all these efforts would lead to desired awareness and as a result, 'healthy environment' would become a reality in the near future.

The task of conserving environmental quality with sustained agricultural production is of two fold: (i) to manage healthy environment without any further degradation through adoption of sound agricultural practices, and (ii) to improve the agricultural practices by adopting suitable technologies like integrated plant nutrient supply system and balanced fertilizer application. Any method suggested for maintaining good environment with sustained fertility and productivity should be cost effective, eco-friendly and scientifically sound for food security and human welfare. The vigorous and faithful accomplishment of this primary goal by the agriculturists especially soil scientists will be the best service to the country. This is high time to think and take necessary steps in this matter, with remembering the words of our nation's founder Prime Minister, Pandit Jawaharlal Nehru, "Everything else can wait, but not agriculture".

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DISCUSSION SESSION

1. Whether the data on LTFE results are on a same crop?

✓ The LTFE was conducted in 6 centres in the initial stages. Then it is increased to 4 more centres. The experiments are conducted in rice crops in various parts of the country and different soil types.

2. Which are the research stations where LTFE is going on there in Kerala?

✓ Regional Agricultural Research Stations at Pattambi and Kayamkulam.

3. The mining of nutrients are mainly depending on plant factor or soil?

✓ It depends on both soil and plant characters. If the crop can give good response to N, the corresponding loss of other nutrients also will be taken place.

4. Why is methaemoglobinemia called as 'blue baby syndrome'?

✓ Since the haemoglobin in the blood is contaminated with nitrite, it can't carry oxygen. The blood becomes blue colour.

5. How can we control Cd content in organic manures?

✓ It can be controlled by growing plants which can absorb large quantity of cd in the bodies.

6. Soil conditions may also affect the uptake of nutrients?

✓ In the alkaline conditions most of the nutrients are unavailable. In slight acidic or neutral conditions, the nutrients are available to the crops.

7. Are we using the Purulia rockphosphate in Kerala?

✓ No. Here we are not using it.

8. Can we increase usage of fertilizers for increasing food grain production?

✓ We can increase it by increasing nutrient use efficiency by adopting IPNS technologies. We have to consider the conditions of the soil also.

9. Whether the fertilizer application is harmful to earthworms?

✓ In an experiment conducted at KAU, showed that superphosphate can be increased to enrich the nutrient status of vermiwash.

10. Can we go for organic farming due to reduce the fertilizer impacts?

✓ Organic farming practices will improve the environmental health definitely, but it will take much time. The productivity of the crop will be reduced to a drastic range when wee adopt organic farming at present system. Since the soil is deteriorated to that extent, this will affect the food grain production. So it is better to adopt IPNS and then slowly to organic farming when soil attains enough production capacity.

11. What are the modifications to be adopted in the present fertilizer recommendation?

✓ The present recommended dose of fertilizers itself can be applied without reducing it. But nutrient use efficiency also is taken into consideration. The accepted ratio of 4:2:1 can be maintained.

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KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara

Ag. Chem. 752 Seminar

Topic: ENVIRONMENTAL IMPACTS OF CHEMICAL FERTILIZERS

Student: Sajnanath K. (2006-21-111)
Time: 11.15 a.m., 07-07-2007

Venue: Conference Hall,
College Library

Abstract

The use of mineral fertilizers in India started during the late 18th century. For the first 40 years, after the initiation of single super phosphate manufacturing, these were used mainly for plantation crops. Undoubtedly, India is proud of its 'Green revolution' since it enabled to attain a four fold increase in food production in the last 50 years. But this achievement has been followed by indiscriminative use of chemical fertilizers that led to a decline in crop production and detrimental impacts on environment. The present consumption of fertilizer in India is 106 kg NPK per hectare area (Chanda, 2006).

The losses of the chemical fertilizers from the soil occurred by leaching, run off, volatilization, fixing or erosion. The adverse effects of these chemical inputs on soil environments are nutrient mining, soil degradation, heavy metal contamination, etc. The results of Long Term Fertilizer Experiments have shown that the continuous cropping with fertilizer application alone leads to a decrease in soil health (Nambiar, 2002). It also shows that the availability of nutrients and the uptake of the same were less in the treatments with chemical fertilizers alone. The excessive use of nitrogenous fertilizers resulted in the leaching of nitrate to the ground water and other water bodies (Palaniappan, 1995). Eutrophication and methaemoglobinaemia are the resultant effects of the contaminated water bodies. The application of phosphatic fertilizers leads to contamination of soil with heavy metals. Mathew (2003) reported that the use of urea favours methane emission more than that of factamphos in rice fields. The nitrous oxide content in the atmosphere increases annually by 0.5% (Roy, 2004).

The task of conserving environmental quality with sustained agricultural production is of two fold. (i) to manage healthy environment without any further degradation through adoption of sound agricultural practices, and (ii) to improve the agricultural practices by adopting suitable technologies like integrated plant nutrient supply system and balanced fertilizer application (Tiwari, 2007).

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EARTHWORMS AND PEDOGENESIS

By

C.J.BINDHU
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Ph. D Scholar
Soil Science & Agricultural chemistry

SEMINAR REPORT
Submitted in partial fulfillment for
the requirement of the course

Ag.Chem.751-Seminar

DEPARTMENT OF SOIL SCIENCE & AGRL.CHEMISTRY
COLLEGE OF HORTICULTURE
KEARALA AGRICULTURAL UNIVERSITY
VELLANIKKARA-680 656
THRISSUR

DECLARATION

I, C.J.Bindhu (2006-21-112) hereby declare that this seminar report entitled "**EARTHWORMS AND PEDOGENESIS**" has been prepared by me, after going through the various references cited at the end of the report and has not been borrowed from any of my fellow students.

Vellanikkara
02.06.07

C.J.Bindhu
(2006-21-112)

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CERTIFICATE

Certified that the seminar report entitled "**EARTHWORMS AND PEDOGENESIS**" for the course Ag.Chem.751 has been prepared by C.J.Bindhu (2006-21-112), after going through the various references cited here under my guidance and supervision and she has not borrowed from any of her fellow students.

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1. INTRODUCTION

Soil is an integral part of ecosystem, which is the basic unit of ecology. The interest in soil as a natural body originated from its ability to produce and sustain crops. Soil is effectively a non-renewable resource and it takes a great deal of time, i.e. thousands or even millions of years to develop. The organisms in an area will contribute to soil formation by facilitating the disintegration process as they live and by adding organic matter as they die. In the dynamics of litter layer and the eventual incorporation in to deeper horizons lay the principal regulating mechanisms that form and maintain soil profiles. The disintegration, decomposition and incorporation of litter result from a combination of dissolution by percolating rainwater, a minor component of atmospheric oxidation, but most importantly from the activities of the decomposers. Earthworms are important contributors to the decomposer industry; they particularly affect the disintegration and incorporation in to underlying soil horizons of forest litter. The mixing of soil horizons by earthworms results in a deepening of the humified zone of the soil profile. Deliberate or accidental introduction of surface-feeding or near surface feeding, burrowing earthworms in to soils that lack them, but have no inherent limitations for earthworms, may result in dramatic changes in pedogenesis. The external abiotic parameters and the poor nutritive resources of the soil system appear to be the controlling factors for earthworm population. In the tropical regions, humus feeders or burrowing earthworm species dominate over the organic matter feeding or composting worms. The pigmentation, quick response to stimuli and high regeneration capacity enable their survival in unstable environment.

2. Pedogenesis

Soils are formed as a result of weathering of rocks ad minerals. It is the disintegration and decomposition of rocks and minerals by physical and chemical processes. Weathering includes two processes; geochemical weathering or geogenesis and pedochemical weathering or pedogenesis. Geochemical weathering is the physical and chemical breakdown of rocks and minerals in to simpler molecules and will lead to the formation of parent material. Pedochemical weathering is the chemical decomposition of parent material to form the product, soil. The evolution of true soil from parent material is through the combined action of soil forming factors and processes. Weathering is a

destructive process whereas pedogenesis is a constructive process, which is the process of soil formation that will lead to the formation of a soil profile. The two stages of weathering and soil formation merge in to each other and no sharp boundary can be drawn between them (Sehgal, 2005). The soil forming processes which lad to the formation; transformation and rearrangement of soil materials in a soil body leave their imprint on the different genetic soil horizons, which constitute the soil morphology.

Soil production is a very slow process, which will take about 4000 years to develop 100 mm of soil. The soil production rate is high in valley than in hills due to the additional water collected as run off.

Physical and chemical changes of soil formation

The basic processes involved in soil formation, according to Simonson (1959), include:

- Gains or additions
- Losses
- Transfers
- Transformations

Pedogenic addition of mineral materials, there will be discontinuities and buried surfaces that are considered as soil horizons. The potential effect of this addition of mineral material on soil genesis appears to be equally important in both submersed and terrestrial environments. The presence of calcium carbonate and organic fragments are biogenic and are added to the profile as a result of in situ organisms.

Pedogenic losses or removals of surface materials occur mainly through the processes of leaching, seepage, erosion and organic matter decomposition. Thus, continuous pedogenic addition and loss of organic matter is responsible for the development of surface layers with relatively low but stable organic carbon levels.

Pedogenic transfers are mainly by eluviation, diffusion and bioturbation. Diffusion occurs when dissolved ions move from zone of higher concentration to zone of lower concentration leading to a build up of the substances in a given part of the soil. The process of diffusion alone would be of very limited magnitude in the formation of soil horizon. But, bioturbation will work together with diffusion process in promoting horizon differentiation. In terrestrial systems, bioturbation occurs by the activity of soil organisms such as earthworms, ants and termites.

Pedogenic transformations are conceptualized as changes in either the organic or mineral fractions. The organic transformations can be documented by the use of C: N ratio. Decomposition of organic residues in soils will result in a lowering of C: N ratios. So, the lowering of C: N ratio is an indicator of the transformation of fresh organic matter to other humic substances (Demas and Rabenhorst, 1998).

Transformation processes:

The fundamental processes of transformation are,

1. Podzolization: Chelated Fe, Al and organic matter that are eluviated from upper horizons will get illuviated or accumulated in the lower horizons like B horizon (Gupta and Tripathi, 1992).

2. Desilication or Laterization: Also called as Ferrilization, is the loss of Si, leaving the Fe oxides at the surface. It occurs at high temperature and rainfall and will lead to the formation of plinthite and iron stone (Siever, 1962).

3. Pedoturbation: It is the biological or physical churning and cycling of soil materials and may even evert soil layers and form discontinuous horizons.

Horizons (layers) result from soil formation

The different soil layers in a soil profile and their characteristics are given below.

Horizon	*Characteristics*
O	Organic (litter)
A	Mineral soil high in organic matter
E	Eluviated (leached, loss of clay)
B	Accumulation (Fe, Al oxides, clay)
C	Fractured parent material

Soil development:

The basic unit of a soil is a pedon. Soil development refers to the development of pedon.

Pedon is the smallest volume that can be recognized as a soil individual.

Pedogenesis and soil fertility result from a combination of abiotic factors including

i. the composition of the parent material
ii. physical and chemical weathering
iii. temperature regimes

iv. hydrology

v. landscape stability and

Biotic factors including

i. Uptake by plants of water and nutrients

ii. Photosynthesis

iii. Litter fall

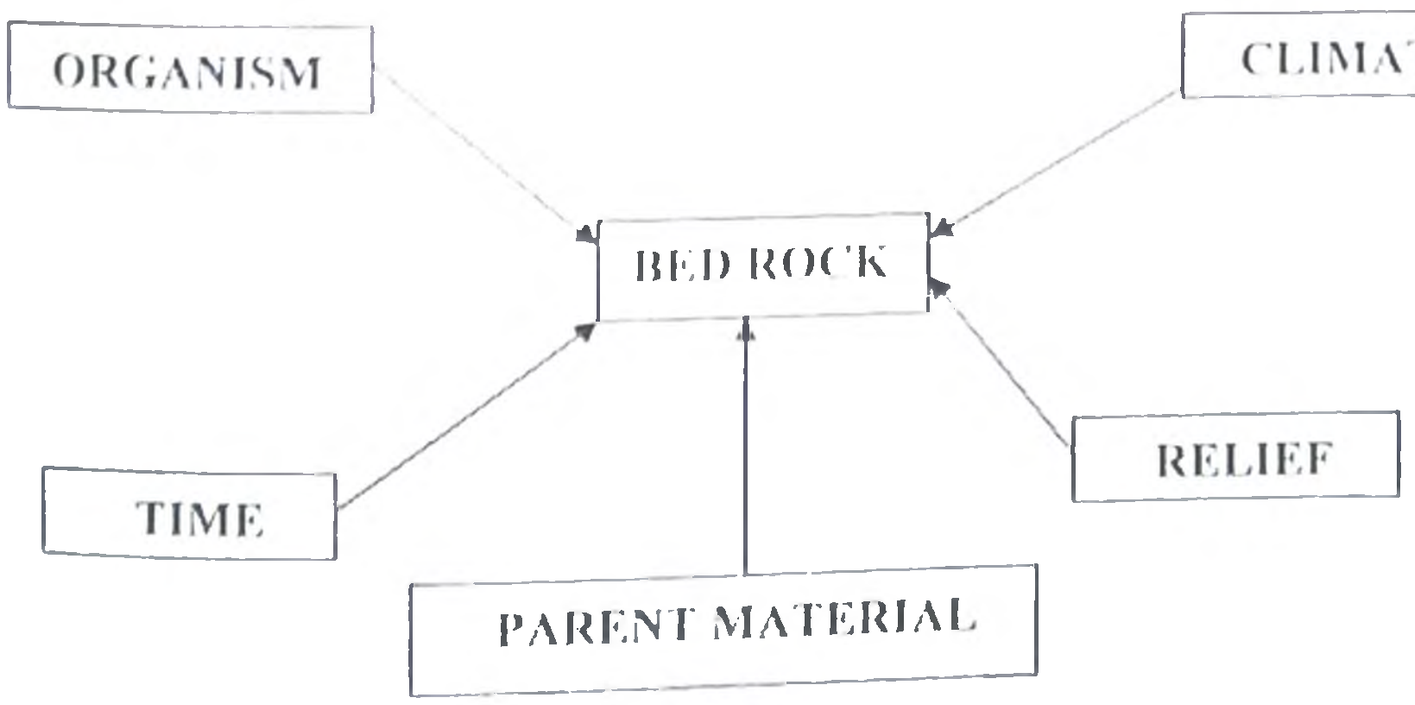
iv. Decomposition of litter and dead root material.

Darwin (1881) recognized that earthworms were particularly significant contributors to the biotic components of soil processes, and his initial findings and observations have been confirmed and amplified by many subsequent workers.

Factors of soil formation

The five soil forming factors, acting simultaneously at any point on the surface of the earth, to produce soil. The soil forming factors are grouped into two broad categories, viz. passive and active factors. The passive soil forming factors are those, which represent the source of soil-forming mass and conditions, affecting it. These provide a base on which the active soil forming factors work or act and develop different soils. Such factors are parent material, relief or topography and time.

The active soil forming factors are those which supply energy that acts on the mass., eg. parent material, for the purpose of soil formation. Such factors are climate and organism.



Macro organisms and soil formation

The macro organisms that inhabit the soils are rodents, moles, millipedes, centipedes, nails, earthworms and termites. Owing to their burrowing habits, they burrow deep in to the soil, causing considerable mixing of the materials of the lower with the upper layers and even bring sub soil to the surface.

Earthworms are soil invertebrates, which play a key role in soil biology by serving as versatile bioreactors. Although one acre of soil may hold up to 8 million earthworms, most people pay little attention to this productive and beneficial organism. They mostly go unnoticed from day to day.

3. Systematic position of earthworm

Kingdom : Animalia

Phylum : Annelida

Class : Clitellata

Subclass : Oligochaeta

Order : Haplotaxida

Suborder : Lumbricina

Ecological functions of earthworms

The functions of earthworms include

i. Effect on key soil ecosystem processes such as decomposition of soil organic matter and nutrient recycling
ii. Effect on soil physical and chemical properties
iii. Interaction with plants, microorganisms and other animals

Earthworm activities and their functions in ecosystem are determined by various factors such as ecological groups of earthworms, population size, vegetation, parent material of soil, climate, time scale and history of soil utilization. The earthworms can effectively harness the beneficial soil micro flora and destroy soil pathogens and convert organic waste in to valuable products such as vitamins and growth promoting substances like gibberellins. They contribute to soil formation by the physical and chemical effects of their casts and burrows. Earthworm casts consisting of waste excreted after feeding, which composed mostly of soil. As plant materials and soil particles passes through an earthworm's digestive system, its gizzard breaks down the particles into smaller

fragments. These fragments once excreted are further decomposed by other microorganisms. Earthworm casts can contribute to 50 % of soil aggregates in some soils.

Classification of earthworms based on ecological strategies:

A classification has been proposed by Bouche (1977) laying stress on ecological strategies. He classified earthworms in to epigeics, endogeics and anecics. The epigeics have no effect on the soil structure, as they generally cannot dig. They are efficient agents of combination and fragmentation of leaf litter. Eg. *Perionyx excavatus*

The endogeics are sub surface feeders and feed mainly on soil and are effective in bringing about soil formation. Eg. *Lumbricus terrestris*

The anecics feed on leaf litter mixed with the soil of the upper horizons. They may also produce surface casts. These are called geophytophagous earthworms. Eg. *Nicodrilus nocturnus*. Geophages worms feed deeper beneath the surface, ingesting large quantities of organically rich soil. These worms are generally called as humus feeders and comprise of the endogeic worms

Dominate earthworm species in tropical and temperate zones

Dash et al (1980) identified the important species of burrowing earthworms and their main substrate materials are given in table. 1.

Table.1. Important burrowing earthworms& their substrates

Earthworm species	Substrate	Consumption rate (mg/g wt/day)
Allolobophora sp	Soil	200-300
Lampito sp	Soil	700-1800
Lumbricus sp	Soil &litter	27-80
Millsonia sp	Soil&litter	4000-7000
Octolasium sp	Soil&humus	29-60
Aporrectodea sp	Soil&litter	40 -70

Temperate zone: Lumbricids, Megascolecids, Octochaetids, and Sparganophilids

Tropical zone : Eudrilids, Eisenids

The burrowing earthworm species, which are found commonly in South India, is given in table.2. Most of the burrowing species of earthworms belong to the family, Megascolecidae. The members of this family are mainly soil or humus feeders and are

stouter than the composting worms. In general these earthworm burrows and structural aggragation due to their castng activities promote water entry in to the soil and therefore enhance structural stability of soil.

Table.2. Important species of burrowing earthworms common in South India

Name of the species	Family
Hoplochaetella kempi	Octochaetidae
Perionyx sp	Megascolecidae
Megascolex konkanensis	Megascolecidae
Metaphire houlleti	Megascolecidae
Amanitas alexandrines	Megascolecidae
Pontoscolex corethrurus	Glossoscolecidae
Drawida kanarensis	Megascolecidae
Dichogaster affinis	Megascolecidae

(Barnes & Ellis, 1979)

The important species of burrowing type of earthworms common in Kerala are.

Lumbricus sp, Nicodrilus sp, Lampoon sp and Aporrectodea sp.

Factors affecting the distribution of earthworms in soil:

Talashilkar and Dosani (2005) identified the important factors that affect the distribution of earthworms in soil as,

- Soil texture and acration
- Temperature
- Moisture
- pH
- Inorganic salts
- Organic matter
- Litter
- Reproductive potential
- Dispersive power of species

4. Earthworms and pedogenesis

Soil formation is essentially a process of decomposition of mineral and organic matter. Earthworms play a multifaceted role in the process of soil formation (Sivapalan *et.al*, 1993). Earthworms may participate in this soil forming process in five ways:

- Through their influence on soil pH
- As agents of physical decomposition
- By promoting humus formation
- By improving soil structure
- By enriching the soil

a. Influence on soil pH

The pH of the intestinal contents of earthworm is remarkably stable around neutral to slightly alkaline. This can have a profound effect on the overall level of soil pH and on the course of organic decomposition. In neutral or slightly alkaline conditions, bacterial activity is favoured. Bacteria effect a more complete breakdown of organic compounds, producing mull-type humus, than soil fungi that flourish under more acidic conditions, and produce mor-type humus.

Chemical composition of worm cast

The chemical composition of worm cast is given in table.3.

Effect of earthworms on soil pH & litter calcium in the 'o' horizon was given by Reich et al, in 1998 by his experiments at Siemianice experimental forest at Poland which inherently devoid of earthworms (Table.4). He introduced the burrowing species of earthworm, *Nicodrilus nocturnus* which is an anecic type of earthworm.

Table.3. Chemical composition of worm cast

Property	Value
pH	7.1
EC (dS/m)	1.0
Org. C (%)	4.0
Nitrogen (%)	0.5
Phosphorous (%)	1.2
Potassium (%)	0.1

(Talashilkar, 1986)

Table.4. Effect of earthworms on soil pH & litter calcium

Earthworm biomass (g/m2)	Soil pH	Litter Ca (mg/g)
0	4.0	2.2
2	4.3	5.3
4	4.8	8.8
6	5.5	10.3
8	6.0	15.6

Earthworm affect soil structure by burrowing through soil, by mixing organic residues with the soil and by providing casts. The burrows increase macro porosity and number of biopores in the soil and also keep the soil and roots well aerated. Earthworm burrows not only provide channels for root penetration of the soil but also contribute to improve root growth. A matted layer of roots, leaves and other organic debris can accumulate beneath pasture and interfere with water infiltration, increase acidity and reduce productivity. However earthworm, that feed on litter and animal dung and can burrow into underlying soil, help to incorporate the mat in to the soil. Further, earthworms have a capacity to incorporate not only root mats but also agricultural chemicals lay fertilizers and pesticides.

Vermicastings are the excreta of earthworms, together with their cocoons and undigested feed. Earthworms eat soil and various organic matters, which undergo complex biochemical changes in the intestine and are excreted in the form of granular castings. These castings are found to have quite a favourable effect and help to change productivity scenario in agricultural sector.

b. As agents of physical decomposition:

The passage of organic material through the earthworm gut results in the physical decomposition due to the muscular grinding action of the gizzard, aided by the ingestion of silica granules. This provides considerably enhanced surface area for microbial decomposition. Kale *et al* (1991) estimated the extend of decomposition of cellulose and lignin present in the soil as agricultural wastes. He used two earthworm species, *Eudrilus euginiae* and *Perionyx excavatus* and is presented in table.5.

Table.5. Utilization of cellulose and lignin by earthworms

Utilization of cellulose and lignin	Soil without earthworms	Soil worked by *E. eugineae*	Soil worked by *P.excavatus*
Total microbial population	4.6	6.6	4.3
Cellulolytic organisms (x 104/g)	1.96	4.0	3.1
Lignolytic organisms (x 103/g)	1.40	2.7	2.8
Residual cellulose (mg/g)	465.0	110.0	97. 5
Residual lignin (mg/400 ml)	71.1	36.4	31.7

Stockdill in 1982 investigated the pedological effects of earthworm introduction in a soil site at upland area in South New Zealand with no inherent earthworm population. He introduced the earthworm species. *Aporrectodea caliginosa* in to this site. After five years, he found that the thick mat on soil surface has disappeared and a deep humified A horizon was formed and there was an overall improvement of soil fertility status. Nielson & Hole (1994) compared the litter horizon depth at two forested sites in US

Table.6. Litter horizon depth at two sites with and without earthworms

Sites	Litter horizon depth (cm)	EW density (per m2)
Site with earthworm	1.6	27
Site without earthworm	4.5	-----

c. By promoting humus formation

The process of humus formation is often characterized by the selective breakdown of cellulose. The end product is a complex mixture of various organic acids, amino acids,

present in raw humus and peat but are degraded to polyphenols in well-decomposed humus. The presence of cellulose in the intestine of the earthworms suggests that these organisms may play an active role in humus formation.

Muller (1878) identified two humus types in forests as mull and mor based on chemical composition of the litter and acidity.

Mull: Because of more mixing, the organic forest floor is mixed with mineral soil and exists a wavy or diffused boundary between the two. High pH and earthworm predominates.

Mor: There is a sharp boundary between forest floor and mineral soil. Fungi predominate because of acidity.

In addition to these, there exists **duff mull**, which is intermediate between mull and mor. There will be an organic rich A- horizon like mull but distinct boundary like mor.

The difference between mull and mor is not attributable simply to the presence or absence of earthworm casts. It is related also to the chemical composition of the litter, especially whether it contains much polyphenolic material, and this is dependent on interactions between forest composition, temperature, rainfall, the concentration of some elements, especially calcium, in the underlying soil horizons, the leaching products dissolved from the litter by percolating rainfall, especially whether or not these are strongly acidic.

The earthworms consume the soil organic matter and convert it into humus within a short period of time and thereby increase the soil fertility. The earthworm casts are coherent because of the gluing effect of gums produced by bacteria or because they are held together by the hyphae of fungi that grow within them. Earthworm activities are direct actions of feeding and burrowing along with related biological activities. Earthworm eats its way through the soil and organic humus. In this process, it continuously keeps its body moist and passes out urine. Thos it keeps on adding micro quantities of humidity and nitrogen. Burrowing of earthworms brings about tillage of soil up to 3 m without adversely affecting the plants in any manner.

Didden *et al* (1970) identified the different species of earthworms in different layers of humus in the soil from central European forest ecosystem (Table.7)

Table.7. Earthworm species in different layers of humus

Humus layer	Dominant earthworm species	Mean abundance (No./m2)
S1	*Octolasion tyrtaeum* (Epigeal)	5.4
S2	*Octolasion tyrtaeum* (Epigeal)	3.2
S3	*Lumbricus terrestris* (Epianecic)	20.3
S4	*Nicodrilus nocturnus* (Anecic)	13.5

d. By improving soil structure:

The physical comminution of organic particles, the amelioration of soil pH, the enhancement of microbial decomposition activity-all these results of earthworm activity contribute to soil fertility. All these effects are reinforced by mixing of soil from different strata in the profile. Burrowing species are instrumental in this mixing process and they act, in this respect, at two levels (Edwards, 2004). Firstly, by ingesting a mixture of organic and mineral particles. They promote the formation of organo-mineral complexes. These complexes are formed in various ways, notably through the agency of organic and inorganic cements. Electrostatic bonding may also occur between negatively charged organic particles and cation, such as calcium. Organomineral crumbs may be formed in this way, and these improve the structure or tilth of the soil. Additionally, these complexes incorporate a pool of metallic ions that are held in the soil, and are not lost by leaching. Crumb formation is also promoted by the secretion of a thin, translucent peritrophic membrane by the anterior part of typhlosole. This provides an envelope within which faecal particles are packaged before being discharged from the body as casts. This discrete packaging of soil materials improves the soil porosity y increasing the diameter of soil spaces, thereby improving the aeration and drainage qualities, which are further enhanced by the creation of burrow systems. Secondly, by castings at the surface, earthworms bring organomineral crumbs from the deeper parts of the profile to the

surface. Deep-burrowing species may also draw fragments of organic material from the soil surface into their burrows in the mineral soil. This two-way interchange of organic and mineral material prevents the accumulation of a surface layer of acid humus and promotes the dispersion of finely decomposed mull humus down the profile. The amount of soil brought to the surface by castings can be of the order of 100 tons per hectare per year (10 mm per year), from the earthworm biomass of 1 ton per ha.

The locomotion in earthworms is of great influence on the soil mixing. It is effected by the contraction and relaxation of the muscular body wall, aided by the turgescence of the coelomic fluid, which has been called the hydraulic skeleton. As the earthworm moves, the circular muscles contract first making the body thinner and longer. This is followed by the contraction of the longitudinal muscles, which brings about the shortening of the body. With the front portion of the body fixed by the points of support, the hind part is drawn up. The body setae enable the worm to get firm grip on the ground during movement (Kale, 1979). The soil mixing rate by earthworms is about 25-50 times of casting rate and 2-3 orders of magnitude greater than soil production rate from his experiments in soil site at Ivory Coast.

Graham and Ervin (1995) listed out the top four ranked macro faunal species in soil mixing at different sites (Table.8) and the Oligochaets (the sub class to which earthworm belongs) are in the first four ranks in all the three margins.

Table.8. Top four ranked macrofaunal species in soil mixing

Rank	Oman margin	Peru margin	Santa barbara basin
1.	*Prinospio* sp (Polychaeta)	*Olivia's sp* (Oligochaeta)	*Lumbricus sp* (Oligochaeta)
2.	*Aphelochaeta sp* (Polychaeta)	*Protodorvillea sp* (Oligochaeta	*Pogonophorana sp* (Polychaeta)
3.	*Lumbricus sp* (Oligochaeta)	*Astrys sp* (Gastropoda)	*Olavius sp* (Oligochaeta)
4.	*Cossura sp* (Polychaeta)	*Sigambra sp* (Polychaeta)	*Protodorvillea sp* (Oligochaeta)

Lee (1992) studied the effect of earthworms on aggregate stability in four soils and found that the mean abundance was more important in improving the soil aggregate stability rather than their biomass (Table.9)

Table.9. Effect of earthworms on soil aggregate stability

Soils	Abundance of EW (No./m2)	Mean biomass of EW (g/m2)	Aggregate stability
1.	51.0	13.4	Less
2.	133.0	22.7	Very high
3.	93.0	44.3	Medium
4.	112	81.7	High

Hopp (1975) investigated the effect of earthworms on soil surface run off and erosion at Ithaca, USA and found that in continuous pasture and virgin ground where there was a relatively high abundance of earthworms, the annual soil loss through erosion and surface run off were negligible or even nil (Table.10)

Table.10. Relationship between EW population, annual loss of surface soil by sheet erosion and annual surface runoff

Treatment for previous 10 years	Earthworms (No./m2)	Annual erosion (t/ha)	Surface runoff (mm)
Continuous cultivation	Nil	75	45
3-year rotation	23	13	10
Continuous pasture	76	nil	5
Virgin ground	200	nil	7

e. By enriching the soil:

Earthworms that burrow deeply in to the mineral strata and return periodically, to cast faecal material at the soil surface may facilitate the transport of certain elements to the surface litter from deep in the profile. There is abundant evidence that concentration of

exchangeable calcium, sodium, magnesium, potassium and available phosphorous and molybdenum are higher in earthworm casts than in the surrounding soil. In addition to the physical mixing of the soil by burrowing activities, soil enrichment is achieved by speeding up mineralization of organic matter 2-5 times by the earthworms.

- Earthworm ingestion causes an increase in surface area of the organic wastes.
- Ingestion removes senescent bacterial colonies and stimulates new bacterial growth.
- Nitrogenous excretion from the worms enriches the soil formed form organic wastes.
- Earthworm burrowing enhances the oxygen penetration.
- Mineral nutrients are released through enhanced microbial mineralization.
- Earthworm feeding increases the interaction among micro flora, improving the flow and exchange of nutrients
- Earthworms eliminate pathogens in the wastes. Two mechanisms are stipulated:
    - The earthworm's gut micro flora out compete the pathogens
    - The earthworms produce bacteriostatic substances.

In addition to nutrients, several valuable compounds are produced through the earthworm-microbial interaction. These include vitamins and plant growth hormones like gibberellins.

Earthworms can also be used for water management. Earthworms enhance water infiltration. Earthworms, numbering 0.2-1 million per ha make permanent, structurally stable burrows in 5the soil. These can extend up to 3m in depth and with complex network of burrows allow water infiltration upto 120mm per ha. Hence, inspite of a heavy spell of rain, there is hardly any run off and soil erosion, as each burrow acts as a micro dam.

Earthworm castings are structurally stable. Earthworms excrete granular, structurally stable castings (vermicastings) on the surface. These do not disintegrate into micro-particles whether dry or wet and hence there is no soil loss due to wind or water. Water run ff, if any, is clear. Vermicastings absorb moisture from the air. Vermicastings, being granular and with enhanced internal porosity and water absorption capacity, absorb

moisture particularly during night and hold it effectively for releasing it to micro-roots of the vegetation.

Earthworms act as a bio-pump. Each earthworm burrow enhances water infiltration and water storage over a considerable depth of soil. Earthworms help to bring this moisture to the upper layer by acting as a bio-pump. They also release 5the water slowly according to the water requirement of the plants.

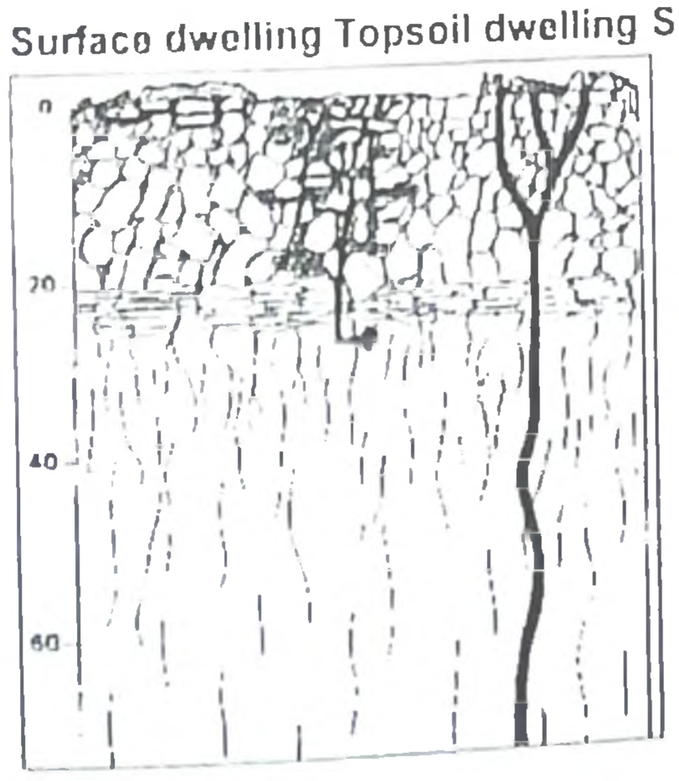
Earthworms produce biological water during the decomposition of organic matter. The roots effectively utilize this slow-release water.

Earthworm burrows

Earthworms form two types of burrows, viz., temperory burrows and permanent burrows. Earthworms moving from one feeding site to another make temporary burrows. Permanent burrows are homes to individual worms, usually more extensive and are open to the surface allowing the resident earthworm to select the most favourable microenvironment for feeding (Figure.9.). The permanent burrows are more effective in bringing about soil mixing and genesis (Sahai, 1990)

Burrowing by different types of earthworms

According to Groffman and Bohlen (1999), the different species of earthworms are different in their burrowing or dwelling activities. Some are surface dwellers; some topsoil dwellers while some others are sub soil dwellers (Figure.10). Among these, the sub soil dwellers are more efficient in pedogenesis.



Different types of dwelling by earthworms

Earthworm maths: Jegan & Kumar (2005) calculated the average population of earthworms and their cast production rate and nutrient content as,

25 Earthworms/sq.ft: 1 t of worms/acre

1 t worms: 100 t of castings/acre

Add 4 lbs of nitrate N, 30 lbs of P, 72 lbs of K, 90 lbs of Mg & 500 lbs of Ca

Kang et al (1994) analyzed the chemical contents of surface (0-15 cm) soil and worm casts collected under two woody species in alfisol (Table.11) and he found that the pH and the content of all nutrients except Na was high in casts than in soil in both the tree species.

Kale et al (1992) examined the micro flora of worm cast and found that bacteria are the dominant group present in worm cast, followed by actinomycetes and fungi. The presence of these microorganisms will help in the organic matter decomposition (Table.12)

Table.11. Chemical contents of surface (0-15 cm) soil and worm casts collected under two woody species

Particulars	Leucaena sp		Glyricidia sp	
	Soil	Casts	Soil	Casts
pH	6.2	6.6	6.0	6.6
Org .C(g/kg)	14.8	44.4	11.4	47.7
Ca(C mol/kg)	1.86	8.06	1.9	7.48
Mg(C mol/kg)	0.43	1.66	0.42	1.58
K (C mol/kg)	0.29	0.86	0.22	0.84
Mn (C mol/kg)	0.01	0.13	0.01	0.18
Na (C mol/kg)	0.34	0.34	0.31	0.52

Physico-chemical analysis of soil bed with and without earthworms was done by Ramesh (1995) during kitchen waste composting and found that the nutrient content was high in soil bed with earthworms than that without earthworms. Also the pH was almost neutral in earthworm worked soil. The C/N ratio was narrowed due to the activity of earthworms, which will favour mineralization, and availability of nutrients to plants (Table.13)

Table.12. Microflora of worm cast

Microflora	Abundance (per gram of cast)
Bacteria (x103)	120.0
Actinomycetes (x105)	46.6
Fungi (x103)	30.3

Reddy and Reddy (1998) investigated the changes in nutrient content of soil upon ingestion by earthworms. He reported that, in the red soil and black soil with earthworms, the content of organic carbon, total nitrogen, exchangeable phosphorous and exchangeable potassium was high than that without earthworms (Table.14). This is due to the more mineralization due to the activity of earthworms in soil and also the nitrogen enrichment that occurs in the worm gut.

Table.13. Physico-chemical properties of soil bed wit and without earthworms

Parameter	After 40 days		
	Initial	Without worms	With worms
pH	5.4	8.1	7.6
Organic C (%)	1.7	3.2	3.4
Total N (%)	0.14	0.28	0.31
Avg P (mg/100 g)	1.83	36.7	58.6
Avg K (mg/100 g)	6.0	102.0	89.0
Avg Ca (mg/100 g)	167.7	236.6	297.5
Avg Mg (mg/100 g)	22.5	38.8	42.0
C: N	12.0	11.4	10.8

Table.14. Effect of soil ingestion by earthworms on nutrient content (28 days after incubation)

Treatment	Org.C (g/Kg)	Total N (mg/Kg)	Extractable P (mg/Kg)	Exchangeable K (mg/Kg)
Red soil				
With worms	20.3	2730	12.2	107.0
Without worms	15.1	1240	6.4	84.0
Black soil				
With worms	15.0	1840	15.3	145.0
Without worms	7.4	760	5.5	118.0

Bioturbation: The overall effect of earthworms on pedogenesis is by the process of bioturbation. It is defined as the actions of animals and other creatures disturbing the depositional layers of soil. By this process, organisms like earthworms will aid the soil horizon development through

- Direct transfer of O2 by the organism
- Intensive biogenic mixing
- Formation of burrows

The factors affecting bioturbation are topographic position and type of earthworms.

5. How to attract and promote earthworms

The earthworms can be attracted and promoted by adopting measures like.

- Inoculation with vermicastings
- Mulching with organic mulches
- Zero cultivation
- Minimum tillage systems
- Avoidance of dangerous synthetic chemicals

It is not practical to pick and transport worms from one area to another, since it will cause transportation shock. But we can inoculate the soil with vermicastings, which contain worm cocoons. It also contains beneficial microflora, humus and undigested organic matter. Use castings of burrowing type earthworms like *Lumbricus* to inoculate new

fields. Then mulched with organic mulches like compost. Earthworms hatched in the newly triggered environment are able to adapt effectively.

Avoid synthetic chemicals like fertilizers and pesticides since they will lead to the ridding of the skin of earthworms and dehydrate them. Acid forming fertilizers like ammonium sulphate and urea and super phosphate will reduce the population of earthworms since they can't tolerate acidity.

6. Dating techniques of pedogenesis

A number of dating techniques are becoming increasingly accessible that can be used to estimate soil age and quantify pedogenic processes with in the soil profile. Until recently, this exercise was largely restricted to evaluating soil formation rates in deposits (Birkeland, 1999) and less commonly *in situ* soil focusing on extrusive rocks such as basalts (Pillans, 1997).

The role of bioturbation (mixing by biota) within the soil profile has proved difficult to quantify, and hence its role in pedogenesis has yet to be fully established. The vertical mixing with in soil profile can now be evaluated over long time scales using OSL (Optically Stimulated Luminescence) and TCN (Terrestrial in situ Cosmogenic Nuclide) datings.

OSL dating:

OSL dating uses a beam of light to release a luminescence signal with in particular mineral grain (Quartz or feldspar), which is measured using a photomultiplier (Duller, 2004). OSL dating differs from TCN dating in that latter uses heat to stimulate the luminescence. The OSL signal, which is proportional to the time elapsed since the grain was last exposed to sunlight, accumulate in the crystal structure of minerals as a result of exposure to cosmic rays and ionizing radiation from radionuclides like Thorium and Uranium in adjacent soil. This radiation flux is measured and termed as the dose rate. The maximum measurable and saturation age depends on the dose rate and crystal characteristics of weathering and it appears intuitive that soil production is maximized under a finite soil cover.

TCN dating:

The principle behind this method is that cosmic rays penetrating the Earth's atmosphere bombard elements in the atmosphere and geosphere where they alter atomic structures,

thereby producing cosmogenic nuclides. Meteoric or atmospheric cosmogenic nuclides are produced in the atmosphere while TCN are produced with in the soil and rocks at the earth's surface.

Commonly used TCN include He^{3}, C^{14}, Ne^{21} and Cl^{36} and a wide variety of minerals are possible targets, such as quartz and olivine. Since the mineral exposure time to cosmic radiation flux is proportional to TCN concentration, the latter can be measured to quantify exposure. TCN production decreases exponentially with depth of rock due to attenuation. In soil production studies, the lowering rate of the surface underlying the soil mantle, usually the bedrock is equated to the soil production rate. It is assumed that soil cover shielding remains constant for the time period that the TCN are produced in the sampled surface, which is the time, required to convert to soil. This is also the time period over which the soil production rate is averaged.

Humped model of TCN:

In the humped model, suggested by Dietrich et al (1995), it is assumed that physical disruption of bedrock can result from biota and other processes. Macrofauna require at least a moderate mantle to exploit its habitat and in doing so, assist soil production. Plant roots and burrowing macrofauna at the soil column disturb the soil-bedrock interface, thereby altering the fabric of bedrock. This disturbance creates voids, which may be filled with water, gases or soil, enabling further access in to the profile by weathering agents. Furthermore, the disturbances increase the surface area of bedrock exposed to chemical weathering. Plant and animals also penetrate beyond saprolite into unweathered bedrock.

7. Future line of work

Detailed studies are to be done on anecic earthworms, which are active factors of soil formation. Also, new technological approaches should be employed for soil microstructure studies for the better understanding of earthworm ecology and their ecosystem functions.

8. Conclusion:

The ancient agricultural practice of mulching, where animal dung and or plant residue are spread on the soil surface, has the dual purpose of insulating the soil from extremes of temperature and moisture and of providing plant nutrients and humus materials for incorporation in to the soil from the decomposition of the added organic matter. These

materials provide food for earthworms as well as surface insulation, are very effective. The deep burrowing species of earthworms, which are very effective for pedogenesis, can work efficiently, even without irrigation. However, a combination of irrigation and mulching is necessary to maintain a high population.

As one of the key soil invetebrates, earthworms can greatly impact soil processes and are ecosystem engineers. They act as consumer, decomposer and modulator in an ecosystem. So the population of these 'soil formers' is to be conserved and promoted to protect our most non-renewable resource, soil.

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Discussion:

1) Whether composting worms can be used for soil mixing and horizon development?

   Composting worms are usually surface feeders, which feed mainly on surface litter, but they have some effect on soil formation through their nutrient rich cast production. While the field worms are sub-surface or deep burrowers and feed on soil and humus and are more efficient in soil mixing and horizon development.

2) What is the adverse effect of fertilizers on earthworms?

   Most of the chemical fertilizers are salt based and are acidic in nature. So when it comes in contact with the earthworm's skin, it will cause the ridding of the skin, which in turn will lead to the dehydration and finally the death of the organism.

3) Earthworms or termites, which are more efficient in soil mixing process?

   Earthworms are more efficient in soil mixing process than the termites since the former can make permanent burrows, which will retain up to 2 years.

4) Which is the soil order that is developed as the result of pedoturbation?

   Vertisol.

5) How earthworms help in elimination of pathogenic soil organisms?

   While ingesting the soil, the earthworms also take the pathogenic organisms in the soil which then get eliminated in the gut of earthworm in which lot of microflora are present.

6) Which are the common microflora associated with earthworm cast?

   Bacteria, fungi and actinomycetes.

7) What is the ideal pH requirement of earthworms to grow and multiply in soil?

   The ideal pH for growth and multiplication of earthworms is near neutral, i.e. near 7.0. But there are certain burrowing earthworm species that can grow in acidic pH (5.4) like *Lumbricus terrestris*.

8) Are the burrowing earthworms available in the market?

   No. The burrowing earthworm species are not commercially available. But we can establish these worms by inoculating the castings of these worms.

KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara

Ag.Chem.751-Seminar

Topic: Earthworms and pedogenesis

Student: C.J.Bindhu (2006-21-112)
Time : 10.15 am, 11.05.2007
Venue: Audio Visual Laboratory,
Dept.of Pomology &Floriculture

Abstract

The soil forming processes or pedogenic processes are constructive and result in a soil profile that has been developed from the surface few feet of parent material. The biochemical processes of soil formation are inherent to all soils (Sahai, 1990). The active soil forming factors are climate and organism and the passive factors are parent material, relief and topography. Earthworms are significant contributors to the soil formation as an active factor. Biomantle formation by soil fauna like earthworms actually can serve to create useful stratigraphic ordering during interim stages of pedogenesis and will lead to the development of distinctive layers and horizons (Sehgal, 2005).

Earthworms may participate in the soil forming processes in five ways: (1) through their influence on soil pH (2) as agents of physical decomposition (3) by promoting humus formation (4) by improving soil structure and (5) by enriching the soil (Sivapalan *et al.*, 1993). The mixing of soil horizons by earthworms result in a deepening of the humified zone of the soil profile. The deep burrowing species of earthworms, are very effective for pedogenesis, and can work efficiently when environmental conditions are favourable. Earthworms contribute to soil formation by the physical and chemical effects of their casts and burrows.

Reduction in the number of earthworms, whether intentional or not can have a detrimental effect on both physical and chemical properties of the soil. Pedogenic processes with in the soil profile can be quantified by using Optically Stimulated Luminescence (OSL) and Terrestrial in situ Cosmogenic Nuclide (TCN) dating techniques (Pillans, 1997).

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BIOMAGNIFICATION BY CHLORINATED HYDROCARBONS AND HEAVY METALS

By

C.J.BINDHU
(2006-21-112)
Ph. D Scholar
Soil Science & Agricultural chemistry

SEMINAR REPORT
Submitted in partial fulfillment for
the requirement of the course

Ag.Chem.752-Seminar

DEPARTMENT OF SOIL SCIENCE & AGRL.CHEMISTRY
COLLEGE OF HORTICULTURE
KEARALA AGRICULTURAL UNIVERSITY
VELLANIKKARA-680 656

375

DECLARATION

I. C.J.Bindhu (2006-21-112) hereby declare that this seminar report entitled "**Biomagnification by Chlorinated hydrocarbons and Heavy metals**" has been prepared by me, after going through the various references cited at the end of the report and has not been borrowed from any of my fellow students.

Vellanikkara
18.08.07

C.J.Bindhu
(2006-21-112)

CERTIFICATE

Certified that the seminar report entitled "**Biomagnification by Chlorinated Hydrocarbons and Heavy metals**" for the course Ag.Chem.752 has been prepared by C.J.Bindhu (2006-21-112), after going through the various references cited here under my guidance and supervision and she has not borrowed from any of her fellow students.

Vellanikkara
18.08.07

Dr. P.K.Sushama
Associate Professor
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INTRODUCTION

Chemicals like pesticides are very necessary to protect a basic food supply and to protect human health. Organisms including human beings are exposed to a myriad of chemicals in their environment. Some of these chemicals occur in trace concentrations in the environment and yet they may be selectively accumulated by organisms to much larger concentrations that can cause toxicity. This tendency is nothing but biomagnification. Biomagnification is the bioaccumulation of a substance up the food chain by transfer of residues of the substance in smaller organisms that are food for larger organisms in the chain. It generally refers to the sequence of processes that result in higher concentrations in organisms at higher levels in the food chain. These processes result in an organism having higher concentrations of a substance than is present in the organism's food. Biomagnification can result in higher concentrations of the substance than would be expected if water were the only exposure mechanism. Accumulation of a substance only through contact with water is known as bioconcentration.

BIOMAGNIFICATION

Biomagnification is a process that results in the accumulation of a chemical in an organism at higher levels than that are found in its own food (Stiling, 1999)

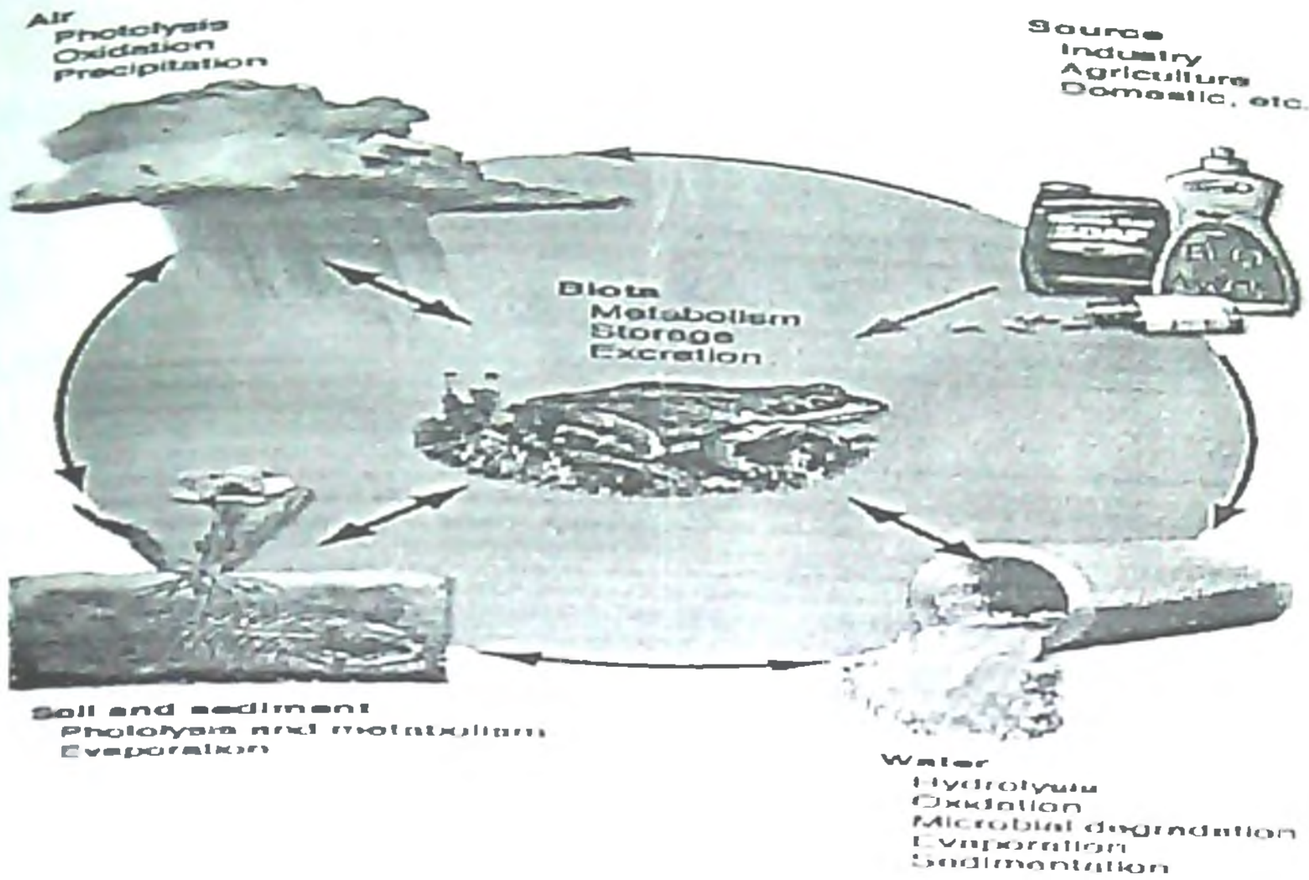
Concept of Biomagnification

The concept of biomanification originated from Rachel Carson's book, Silent_Spring. In this book, she catalogued the environmental impacts of the indiscriminate spraying of pesticides. The book questioned the logic of releasing large amount of these chemicals in to environment without fully understanding their effect on ecology and human health. Its publication was one of the signature events in the birth of the environmental movement and led to the banning of the most effective anti-malarial insecticide, DDT.

Secondary Poisoning

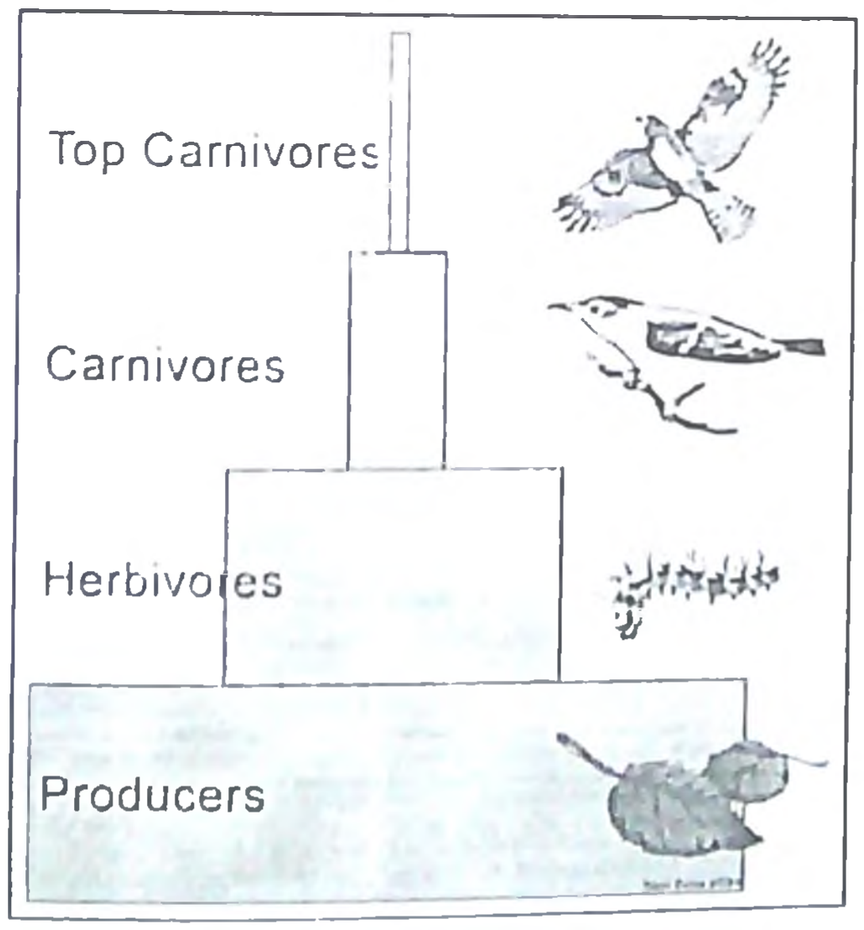
Biomagnification is a case of secondary poisoning. The predators become sick after feeding on dead or dying animals poisoned by chemicals. Then the chemical residues move up the food chain (plants eaten by plant feeding animals which in turn are eaten by predators) (Hedland and Ohrn, 1990)

Exposure of organisms to pollutants



Food chain

To study biomagnifications, one should know what a food chain is. Most of the food chains, whether aquatic or terrestrial, consist of four trophic levels as producers, herbivores, carnivores and top carnivores.



(Lutz et.al. 1992)

How do the pollutants affect the environment?

The pollution can be of two types as,

Point source pollution: contamination that comes from a specific, identifiable place (a point)

Non-point source pollution: contamination that comes from a wide area

Environmental Impact of chemicals in Soil

The chemicals can move in the environment via the soil by two methods:

1. Erosion
2. Leaching

The chemical properties that affect leaching are solubility, adsorption, and persistence

Movement and Distribution of chemicals

Based on solubility, the chemicals are grouped in to two as,

- Chemicals which dissolve more readily in water and,
- Chemicals which dissolve more readily in oil

Conditions for biomagnification

For biomagnification to occur, the Pollutant must be long lived, mobile, soluble in fats and biologically active (Palmer et.al. 1994). If the pollutant is not long-lived, it will break down before it can become dangerous. If not moble, it will stay in one place and is unlikely to be taken up by organisms. If it is soluble in water, it will be excreted by the organism. Pollutants that dissolve in fats however may be retained for a long time. If the pollutant is not active biologically, it may biomagnify, but we really don't worry about it much, since it probably won't cause any problem.

Persistent Organic Pollutants

Persistent organic pollutants are chemicals which are very stable, and do not degrade rapidly under most conditions, thus their concentrations will persist for long time. They are characterized by high toxicity, persistence, fat soluble and ability to travel long distances.

Processes in biomagnficaton

The important processes in biomagnification are adsorption and lipid partitioning. At first, the chemical will get adsorbed on to the skin of organisms and then get their way in to the system by lipid portioning because of their lipid soluble nature.

Substances that have the potential for biomagnification

Not all the chemicals have the potential for biomagnification. But there are certain organic and inorganic substances that have very high potential for biomagnification.

Organic substances

- DDT
- PCBs
- Toxaphene

Inorganic substances

- Mercury
- Arsenic
- Cadmium

Organic substances

The organic substances which will biomagnify are mainly the organochlorine group of pesticides. The organochlorines are characterized by,

- low volatility
- chemical stability
- lipid solubility
- slow rate of biotransformation and degradation

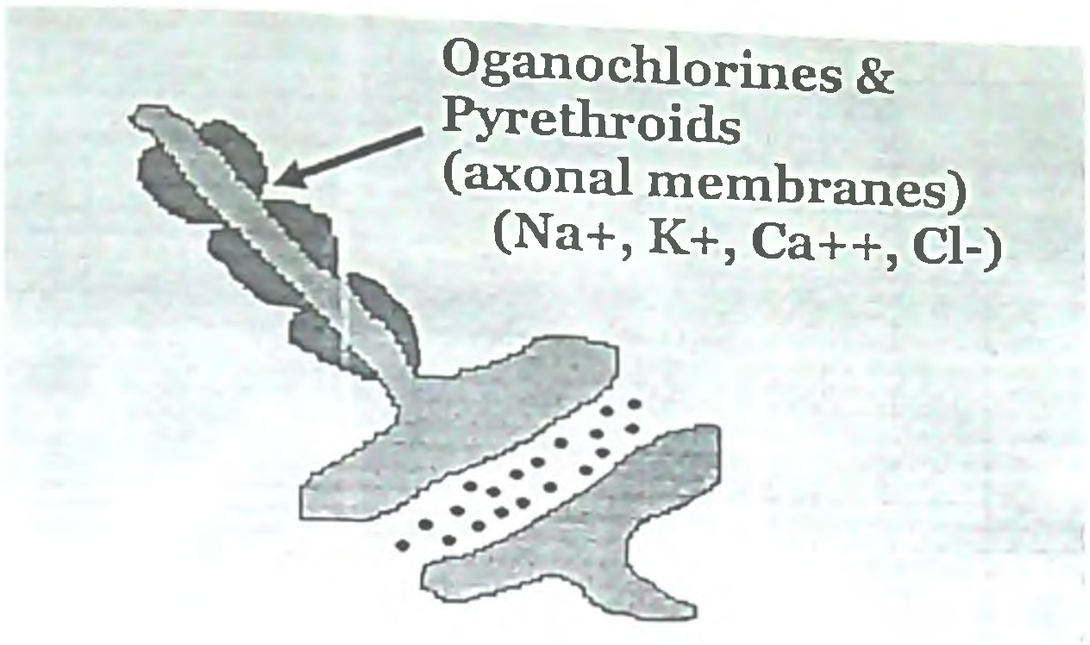
These properties which made them good insecticides itself lead to the demise of these powerful insecticides.

Mode of action

The organo chlorines mimic the action of the chemical, picrotoxin.

1. They results in only partial repolarization of the neuron and a state of uncontrolled excitation.
2. Inhibitors of Na/K ATPase and Ca/Mg ATPase that is essential for the transport of Ca++ across membranes.

Mechanism of Action of organochlorines



Residues in soil and water

Environmental persistence of organochlorines

Table.1. Half-life of some organochlorines in soil

Insecticide	Half-life in soil (year
Aldrin	1-4
Chlordane	2-4
DDT	10-15
Dieldrin	1-7
Endrin	4-8

(Langeland, 1990)

DDT

DDT is also called Guesarol. It is a ppersistent organic pollutant with a half life of 15 years The Loss and degradation of DDT in soil include runoff, volatilization, photolysis and biodegradation. DDT was accumulating in the fat of humans and all other animals. It is banned for sale in U.S. on January 1, 1973. Bit in 1993, DDT was the most frequently detected pesticide on produce entering the U.S. indicating the high persistence of DDT even after 20 years.

Breakdown of DDT

DDT on dehydrohalogenation converted to DDE

(Dichlorodiphenyldichloroethylene) which on metabolism, converted to DDD

(Dichlorodiphenyldichloroethane). Both these compounds are also as persistent as DDT.

Breakdown of DDT over years

Table.2. Breakdown of DDT

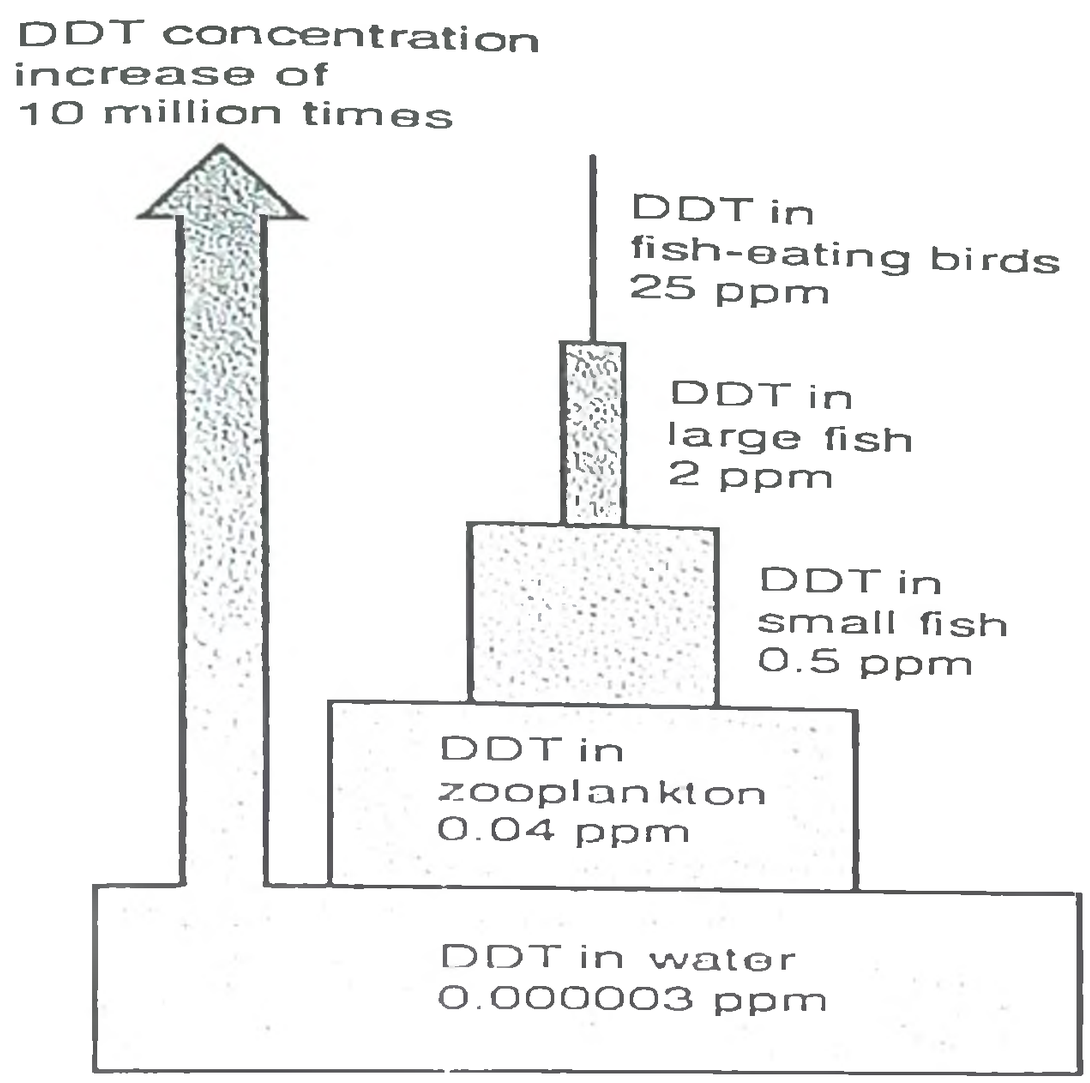
Year	Amount remaining (Kg
0	100
15	50
30	25
45	12.5
60	6.25
75	3.13
90	1.56
105	0.78
120	0.39

Table.3.Biomagnification of a Persistent Pesticide – DDT

Source	DDT Residue (ppm)
Water	0.00005
Plankton	0.04
Silverside Minnow	0.2
Sheepshead Minnow	0.9
Tern (Bird, feeds on small animals)	3.9
Herring Gull (Scavenger)	6.0
Fish Hawk (Osprey egg)	13.8
Merganser (Fish eating duck)	22.8

(Le Blanc, 1972)

DDT in Food Chain



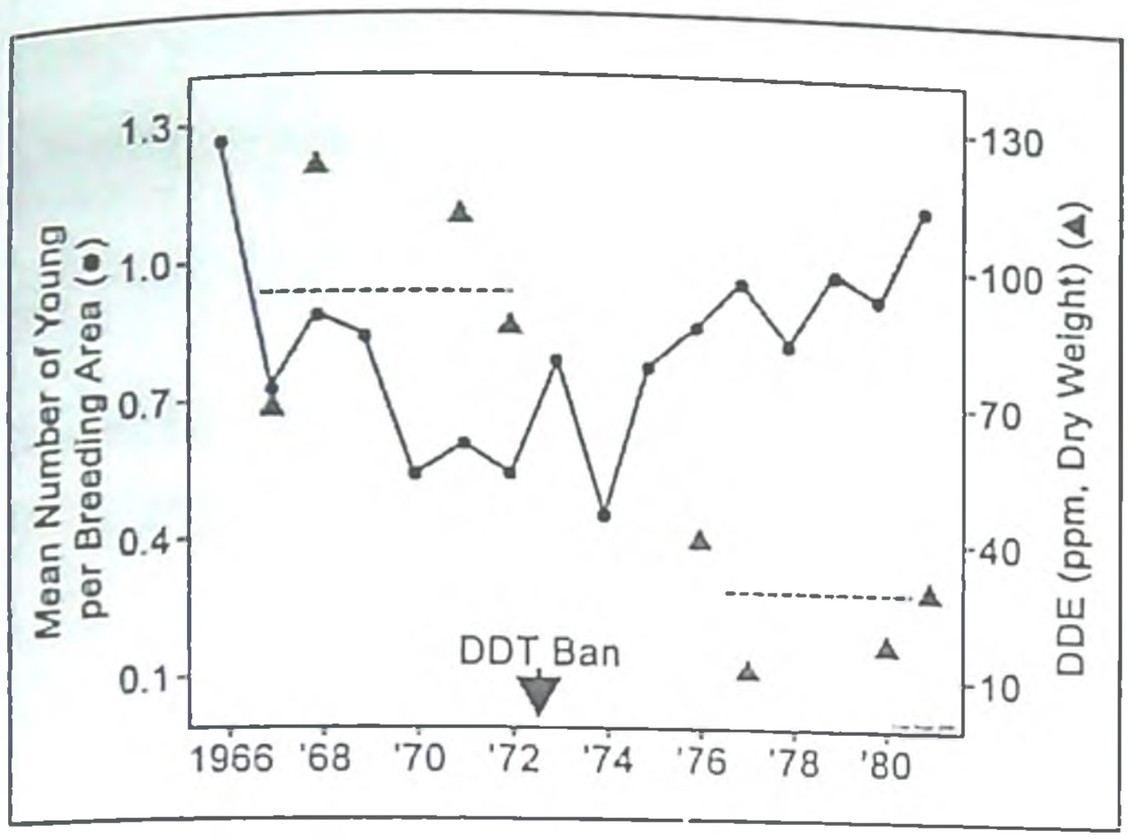
Classical examples of biomagnification (DDT application rates: 0.0200 ppm)

Table.4. Biomagnification by DDT

Organism	Residue (ppm)
Plankton	5.3
Small fish	80
Predatory fish	196
Grebe	500-2500

(Hassall & Rushton, 1982)

Eagle reproduction before and after DDT ban



Why DDT become concentrated in tissues of organisms?

DDT get accumulated in the tissues of organisms due to its.

- Slow metabolism and excretion
- Accumulation in fatty tissue
- DDT ingested still present in net pro !uction

Table.6.Residues of DDT in eggs of museum specimens

Specimen	Residue (ppm)
Pelican	0.5
Falcon	2.0
Brown pelican	4-5
Cormorants	20

(Laakso & Setala., 1972)

DDT & egg shell thinning

1. in Hawks when DDT is given at a Dietary level of 10 ppm, the eggs showed a concentration of 32.4 ppm and the shell thinning was found to be 9.7% (Moore et. al.,2003)
2. In black ducks & owls when dietary level is 10 ppm, the eggs contain 46 ppm of DDT and the shell thinning was 12.2% (Santos et.al., 1981)

Table.7. Egg shell thinning in American sparrow hawks by Dieldrin residues

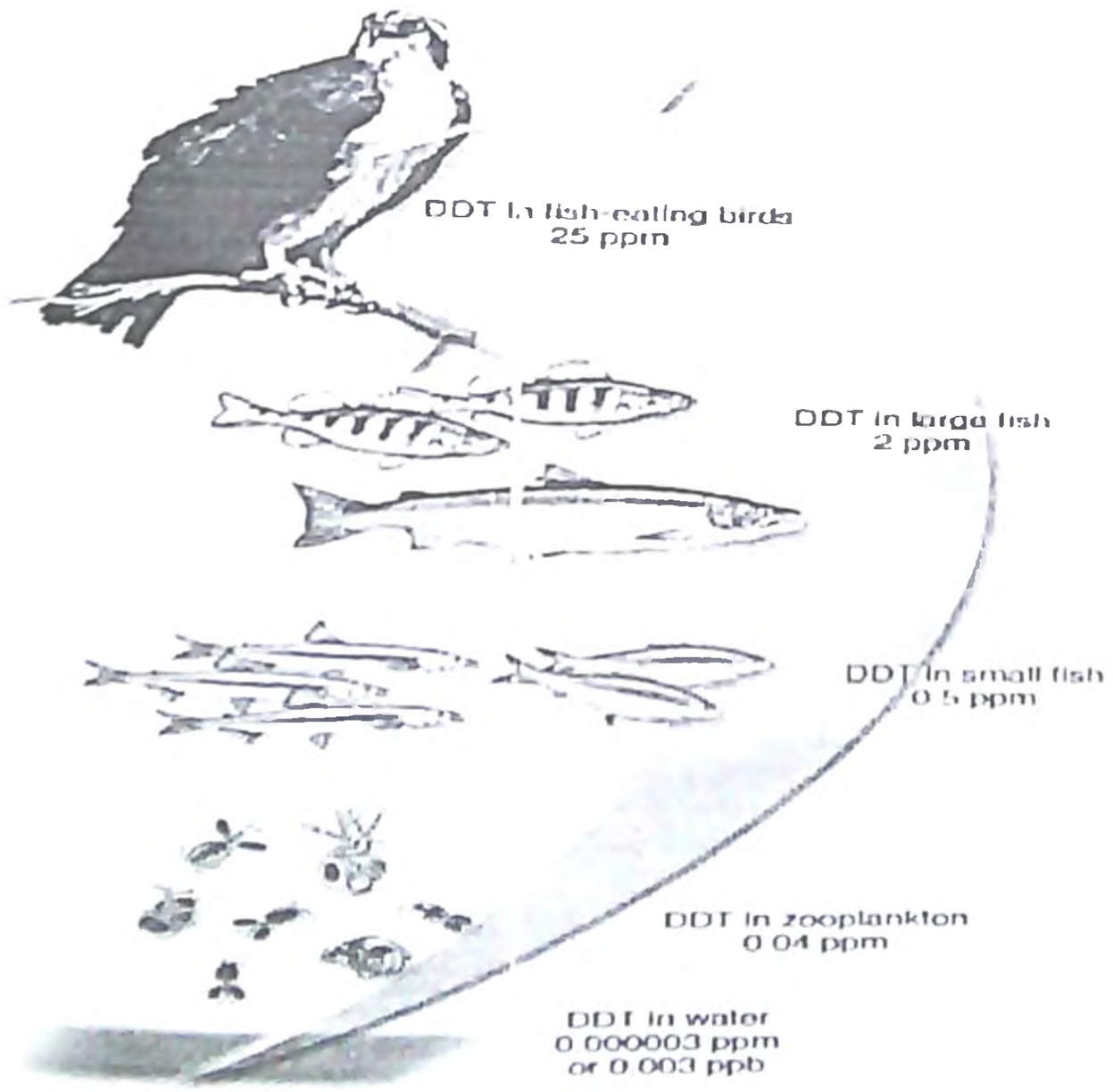
Residue (ppm)	Egg shell thinning (%)
1	11
3	17
5	28
15	50

(May, 1973)

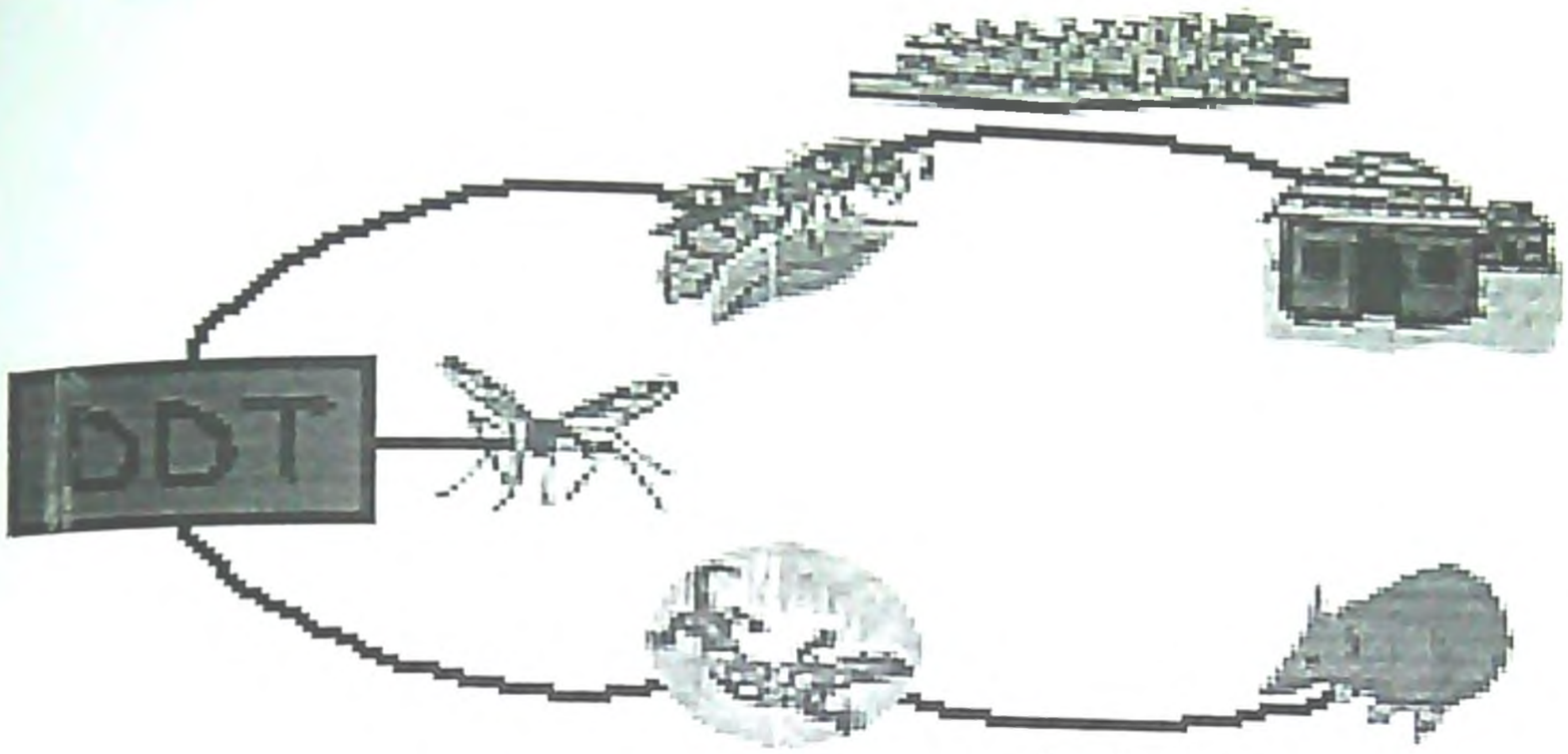
The egg shell thinning action of chlorinated hydrocarbons is due to their,

- Direct estrogenic action
- Inhibition of carbonic anhydrase
- Inhibition of Ca metabolism

DDT IN AQUATIC FOOD CHAIN



Route of DDT in terrestrial food chain

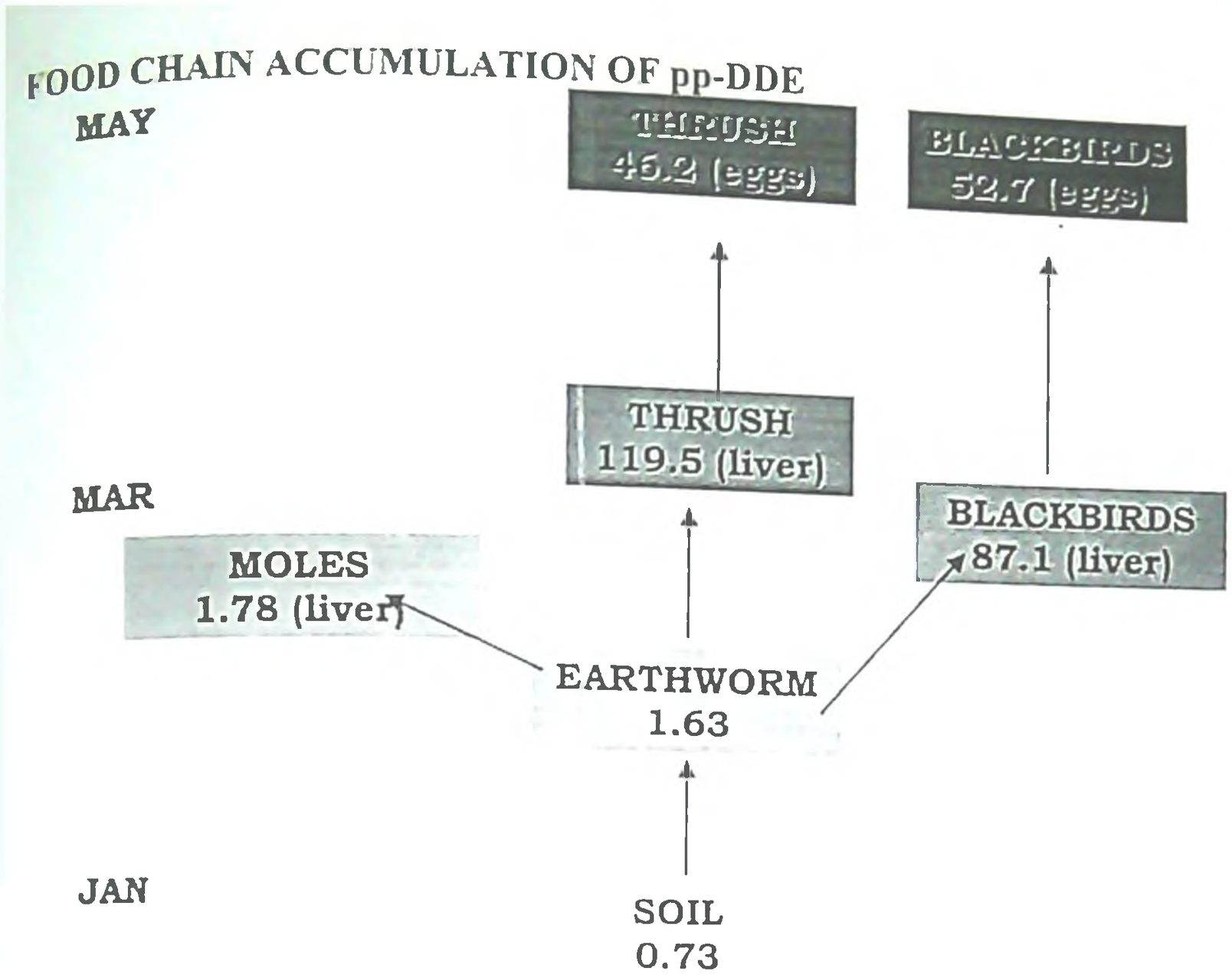


- Studies in wildlife species demonstrated potent estrogenic and enzyme-inducing properties, which interfere with fertility and reproduction.
- In birds, the interference is related to steroid metabolism and the inability of the bird to mobilize sufficient calcium to produce a strong eggshell.

Biomagnification study of DDT

- soil level of DDT: 10 ppm
- earthworms : 141 ppm
- robins : 444 ppm

(Johnson, 1973)



A biomagnification study conducted at Kuttanad backwaters revealed that the main source of DDT was rice fields and the major metabolite was DDE. This high concentration of DDE is due to the continuous use of DDT in the anti malaria eradication programme (Spacie et.al, 1995).

Table.8. Recovery percent of organochlorines in different samples

Sample	Fortification level (ppm)	Recovery %
Soil	0.0005	75-95%
Plant	0.0005	70-85%
Water	0.0002	85-98%
Fish & Clam	0.001	75-96%

(Gray, 2002)

Table.9.Residues of different organochlorines detected in rice grain & straw, fish and clam (µg /g)

Residue	Straw	Grain	Fish	Clam
DDT	0.01	0.002	---	---
DDD	0.003	0.001	0.0012	0.001
DDE	0.007	---	---	---
Aldrin	---	0.0007	---	---
Dieldrin	0.013	0.0008	---	---
Endosulphan	0.019	0.001	---	---

(Gray, 2002)

Metabolism of Chlorinated hydrocarbons

The very slow rate of degradation of DDT is due to the complex aromatic ring structure and chlorination. Half-life is 335 days in cattle. DDT can be broken down to DDE both non-enzymatically and by cytochrome P450 reductive dechlorination. All the metabolites formed are highly lipophilic and have very high potential for biomagnification.

Toxaphene

Toxaphene (CAS 8001-35-2) is an organic insecticide. When inhaled or ingested, it will damage the lungs, nervous system, and kidneys, and may cause death. Here, the mode of action is similar to DDT

Herbicides and biomagnification

2, 3, 6- TBA —Metabolized→ 3-chlorobenzoic acid

The chemical absorbed and metabolized in the plant system and the organic residues circulates through the system, retained in edible portion of plants and goes in to food chain (Singh, 2001)

Table.10.Persistence of 2, 4-D in different components of aquatic system

Components of aquatic system	Mean radioactivity (cpm ml-1 or cpm g-1)			
	1 (DAS)	30 (DAS)	60 (DAS)	120 (DAS)
Water	1862.9	77.8	56.2	26.0
Fish	664.8	83233.1	97768.9	26479.1
Salvinia	8946.9	1192.5	2790.3	Decomposed completely

2,4-D level:2 kg/ha

(Landrum et.al, 1996)

Table.11. 2, 4-D residues in soil and grain & straw of rice (µg g-1)

Soil	Plant			
I DAS	30 DAS	Harvest	Grain	Straw
0.480	0.016	---	---	---

2, 4-D @ kg a.i/ha

(Suedel et.al, 1994)

Poly Chlorinated Biphenyls

The Polychlorinated biphenyls (PCBs) are with a general chemical formula of C12H10-xClx. They have low water solubility and low vapor pressure. They are soluble in most organic solvents, oils, and fats. Very stable and do not degrade readily and their half-life range from <1 year to 71 years.

Sources of PCBs

The PCBs were found in significant quantities in transformers and capacitors, heat transfer applications, insulating tapes and protective lacquers, epoxy resins, carbonless carbon paper, hydraulic fluids, and plasticizers.

Degradation products of PCBs

They produce extremely toxic dibenzodioxins and dibenzofurans through partial oxidation. The degradation proceeds quite slowly relative to most other compounds. One of the most toxic compound of degradation is, 2, 3, 7, 8-tetrachlorodibenzo[p]dioxin

Mode of action

The toxicity of coplanar PCBs (such as dioxin) and mono-ortho-PCBs are mediated via binding to aryl hydrocarbon receptor (AhR). The di-ortho-substituted non-coplanar PCBs interfere with intracellular signal transduction dependent on calcium; this may lead to neurotoxicity. Ortho-PCBs may disrupt thyroid hormone transport by binding to transthyretin.

Effect of PCBs on animals

The Polychlorobiphenyls causes anaemia. liver, stomach, and thyroid gland Injuries (including hepatocarcinoma). The other effects of PCBs in animals include changes in the
immune system, behavioral alterations, and impaired reproduction.

Biomagnification by PCBs

The shorter the food chain in the lake, the lower the concentration of PCBs in the tissue of trout. which feed at the top of the chain .The amount of PCB in tissues increased 3.5 times per trophic level.The amount of lipids as a proportion of tissues increases much less, only 1.5 times per trophic level (Rasmussen et al., 1990).

Some findings on PCB contamination

Many of the fish stocks of the Great Lakes and numerous of the nation's river systems became too contaminated to eat because they contained more than 2 ppm. The contamination levels as high as 7.5 ppm of PCBs compared to the 2 ppm limit in Hudson River and Lake Ontario.

Health Effects due to chlorinated hydrocarbons

The ill-effects of PCBs on human beings can be grouped in to two as acute and chronic disorders.

Acute disorders

The disorders will vary with the organ it affects.

Central nervous system: dizziness, headache, tremors, seizures and coma.

Cardio Vascular System: Cardiac irregularities. hypertension

Respiratory system: Difficulty of breathing

Gastro-Intestinal Tract: Nausea, vomiting, diarrhea, salivation

Integument: Rashes, sweating

Chronic disorders

The chronic disorders include.

Endocrine disruption, cardiovascular (hypertension), CNS (neuropathies), cancer and Skin lesions.

BIOMAGNIFICATION BY HEAVY METALS

Mercury (Hg)

Mercury is a persistent, bioaccumulative toxin (PBT) and is highly mobile. Mercury and its vapor are very difficult to remove and its half-life is 44 and 80 days. Mercury is absorbed in the form of methyl mercury which will readily crosses the placental barriers.

Sources of Hg

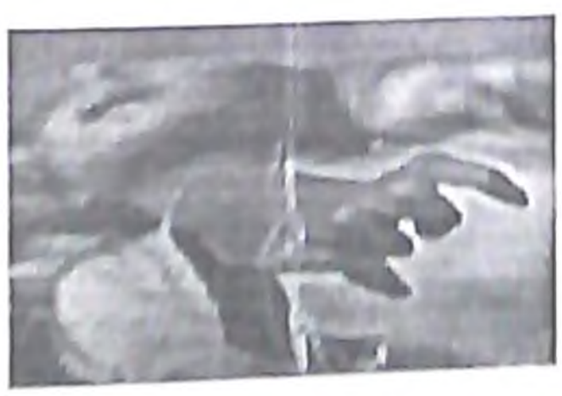
1. Metallic mercury is used in gold mining and in the production of chlorine gas and caustic soda.

2. It is a component of any thermometers, barometers, electrical switches, and batteries.

3. 50% of the content of dental fillings, crowns, etc., is mercury.

Effect of Hg

Organisms (including fish) are most often exposed to mercury in its methyl mercury form. They may experience death, reduced reproductive success, impaired growth and development, or behavioral abnormalities. Top-level predators are at risk for mercury exposure than organisms existing lower on the food chain. The most dangerous effect of mercury on human beings is the Minamata disease. It is a neurological disorder, caused due to severe methyl mercury poisoning. The main source of Hg in aquatic systems is industrial pollution. It was first reported in Japan in 1956. The characteristic symptoms are ataxia, numbness in hands and feet, general muscular weakness, narrowing of field of vision and damage to hearing and speech.



Toxicity due to Hg

The most of the risk from mercury exposure is due to methyl mercury exposure from fish consumption. The level of risk from mercury depends on exposure, including the dose, duration, and type of contact.

Fate of Hg in environment

The natural processes such as ore erosion and volcanic activity release mercury into the atmosphere. The microscopic organisms metabolize the mercury and release methyl mercury. It gets quickly taken up into higher organisms through the food chain. The Levels of methyl mercury in fish are typically hundreds of thousands times greater than those levels in surrounding water.

Regulation against Hg

The Environmental Protection Agency has set a mercury limit for drinking water of 2 ppb and the level of mercury in rivers, lakes, and streams are no greater than 0.144 ppb. They issued guidelines for fish consumption advisories for methyl mercury and have set a maximum methyl mercury level for seafood of 1 ppm.

Methylation of Hg & Biomagnification

The Methylcobalamin, methylate "inorganic" mercury compounds to CH_3Hg^+ (aq).

Then the CH_3Hg^+ (aq) ion is absorbed by plankton, is eaten by small fish. Then they excrete the mercury so slowly, and gradually build up in their systems. The concentration of mercury in the organism increases each time. The final concentration in animals higher up the food chain) can be thousands of times larger than that in the water

Biomagnification by Hg

Mercury is present in small amounts in seawater. It is absorbed by algae (generally as methyl mercury) but only very slowly excreted by organisms (Shurinet al, 2005). The bioaccumulation and biomagnification result in buildup in the adipose tissue of successive trophic levels. For example, herring contains mercury at approximately 0.01 ppm and shark contains mercury at greater than 1 ppm (EPA 1997).

Toxicity of methyl mercury

Methyl mercury readily crosses the blood-brain barrier, due to its formation of a methyl mercury-cysteine complex. It will thus attack the thiol groups of enzymes and inhibit them. It is a cumulative poison, being very slowly removed (excreted) from the body.

Arsenic and biomagnification

Arsenic is called the 'King of poisons' and 'the poison of kings' because, in earlier times, the emperors are used arsenic for suicide. Lead hydrogen arsenate used as pesticides. Its half-life is 1.0778 days. It get oxidized to arsenic trioxide.

Mode of action

1. Arsenic disrupts ATP production by inhibiting enzymes in glycolysis & citric acid cycle
2. Inhibit reduction of NAD+
3. Production of more reactive oxygen species

Effect of Arsenic poisoning

Arsenic causes multisystem organ failure, haemorrhage, and arsenicosis

Cadmium and biomagnification

Cadmium is having very high half-life of 2.9-7.7 years and has very high potential for biomagnification. Inhalation of Cd containing fumes cause severe health problems including cancer. Continuous exposure to cadmium causes Itai-Itai disease, proteinuria, and glucoseuria. The first documented case of mass cadmium poisoning and the occurrence of Itai-Itai disease were reported in Japan. The main symptoms are softening of bones and kidney failures. The meaning of the Japanese word, Itai is pain and the disease is characterized by severe pain in joints and spine.

Symptom of Itai-Itai disease

Mode of action of Cadmium

Cadmium is carried to body through Zn binding proteins and replaces Zn from biological systems. It can bind 10 times stronger than Zn and degrade very slowly.

Table.12.Accumulation of Hg & Cd in gill and liver (mg/g) of *Mugil cephalus*, Cuddalore

Heavy meta	Gill (mg/g)	Liver (mg/g
Hg	0.125	0.053
Cd	0.047	0.042

(Priesser and Strong, 2004)

Table.13. Accumulation of heavy metals in different samples

Heavy metal	Soil (µg g-1)	Sediment (µg g-1)	Straw (µg g-1)	Grain (µg g-1)
Hg	2.6B	1.5	19.6	29.7
As	8.4	10.9	20.9	36.4
Cd	2.1	2.6	32.4	49.4

(Gray, 2002)

Table.14. EPA limits of biomagnifying chemicals in drinking water

Chemical	Allowable limit
Chlorinated hydrocarbons	0.0005 mg/L
Mercury	0.002 mg/L
Arsenic	0.001 mg/L
Cadmium	0.01mg/L

(EPA, 1997)

Regulatory Status

1. Federal Insecticide, Fungicide, and Rodenticide Act
2. Environmental Protection Agency (EPA)

   EPA will fix the residue limit of pesticide or toxicant in every food commodity. The petitioner or person who requires registration should submit the analytical method also for the quantification of residue. EPA takes over FIFRA – expanded registration and safety requirements
3. Food and Drug Administration (FDA)

   FDA is the apex federal agency for the development of pesticide analytical methods specifically for food.
4. Centre for Science & Environment
5. State Pollution control board
6. Food testing laboratories

Why Biomagnification?

The lipid contents of organisms increase with the trophic level . At the same time, the elimination efficiency of the substances decreases with trophic level. The pattern of increased tissue concentration with higher trophic levels could be due to the differences in bioaccumulation.

Management strategies

The management strategies to reduce the residual effect of pesticides and heavy metals include ,

1. Decreased dependency on chemical methods of plant protection: The dependency on the chemical s for pest control is to be reduced and should rely on other methods of plant protection like biological control and integrated management approaches.
2. Banning of pesticides: The pesticides that are harmful to human beings, animals and beneficial organisms are to be banned by law.
3. Eliminate obsolete pesticide stockpiles: Not only the banning of harmful chemicals enough but the obsolete stockpiles of pesticides in the pesticide depot are to be eliminated.
4. Efficient monitoring system: Before recommending for wide use of a Particular chemical, its residual effects on living organisms are to be monitored.

CONCLUSION

1. Not all the pollutants have the potential for biomagnification.
2. The pollutant should be long-lived, bioaccumulated and fat soluble for biomagnification to occur.
3. Chlorinated hydrocarbons and heavy metals have the highest capacity for biomagnification

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DISCUSSION

1. What is the difference between biomagnification and bioaccumulation?

Biomagnification is the accumulation of a chemical in an organism at higher levels than are found in its own food. Bioaccumulation is an increase in the concentration of a chemical over time in a biological organism compared to the chemicals concentration in the environment.

2. Why the milk of mammals is tested to know the biomagnification effects?

Usually the milk of mammals is tested to know the effect because milk is rich in fat and the lipid or fat soluble chemicals have very high potential for biomagnification.

3. Although a heavy metal, Arsenic is not as problematic as mercury and cadmium?

Arsenic is not as persistent as Hg and Cd, because its half life is less i.e. 1.075 days.

4. Why the concentration of certain chemicals increases along the food chain?

The concentration of chemicals increases along the food chain, because there is a lot of energy loss at each level of food chain and therefore, a predator must consume many preys including all of the lipophilic substances.

5. What is meant by lipid partitioning?

Fat-loving or lipophilic chemicals pass into organism's cells through fatty layer of cell membranes more easily than water soluble chemicals. Once inside the organism, these chemicals may move through numerous membranes until they are stored in fatty tissue and begin to accumulate.

6. When do the fat soluble chemicals will become problematic in the animal body?

The storage of toxic chemicals in fat reserve serves to detoxify the chemical or at least removes from harms way. However, when the fat reserves are called upon to provide energy for an organism, the materials stored in the fat may be re-mobilized within the organism and may again be potentially toxic. If appreciable amounts of a toxins are stored in fat and fat reserves are quickly used, significant toxic effects may be seen from re-mobilization of chemicals.

7. What are teratogens?

Teratogens are dangerous at very low concentration far below their direct toxic effect because abnormal cell growth excert a form of biological magnification.

KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara

Ag.Chem.752-Seminar

Topic: Biomagnification by chlorinated hydrocarbons & heavy metals

Student: C.J.Bindhu (2006-21-112)
Time : 11.15 am, 06.07.2007
Venue: Conference Hall
(Attached to library)

Abstract

Biomagnification is a process that results in the accumulation of a chemical in an organism at higher levels than that are found in its own food (Stiling, 1999). The concept of biomagnifications traces back to Rachel Carson's book, 'Silent spring', published in 1962. Biomagnification results from a dynamic equilibrium between exposure from outside environment and uptake, excretion, storage and degradation within an organism (Le Blanc, 1995). In order for biomagnifications to occur, the pollutant must be, (i) long lived (ii) mobile (iii) fat soluble, and (iv) biologically active. Chlorinated hydrocarbons like DDT and certain heavy metals like Mercury (Hg), Arsenic (As) and Cadmium (Cd) possess these characteristics and have very high potential for biomagnification (Fisk *et al.*, 2002).

The elimination efficiency of the substances decreases with trophic level, because larger organisms have relatively less surface area to process and excrete toxic substances. Thus the pattern of increased tissue concentration with higher trophic levels could be due to these differences in bioaccumulation (Gray, 2002).

The heavy metals, which have absolute environmental persistence, generally have shorter biological half-lives, exhibit less biological magnification than DDT, and have produced less injury to wild life. Since each individual requires time to accumulate a substantial concentration of a pollutant and because a particular food chain may involve several species, there may be a delay in the onset of injury associated with biological magnification (Suedel *et.al.*, 1994)

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CROP REGULATION IN MAJOR TROPICAL TREE FRUITS

By

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(2006 – 22 – 101)
Department of Pomology and Floriculture

SEMINAR REPORT

Submitted in partial fulfillment of the requirement for the course

Hort. 751 – Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 650 656, Kerala

2007

DECLARATION

I hereby declare that the seminar report entitled "Crop regulation in major tropical tree fruits" is a record of the seminar presented by me during the course (11.05.2007) and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
20.06.2007

(P.S.Manoj)
(2006-22-101)

CERTIFICATE

Certified that the seminar report entitled "Crop regulation in major tropical tree fruits" is a record of the seminar presented by Sri.P.S.Manoj (2006-22-101) under my guidance and that this report has been prepared by him independently.

Vellanikkara
20.06.2007

Dr.Sarah T George
Major Advisor

CONTENTS

CROP REGULATION IN MAJOR TROPICAL TREE FRUITS

1. INTRODUCTION

India is the leading producer of fruits in the world with an annual production of 43 million tonnes from an area of 4 million hectares contributing 9-10% of world fruit production (Radha and Mathew, 2007). Fruit crops play a significant role in the economic development, nutritional security, employment generation and overall economic growth of our country. As most of the fruit crops are woody perennial having long pre-bearing age, they require intensive cultivation with high investment. The ultimate aim of fruit culture is to increase the production of quality fruits from unit area. This involves regulation of cropping by understanding the physiology of reproduction along with advances in production technology of these crops.

The primary objective of crop regulation is to force a tree for its rest and to produce profuse blossom and fruits during desirable seasons. It also aims to regulate a uniform and good quality fruit to maximize the production as well as profit to the grower (Singh, 2001). Cultural methods, canopy management and chemical means are generally employed to manipulate cropping in tropical tree fruits. Main cultural methods followed include withholding irrigation, root exposure and root pruning, proper nutrition and irrigation, mulching and smudging. Pruning, flower and fruit thinning, high density planting, use of dwarfing rootstocks, bending, pollarding etc. are also utilized to regulate cropping.

Ever since the discovery that the plant hormones have the ability to influence several physiological processes in plants, attempts have continuously been made to manipulate the growth and development process in different fruit plants to streamline the production. As the years passed, besides natural compounds, a number of synthetic compounds were also found to have growth regulatory roles, which are now collectively known as bio-regulators. Because of the diverse effects, growth regulating chemicals can beneficially be utilized at

various stages of plant growth and development. Bio-regulators such as NAA, GA, ethephone etc. are utilized to manipulate the growth and developmental process in different fruit plants (Rao, 2001).

The important crop regulation practices followed in the major tropical fruits of mango, guava, sapota and jack are presented here under.

2. CULTURAL METHODS

2.1 Bahar treatment in guava

There are three distinct flowering seasons in guava 1) Spring (*Ambe bahar*), 2) Rainy (*Mrig bahar*) and 3) Autumn (*Hasth bahar*) with corresponding harvesting period, rainy, winter and spring (Singh and Kumar, 1993; Mitra and Sanyal, 2004). Rainy season crop is rough, insipid, poor in quality and is attacked by several insect pests and diseases. Winter crop is superior is quality, comparatively free from pests and diseases and have long storage life, fetching more price in market. *Hasth bahar* or spring crop is not very common. The fruits harvested during February to April are of good quality but yield in general is very poor (Table 1)

Table 1. Flowering and harvesting seasons in guava

Flowering	Harvesting	Characteristics of crop
Spring (March-April) (*Ambe bahar*)	Rainy season	Rough, insipid, poor in quality, affected by several insect pests and diseases
Rainy (June-July) (*Mrig bahar*)	Winter	Superior in quality, comparatively free from pests and diseases, long storage life, fetch more price in market
Winter (October-November) (*Hasth bahar*)	Spring	Good quality but poor in yield, not very common

Since *Mrig bahar* or winter crop yields fruits of superior quality, this is preferred to rainy season (*Ambe bahar*) crop. To obtain heavy flowering and fruiting for winter crop, certain crop regulation methods are adopted, the practice being called as Bahar treatment. This generally includes withholding of irrigation and root exposure and root pruning.

2.1.1 Withholding irrigation

Withholding irrigation from December to June or until beginning of monsoon depending on prevailing condition at a particular location has been recommended in Southern Peninsular India (Mitra and Sanyal, 2004). This leads to a rest period during which accumulation of food materials take place in branches. During June, the trees are manured and followed by irrigation. The trees thus treated produce profuse shoot growth followed by heavy flowering leading to enhanced yield in winter season.

2.1.2 Root exposure and root pruning

Root exposure followed by root pruning was also recommended to suppress rainy season crop so as to get a good winter crop under West Indian conditions (Kaul, 1974). This operation involves, exposing of feeding roots and pruning fibrous ones to regulate cropping. Upper soil surrounding trunk, about 90 to 120 cm diameter is removed to expose roots. After about 3–4 weeks, exposed roots are covered with soil followed by manuring and irrigation.

2.2 Mulching in mango to reduce fruit crop

In mango, in spite of profuse number of panicles and a very high initial fruit set in the 'on' year, the ultimate retention and harvestable and marketable produce is phenomenally low primarily due to heavy fruit drop (Chadha, 1993). The extent of fruit drop is as high as 90 to 99 per cent which occurs in the initial three to four weeks after set as reported by Iyer and Subramanyam (1972). Sanyal and Maity (1989) and Bhowmick and Banik (2005). The intensity of fruit drop with varietal variation depends, to a major extent, on climatic factors and apparently is of the same magnitude in 'on' and 'off' years. This fruit drop in mango assumes an important facet having a direct bearing on the economics of the crop.

The extent of fruit drop can be reduced significantly by various cultural practices and mulching is one of the effective operations. Sawke *et al.* (1990) obtained yield increase up to 2 per cent by using polythene mulch over control in Alphonso.

2.3 Irrigation in mango to reduce fruit drop

Bhambid *et al.* (1988) observed significant effect of irrigation on the reduction of fruit drop in 28 year old Alphonso. Irrigating the trees twice during flowering with 1500 litres of water per tree significantly increased the yield to the extent of 2.84 per cent.

2.4 Proper nutrition in mango to reduce fruit drop

Yacob (1987) observed that application of fertilizers and flower inducer increased yield in mango. Irrigation further increased yield from 60 to 136 fruits/harvest and decreased fruit drop from 590 to 197 fruits/tree.

2.5 Smudging or smoking in mango to induce flowering

Induction of flowering in mango through smudging (continuous exposure of trees to smoke from burning leaves) is an age-old practice in the Philippines (Gonzales. 1923). Only branches which have attained sufficient age or ripeness to flower respond to smudging (Acala and Pedro. 1935). A mango tree is ready for smudging if it has an appearance of suspended growth. Under Indian conditions. through this has been tried. results are not that much encouraging due to differential varietal response (Cheema *et al*. 1954).

3. CANOPY MANAGEMENT

3.1 Pruning

Specific objectives of tree pruning are removal of surplus. diseased. dead. criss-cross branches and water sprouts. improvement of fruiting wood by regulating floral bud production and opening up of tree centres facilitating sunlight penetration in turn improving fruit colour. Pruning methods are divided into two groups. namely heading back and thinning out. Terminal portion of twigs. shoots or branches are cut in heading back. which stimulates development of more growth. In thinning out. a few twigs. shoots or branches are removed completely. which induces fruit bud formation in remaining plant parts.

Annual regular pruning in all types of fruit crops involve removal of broken. diseased. criss-cross. dried and unwanted shoots. limbs and branches. Methods of pruning depend mainly on nature of trees. precisely the bearing habit. Plants producing flowers and fruits on current season shoots respond positively to

regular pruning whereas those bearing fruits in last season or old shoots should not be pruned regularly since it leads to reduction in flowering and fruiting shoots.

Annual pruning and training of fruit trees have been used as an effective cultural technique for regulating the cropping pattern to increase the fruit yield and to improve the fruit quality of many fruit crops.

3.1.1 Mango

Mango being an evergreen tree, has been assumed to be unresponsive to pruning unlike the grapes or the fig which are pruned regularly to induce and regulate growth, flowering and cropping (Shanmugavelu and Saidha, 1993). Pruning is particularly effective in trees which bear fruits on new shoots and thus it is done to induce healthy current season shoots from older wood. In mango, fruits are borne largely on the previous years' shoot but occasionally, in some varieties, new shoots as well as very old limbs also bear flower panicles. Naik (1948) reported that pruning of mango trees may not be successful to regulate bearing as the new growth turns out to be purely vegetative. However Iyer and Subramaniam (1975) reported that pruning the one year old shoots at the base induced flowering

A large number of trees become unproductive in due course because of dense and over crowded canopies, which reduce light interception and utilization by the photosynthetic surfaces (Jackson, 1980). Favourable effects of different intensities of pruning in mango on light interception, chlorophyll content in leaves and yield have been reported (Rao, 1971; Rao and Khader, 1979; Shanmugavelu and Selvarajan, 1985)

A typical method of severe pruning is recommended from Tamil Nadu Agricultural University for aged unproductive mango trees to make them productive. In this method, opening of the tree centre is done by removing a few internal branches once and annually during August-September, the terminal whorl of shoots are thinned out retaining only one or two healthy ones. This type of pruning promotes increased yield in mango varieties Mulgoa and Baneshan (Rao and Shanmugavelu, 1976). Increased yield as a result of severe pruning, which facilitates light penetration in the canopy during fruit growth was also reported by Durand (1997) and Lal *et al.* (2000). Swaroop *et al.* (2001) reported that pruning in

on year during July-August increased the emergence of more number of shoots. Pruning is practiced for tree size control in high density orcharding. Pruning to a moderate level after harvesting helps to restrict tree size without adversely affecting fruit yield (Pratap *et al.*, 2003).

Lal *et al.* (2000) conducted a study on rejuvenation of old and unproductive Dashehari mango orchard with major emphasis on pruning to rebuild canopies and restore productivity of trees. The experiment was conducted on 45 year old Dashehari trees planted at 8 m x 8 m spacing. Pruning was done in the month of December with five pruning severities i.e. first order branches emerging from main trunk, second order branches emerging from first order branches, third order branches emerging from second order branches, fourth order branches emerging from third order and fifth order branches representing a height of 4 m approximately, which represents terminal branches. The branches were pruned leaving 40-60 cm. Besides, intermingling, dried and diseased branches were also removed.

It was observed that, the growth of emerging shoot on pruned branches was influenced by severity of pruning. Length and growth of emerging shoots were more in first, second and third order pruned trees than in fourth and fifth order treatments whereas it was minimum in the control. Fruit yield was higher in fourth and fifth order pruned trees than the control (Table 2). No fruit yield was obtained in first and second order pruned trees during first and second year of fruiting, but yield was obtained in an increasing trend in subsequent years. Cumulative fruit yield of six years indicated that fourth order pruned trees gave the highest yield whereas it was lowest in the control.

Table 2. Yield pattern of rejuvenated Dashehari trees

Pruning severity	Fruit yield per tree (kg)						Cumulative yield (kg)
	1st year	2nd year	3rd year	4th year	5th year	6th year	
1st order	-	-	27.50	35.70	37.80	33.68	134.68
2nd order	-	-	43.00	37.48	47.42	42.63	171.41
3rd order	29.00	39.00	52.00	42.50	46.30	44.49	253.29

4th order	37.50	42.30	75.00	82.00	57.84	46.28	330.92
5th order	40.60	46.70	59.50	79.50	53.80	41.25	321.35
Control	20.16	24.74	17.32	27.90	23.40	18.29	131.81

3.1.2 Guava

Guava bears flowers on current season's growth. Therefore a light annual pruning is considered to be essential to boost up new vegetative shoot emergence (Mitra and Sanyal, 2004). Bearing trees are pruned to avoid over-crowding in orchard. Root suckers, water sprouts and criss-cross branches are to be pruned regularly (Mitra and Bose, 2001). After harvest, tips of 20 to 30 cm length from past season shoots are pruned, usually during September and February. The length of flowering shoots tended to decrease with delay in time of pruning but increased with the increasing severity of pruning irrespective of season (Bajpai *et al*., 1973; Dhaliwal *et al*., 1998). An increase in shoot length due to severity of pruning might be due to elimination of growing points which in turn encouraged the length of remaining shoots (Dhaliwal *et al*., 1998).

The intensity of pruning has considerable effects on bearing of guava trees. Bajpai *et al* (1973) reported that severe pruning has an adverse effect on productivity. Removal of terminal 15 and 30 cm of branches adversely affected flower production and yield (Sheikh and Hulmani, 1993, 1994). Time of flowering in guava can also be altered by suitable pruning (Sundrarajan and Muthuswamy, 1964). With the increasing pruning intensity, the rainy season yield decreases and one-leaf-pair pruning proved superior to other pruning treatments for flower-bud initiation and fruit yield of the winter crop (Lal *et al*., 1996). Duration from shoot emergence to flower bud emergence and anthesis to fruit maturity decreases with increase in severity of pruning as well as in delaying pruning time. However, duration from flower bud emergence to anthesis decreases with increase in intensity of pruning but remained unaffected by the date of pruning (Dhaliwal *et al*., 1998).

3.1.3 Sapota

Sapota tree has a thick central stem and well balanced distribution of branches. The tree crown grows to a uniform shape. After 6 to 7 years of growth, lower most branches up to a height of one metre are removed. Over crowded, shaded, dried and diseased and criss-cross branches are pruned as per necessity. New growth and flowering occur simultaneously and it is a mixed type of bearing habit. Flowers and fruits appear in the leaf axils on the new growth and hence pruning of branches should not be done (Sulladamath and Reddy, 2002).

3.1.4 Jack

No regular training and pruning is given to jack. But it is desirable to maintain a single stem up to a certain height (Radha and Mathew, 2007). Muthulakshmi (2003) studied the effect of different pruning treatments on the yield and quality of jack. The treatments comprised of removal of 25, 50 and 75 per cent of 2^{nd}, 3^{rd} and 4^{th} order branches. Pruning resulted in reduction in number of days taken for flushing and flowering with decreasing order of severity. On pruned trees, more number of shoots with increased vigour was emerging with increasing severity of pruning. No significant difference in duration of male and female spike development, flowering and fruiting span was noticed among various pruning treatments. However all the pruning treatments showed a reduction in span of flowering and fruiting.

All pruning treatments showed enhanced per cent of fruit set during first and second year of pruning. Medium pruned trees showed maximum increase in fruit set (3-6%) than severe (1-2%) and light pruned trees (2-3%).

During first year of pruning all the pruning treatments showed reduction in fruit number and yield and the reduction was increasing with increasing severity of pruning (Table 3). In the 2^{nd} and 3^{rd} year of pruning, an increase in fruit number and yield was observed.

Table 3. Effect of pruning on fruit yield of jack

Treatment	Fruit yield (kg)			
	Before pruning	1st year of pruning	2nd year of pruning	3rd year of pruning
Removal of 25% of n_4 order	550.49	550.15	555.55	557.59
Removal of 50% of n_4 order	513.17	439.23	555.21	530.75
Removal of 75% of n_4 order	602.28	553.00	776.44	700.57
Removal of 25% of n_3 order	467.81	434.31	488.87	472.23
Removal of 50% of n_3 order	487.64	379.91	652.90	546.27
Removal of 75% of n_3 order	507.86	278.60	460.37	621.79
Removal of 25% of n_2 order	492.42	336.99	699.82	602.87
Removal of 50% of n_2 order	496.02	216.91	419.92	604.03
Removal of 75% of n_2 order	482.92	149.57	385.95	542.83

3.2 Bending in guava

In medium aged trees when the trees reach more height, the branches will grow more or less straight and such upright growing branches will not produce axillary growth leading to reduction in yield. These upright branches should be bent and their terminal portions should be buried inside the soil or tied strongly to pegs driven in the soil around the trees. By this, the dormant buds are accelerated and induced to produce new flush which will result in production of flowers leading to higher yield (Veeraragavathatham *et al*., 2004).

3.3 Pollarding or dehorning

When the trees become very old, there is practically no production of new flush. In such a situation, pollarding is resorted in which big branches are cut back leaving 30 cm length base at the places of their origin. The new growth in the following season will put forth profuse flowering and give high yields (Veeraragavathatham *et al*., 2004).

3.4 Flower and fruit thinning

A proper balance between new shoot growth and flower bud formation is a must for maintaining health of bearing trees. In general, thinning refers to partial removal of flower buds, flowers and small fruits. This practice helps to increase number of marketable fruits, fruit size, improve colour of fruits and eating quality and more over helps to decrease tendency for alternate bearing. Thinning can be practiced either manually or through chemical means.

3.4.1 Guava

3.4.1.1 Manual thinning

Manual thinning of rainy season flowers to promote winter crop though very effective, is not in practiced as it is cumbersome, laborious and uneconomic (Mitra and Sanyal, 2004). It can be done on a small scale, but on a commercial scale, chemical flower thinning is practiced.

3.4.1.2 Chemical thinning

To reduce rainy season crop and to increase winter crop, two sprays with urea (15 %) in April-May at 15 days interval was quite effective (Singh *et al.*, 1994). Among growth regulators, Naphthalene acetamide (50 ppm) and NAA (1000 ppm) were the most effective to regulate cropping in guava (Kumar and Hoda, 1977, Tiwari *et al.*, 1992). Sprays with urea (10%) in Allahabad Safeda and potassium iodide (0.03%) in Sardar guava are the best for inducing heavy winter crop (Singh and Singh, 2000).

The varying degree of success with different chemicals may be due to cultivars, tree condition, soil type and environment. Most of the workers are of the opinion that chemical thinning is economic and it increases the winter yield as well as improves fruit quality.

3.5 High density planting (HDP)

The modern trend in fruit culture is to increase total production per unit area, though individual tree yield gets reduced, due to more number of trees accommodated. Suitability of crops and varieties to HDP should be evaluated and techniques to control size of trees should be followed for adopting this system of orcharding. Using dwarfing rootstocks, adopting appropriate pruning techniques

and using growth regulating chemicals are other practices adopted to control tree size.

3.5.1 Mango

Establishment of mango orchard is a long-term investment and trees continue to produce fruits for longer years than many other fruit crops. Poor yield experienced by the Indian farmers are partly due to the wide tree spacing of conventional orchards with spacing ranging from 10 to 12 m between trees in rows and between rows (Majumdar and Sharma, 1990). This often results in underutilization of the interspaces during the early stage of orchard life. This makes mango orcharding unattractive, particularly on small holdings because of long gestation period before giving returns. High density planting makes maximum use of land to achieve high yields in the early periods of orchard life along with ease in its management (Ram, 1993).

Control of excessive vegetative growth in the tree for increased productivity is the major principle of high density orcharding (Ram, 1996). The horticultural methods most commonly utilized to control tree growth are training, pruning, use of dwarfing rootstock and growth regulators. Training begins when the tree is first planted and continues throughout its productive life. Once the tree is mature, excessive growth can be regularly removed by pruning to provide a short term or immediate benefit. Bearing behaviour of the cultivar, precocity of bearing, planting density, tree form, use of growth retardants etc. are other factors to be considered in HDP of mango (Majumdar *et al*., 1982; Ram and Sirohi, 1991).

Concept of HDP in mango came into practical shape by the advent of a dwarf hybrid Amrapali at IARI and the dehorning technique developed at G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar (Ram and Sirohi, 1989). The spacing recommended for Amrapali is 2.5 m x 2.5 m accommodating 1600 plants per hectare. In the case of Dashehari, a closer spacing of 2.5 m x 3 m (1,333 plants/ha) is suggested from Pantnagar along with the dehorning technique.

In the case of HDP of Dashehari, the first training treatment was given after one growing season. Each plant was allowed to maintain single stem having

upward growth and the top of the main stem was removed by cutting out at 60 cm height from the soil surface in 2nd year. In the 3rd year, 4-5 primary branches were retained on main stem. Thereafter, shoots arising from secondary and tertiary branches were given 5-10 cm deep pruning soon after harvest. No pruning was given in non-flowering and fruiting years. Spray of 1% urea combined with any copper fungicide was done soon after pruning. Shoot pruning and spray of fungicide was not done in normal density orchard. Pruning induced new shoot production within one month. In general, every pruned shoot produced one new shoot but occasionally more than one shoot was also produced. In the 11th year, about 75% branches of tree canopy were dehorned in high density orchard and the remaining 25% branches were dehorned in 12th year after crop harvest. Dehorning was done to avoid overlapping of trees in the high density orchard. Soil application of paclobutrazol was also combined with pruning to induce regular flowering and also for tree size control. In general, 3 m tree height and 10 m circumference was maintained in HDP.

Arka Aruna, Sindhu and Paiyur-1 are some other varieties suitable for HDP. A spacing of 3 m x 3 m accommodating 400 plants per ha is recommended for Arka Aruna and Sindhu.

At GBPUAT, a study was conducted to compare the performance of Dashehari under normal density (12 m x 12 m) and high density (2.5 m x 3 m) system (Ram, 1996). The recommended practices were followed for both the system. Trees in both the densities started fruiting in fifth year of planting and a similar rise and fall occurred in annual yields of trees in both the densities up to nine years. However trees of normal density started producing more fruits per tree than high density from 10th year onwards because of tree size in high density was reduced by dehorning and pruning to avoid overlapping of branches and also to induce growth after crop harvest in fruited shoots. Annual fruit yield per hectare, however, was much higher from high density orchard starting from fifth year itself due to higher number of trees per hectare (1333 tree/ha) than that of normal density (69 trees/ha) (Fig. 1)

The study revealed that, high density planting not only produced higher annual yields per hectare (7.995 to 9.411 t) in the early stage of orchard life but also produced about 11 times higher yields than that of normal density (0.805 to 0.8475 t/ha) (Fig. 1, 2 and 3). There was no difference in the quality of the fruits in both the densities. Thus, high productivity without loss in quality from high density orchard was obtained during 19 years of high density orchard life. The annual fruit yield increased further beyond 16 years of tree age regularly up to 19 years of study as a result of paclobutrazol applications and as high as 32 t (40 kg/tree) fruit yield was obtained from high density trees compared to 5 t/ha from trees of normal density in 19th year.

High density orcharding with regular bearing Amrapali (1600 plants/ha) yielded 11.5 t/ha in the fourth year (Majumdar *et al.*, 1982). The productivity increased to 24.96 t/ha in the 11th year giving average yield of 12.2 t/ha (Table 4).

Table 4. Comparison of varieties under normal and HDP

System	Variety	Spacing (m)	Plant population (plants/ha)	Yield (t/ha)
Normal	Dashehari	12 x 12	69	1.60 (10th year)
HDP	Dashehari	2.5 x 3.0	1333	18.60 (10th year)
HDP	Amrapali	2.5 x 2.5	1600	24.96 (11th year)

Anbu *et al.* (2001c) compared the performance of five planting systems with varied planting densities under HDP in Neelum variety of mango. In the study, square, hedge row, double hedge row, paired row and cluster planting systems were followed (Table 5). During the fifth year of study, there were significant differences in the fruit yield (number of fruits and fruit yield per plot). Highest mean plot yield of 54.2 kg and 70.6 kg was recorded respectively for 5th and 6th year in double hedge row with 20 plants per plot. This was followed by cluster planting (16 plants per plot) with a mean yield of 36.0 kg and 49.0 kg respectively for 5th and 6th year. In all planting densities, fruit yield increased with increase in number of plants per unit area, irrespective of the system of planting.

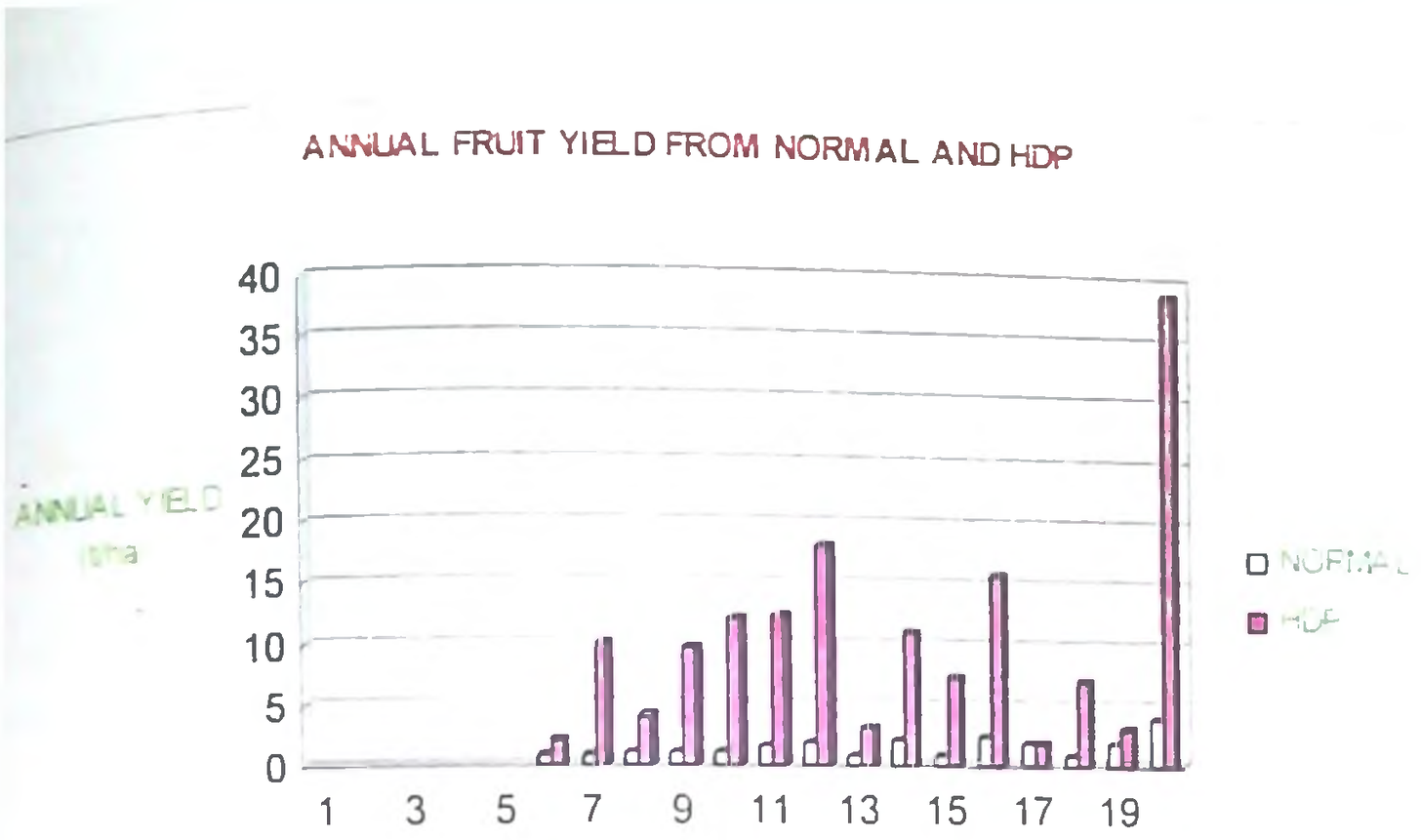

Fig. 1. Annual fruit yield of mango from normal and HDP

Fig. 2. Cumulative fruit yield of mango from normal and HDP

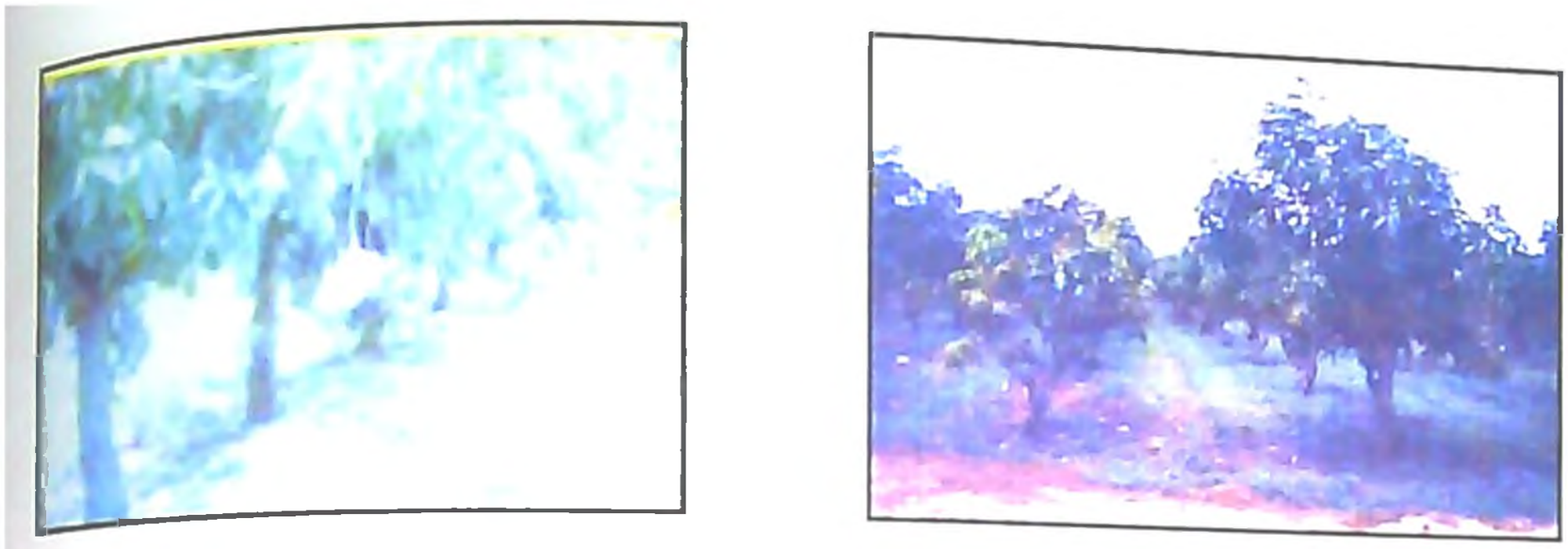

Fig. 3. High Density Planting in mango

Sapota: PKM -3

Sapota CO -3

Jack: PLR - 1

Fig. 4. Varieties suitable for High Density Planting

Table 5. Effect of HDP and planting systems in mango cv. Neelum on fruit yield

Planting system	No. of trees per 30 m x 30 m plot	Yield per 30 m x 30 m plot			
		5th year		6th year	
		No. of fruits	Weight of fruits (kg)	No. of fruits	Weight of fruits (kg)
Square (204 plants/ha)	9	144.60	29.00	183.20	36.60
Hedge row (340 plants/ha)	15	150.60	34.60	220.20	44.80
Double hedge row (453 plants/ha)	20	255.70	54.20	332.60	70.60
Paired row (272 plants/ha)	12	149.20	31.20	197.80	38.20
Cluster planting (365 plants/ha)	16	183.40	36.00	234.40	49.00

3.5.2 Guava

Traditionally guava is planted at a spacing of 5 m x 5 m or 6 m x 6 m accommodating 278 to 400 plants per ha in a square system of planting. The distance between trees has a profound influence on growth, yield, fruit quality and on nutritional level of leaves (Mitra and Bose, 2001).

In guava, the total number of fruits per plant and the number of fruits of different grades were not much influenced at the early stage by plant density, but high density planting had an adverse effect on the quality of fruits. The contents of total soluble solids, total sugar, reducing and non-reducing sugars and ascorbic acid were significantly reduced, while acidity increased with higher plant density. High plant density also caused decrease in fruit weight and size, but the yield per unit area was increased considerably (Kundu *et al*., 1993, Mitra *et al*., 1984). Chundawat *et al* (1992) observed that planting of Allahabad Safeda trees at 6 m x 2 m and managing them by hedge row system produced highest yield per hectare with better quality fruits. However per tree yield was reduced as compared with 6 m x 6 m, while maximum yield per hectare was obtained from trees spaced at 2 m x 1 m followed by those at 6 m x 6 m (Kalra *et al*., 1994). Kumar and Singh (2000) compared different planting system-cum-densities such as square system (400

plants/ha), hedge row (800 plants/ha), double hedge row (530 plants/ha) and paired planting (1000 plants/ha) and concluded that paired planting is most beneficial under rainfed conditions.

In ultra high density planting (73,000 plants/ha), trees grew in height with few laterals, although the lowest density (27,000 plants/ha) produced highest yield (Mohammed *et al.*, 1984). Studies conducted at IARI, New Delhi showed that Allahabad Safeda grafted on Aneuploid No.82 grows dwarf and these are planted at a distance of 3.3 m x 3.3 m (1076 plants/ha) successfully (Sharma *et al.*, 1992).

Pusa Srijan is identified as a potential dwarfing root stock for high density planting in guava using which tree size is reduced to 50% and they show tolerance to guava wilt (Sharma, 2004). Edward and Shankar (1964) reported that tree size in guava could be reduced by employing *Psidium friedrichsthalianum* as a rootstock.

3.5.3 Sapota

The normal spacing followed for sapota is 8-10 m both ways depending on planting materials and soil (Sulladamath and Reddy, 2002).

Sapota varieties PKM-3 and CO-3 released by TNAU, suitable for HDP can be planted at a spacing of 5-6 m (Fig 4). Studies have also indicated that these varieties could be planted at a lesser spacing in initial years and removing alternate rows when canopies overlap.

In a HDP trial in sapota cv. Kalipatti, a closer spacing of 5 m x 5 m produced fruits of 13.61 t/ha/year compared to 5.05 t/ha/year under wider spacing of 10 m x 10 m. In PKM-1 variety, a closer spacing of 8 m x 4 m produced 7113.6 kg/ha of fruits (312 plants/ha) as against 4867.2 kg/ha in 8 m x 8 m (156 plants/ha) and 4720.0 kg/ha in 10 m x 5 m (200 plants/ha) (Veeraragavathatham *et al.*, 2004).

3.5.4 Jack

Jack is normally planted at a spacing of 12-15 m (KAU, 2002). TNAU has developed a high yielding variety of jack, PLR-1, suitable for HDP (Fig 4). It is having medium height and is less spreading also. It also produces off season crop during October-December (Veeraragavathatham *et al.*, 2004).

4. PROPAGATION TECHNIQUES

4.1 Propagation techniques standardized in major tropical tree fruits at KAU

Quality of planting material is one of the factors determining success of fruit culture. Healthy, vigorous, disease and pest free nursery plants of the choice variety should be selected. Rootstock used in grafted plants and quality of mother plants should be ascertained as far as possible. Inorder to achieve these objectives, Kerala Agricultural University has standardized various vegetative propagation techniques for major tree fruits.

4.1.1 Mango

4.1.1.1 Epicotyl grafting

The epicotyl grafting technique in mango was standardized by Ratan (1985) and Radhamony (1987) as detailed below:

- Age of rootstock - 5- 10 days
- Age of scion 3-4 months
- Precuring - 10 days
- Length of scion - 8 cm
- Height of grafting – 6- 8 cm
- Time - July-August

The grafted plants were kept in mist chamber, cambial bridge was established within 45 days and union was completed in 3 months.

4.1.1.2 Softwood grafting

Softwood grafting technique is mango was standardized by Savithri (1990) as detailed below

- Age of rootstock - 2 months
- Age of scion 3-4 months
- Defoliation of scion 10 days prior resulted in maximum survival
- Height of grafting - 10 cm

4.1.1.3 Veneer grafting

Dhungana (1984) standardized veneer grafting technique in mango

- Age of scion - 6 months
- Age of rootstock - 14 months
- Time - September
- Mundappa, Alphonso, Bangalora and Bennet Alphonso are more adapted for veneer grafting

4.1.2 Jack

4.1.2.1 Epicotyl and softwood grafting

Jose (1989) standardized epicotyl and softwood grafting techniques in jack.

- Age of the rootstock
  - epicotyl - 5 days
  - Softwood - 2 months
- Age of scion – 3 - 4 months
- Precuring - 10 days before
- Height of grafting
  - epicotyl - 5- 6 cm
  - softwood - 15 cm

4.2 Propagation technique and yield in mango

Since method of propagation influences yield, a study was taken at Central Institute of Subtropical Horticulture (CISH) to compare the effect of different propagation methods on yield of mango (Table 6).

Table 6. Performance of mango trees under different propagation methods

Propagation method	Cumulative yield per tree in kg (for 13 years)
Inarch grafting	449.09
Veneer grafting	732.28
Patch budding	604.09
Shield budding	627.49

In the study, veneer grafting was found to be best method giving a cumulative yield of 732.28 kg per tree for 13 years (CISH, 2000)

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5. ROOTSTOCK FOR CANOPY MANAGEMENT IN MANGO

Seedlings are used as rootstock in mango. Polyembryony is exhibited by certain varieties mainly confined to west coast region. Exploitation of these nucellar seedlings as rootstock for superior varieties to get uniformity is being tried.

Marie (2001) studied the dwarfing potential of various indigenous rootstocks of mango. Varieties Vellaikolumban, Kalepady and Olour were placed in a group with low growth potential. Moovandan, Chandrakkaran and Mundappa were placed in a group with high growth potential. Velocity of sap flow/transpiration stream in grafts with Moovandan, Chandrakkaran and Mundappa rootstocks was found to be highest and this was found to be lowest in Vellaikolumban, Kalapady and Olour. Babu (2005) also characterized the polyembryonic behaviour of Moovandan and Vellaikolumban varieties.

Effect of various rootstocks on growth of mango was studied by several workers (Table 7). Vellaikolumban, Olour, Langra and Mylpelian rootstocks were found to induce reduction in canopy size of various varieties, while Moovandan was found induce vigorous growth in several varieties.

Table 7 . Effect of rootstock on growth of mango

Variety	Rootstock	Effect	Reference
Dasheharı	Vellarkolamban	Reduction in canopy size	Reddy and Kurien (1993)
Alphonso	Vellarkolamban	Reduction in canopy size	Chadha (2001)
Himasagar	Olour	Reduction in canopy size	Chadha (2001)
Langra	Olour	Reduction in canopy size	Chadha (2001)
Many varieties	Muvandan	Vigorous growth	Pathak and Pathak (2001)
Bombai	Langra	Reduction in canopy size	CHS (2000)
Bombai	Mylepelian	Reduction in canopy size	Singh and Singh (2004)

A study was conducted at CISH, Lucknow to compare the influence of various rootstocks on Bombai variety (Table 8). Maximum reduction in canopy

size was obtained when Langra was used as rootstock. However, highest yield was obtained when Kalapady was used as rootstock (CISH, 2000).

Table 8. Effect of rootstock on growth and yield of mango cv. Bombai

Rootstock	Tree height (m)	Tree spread (m)	Cumulative fruit yield for two years (kg)
Mylpelian	5.91	7.21	232.40
Puttu	5.63	7.60	224.80
Kurukkan	5.70	7.30	202.70
Kalepady	5.90	8.95	237.40
Langra	5.40	5.30	227.20
One tree seeding	6.20	6.81	218.90
Random seedling	5.95	5.56	203.70

5. CHEMICAL REGULATION

Various bio-regulators as well as other chemicals are used in many tropical tree fruits to manipulate flowering, yield and yield attributes. The important accomplishments in this field are presented hereunder.

5.1 Mango

5.1.1 To improve proportion of perfect flowers

NAA 50, 100 and 200 ppm application at flower bud initiation was found to enhance yield in shy bearers (Singh *et al.*, 1965). Kurien and Iyer (1993) observed that soil application of paclobutrazol (10 g a.i/tree) just prior to vegetative flowering improved the number of perfect flowers. Foliar application of boric acid (750 ppm) at late bud swelling stage was also found to increase the proportion of hermaphrodite flowers (Haggag *et al*., 1995).

5.1.2 For flowering and fruit set

Suma (1987) studied the effect of ethephone, NAA and GA on flowering and fruit set in mango. It was observed that 3 sprays of ethephone 200 ppm, at 15 days interval starting from mid September induced early flowering, increased number of inflorescence and yield in Benganapalli, Imam Pasand and

Mundappa varieties of mango. Three sprays of GA 100 ppm at weekly intervals starting from full bloom increased initial fruit set. NAA 40 ppm spray, three times at weekly interval starting from full bloom reduced fruit drop and increased fruit weight.

5.1.3 To reduce fruit drop

Various chemicals as well as plant growth regulators were tried to reduce fruit drop in mango.

5.1.3.1 Chemicals

Urea spray (2–4%) at flowering was found to increase fruit retention in Dashehari mango (Singh, 1977).

5.1.3.2 Growth regulators

The results obtained to control fruit drop by using growth regulators in different varieties at different locations have been summarised in Table 9.

Table 9. Control of fruit drop in mango by plant growth regulators

Growth regulator	Conc. (ppm)	Time of application	Variety	Reference
NAA	20.25	12-25 mm dia fruit size	Alphonso	Gofur *et al.* (1998), Gokhale and Kanitkar (1951)
2,4-D	5, 10, 20, 25, 30, 40, 60	Pea stage	Bombay Green, Dashehari, Langra	Arora and Singh (1964), Gill and Mukherjee (1967), Roy *et al.* (1963), Singh *et al.* (1961)
GA	10	Full bloom	Dashehari	Teaotia *et al.* (1967)
Alar	100	Pea stage	Dashehari	Rao *et al.* (1976)
Planofix	20	Marble stage	Neelum	Aravindakshan *et al.* (1979)
Ethephon	50-200	40 days after flowering	Carbao	Andam (1983)
Paclobutrazol	4 g ai/tree	August	Alphonso	Bhatt *et al.* (1997)

5.1.4 Use of paclobutrazol in mango

Growth retardants, particularly paclobutrazol, are being used to stimulate enhanced or early flowering in mango. Unlike the other classes of growth retardants which are normally applied as foliar sprays, paclobutrazol is usually

applied to the soil due to its low solubility and long residual activity (Davenport and Elisea, 1997). This growth retardant is most efficacious as it reduces shoot elongation and promotes flowering. As a result, paclobutrazol is being promoted to control flowering and vegetative growth in commercial mango orchards of Indo-China, Australia and South Africa (Voon *et al.*, 1991).

The main effects of paclobutrazol are induction of regularity, reduction in tree height, more number of flowering shoots, improved number of perfect flowers, fruit set, retention and growth and ultimately increased yield.

Anbu *et al.* (2001b) studied the effects of paclobutrazol in induction of flowering in mango variety Neelum (Table 10).

Table 10. Effect of paclobutrazol on yield of mango cv. Neelum (12 year old)

Paclobutrazol treatment (days before bud break)	1st year		2nd year	
	Number of fruits/tree	Weight of fruits/tree (kg)	Number of fruits/tree	Weight of fruits/tree (kg)
Control	44.50	10.45	55.00	13.10
5 ml - 120 days	139.50	32.32	225.50	52.60
5 ml - 90 days	380.00	91.65	302.00	72.85
5 ml - 60 days	54.00	12.24	50.50	11.30
10 ml - 120 days	201.50	46.70	259.50	60.25
10 ml - 90 days	316.50	78.80	260.50	64.85
10 ml - 60 days	107.50	24.80	63.50	14.65

Soil drenching of 5 ml of PP^{333}, 90 days before bud break recorded the maximum number of fruits and fruit yield per tree in both the years of application. The next best treatment was 10 ml of PP^{333} applied 90 days before bud break.

Murty *et al.* (2001) studied the influence of paclobutrazol on tree vigour and flowering in mango cv. Alphonso. The treated trees showed inhibition of gibberellin biosynthesis, enhancement in the level of ABA and cytokinin in xylem sap and leaves, increase in phenol content and decline in IAA content of leaves, higher phloem to xylem ratio and decline in xylem sap volume. These results show that paclobutrazol induced inhibition in tree vigour and promotion of flowering in

mango is not only associated with gibberellin biosynthesis inhibiting character of paclobutrazol, but also with its influence on other hormones such as ABA, IAA and also on phenol.

5.2. Guava

Various chemicals used in guava to regulate cropping and to improve quality are summarized in Table 11.

Table 11 . Crop regulation using chemicals

Chemical	Effect	Reference
NAD 50 ppm* and 2,4-D 30 ppm*	Minimise fruit set in rainy season crop, increased fruit weight, number and yield of winter crop	Kumar and Hoda (1977), Mitra *et al.* (1982)
Urea 10%*	Increased fruit weight, TSS, vitamin C and acidity and high yield	Bariana and Dhaliwal (2002)
KI 0.5%*	Reduction in rainy season yield, high winter crop and better fruit quality	Singh and Singh (2000)
DNOC 10 ppm*	Heavy winter crop	Kundu and Mitra (1997)
Ethrel 2000 ppm*	Heavy winter crop	Chandra and Govind (1994)
CCC 500 ppm before flowering	Early flowering, high fruit set, retention and yield	Brahmachari *et al.* (1996)
GA, 30 ppm in January	Increased fruit retention and yield	Rajput *et al.* (1977)

* treatments applied during April - May

5.3 Sapota

In an attempt to get higher fruit set and retention of the set fruits, several growth substances such as GA_3, ethrel, cycocel, planofix and SADH were tried on cultivar Cricket Ball (Das and Mahapatra, 1975). These chemicals were applied in spray solutions before flowering and again at the pea stage. Of all the regulators, SADH at 100 ppm resulted in the highest fruit set and planofix at 300 ppm resulted in the highest fruit retention and largest fruits followed by 100 ppm GA_3.

Kadam *et al.* (2005) studied the influence of growth regulators on flowering behaviour of sapota under semi-arid zone of Maharashtra. Growth

regulators such as CCC, GA and NAA were sprayed on 12 year old trees at fruit bud differentiation, flowering and fruit development stage. Spraying of CCC 400 ppm at fruit bud differentiation stage significantly increased number of flowers per shoot. Percentage of flower and fruit drop was decreased by 100 ppm NAA than 50 ppm GA when sprayed at flowering and pea stages (Table 12). Further, spraying of NAA at pea stage was more effective than spraying at lag phase. Rathod and Amin (1982) have also indicated higher fruit retention with NAA as compared to GA in sapota

Table 12 . Effect of growth regulators on fruit set and yield of sapota (12 year old)

Treatments	Fruit set (%)	Fruit retention (%)	Yield (kg/tree)
Flowering stage			
i) GA 50 ppm	37.64	12.48	35.28
ii) NAA 100 ppm	41.66	12.97	38.23
Fruit development stage			
i) NAA 100 ppm (pea stage)	-	14.38	44.05
ii) NAA 100 ppm (lag phase)	-	11.89	32.07
iii) GA 50 ppm (pea stage)	-	12.90	39.33
iv) GA 50 ppm (lag phase)	-	11.72	31.47

6. CONTROL OF BIENNIAL BEARING IN MANGO

In mango, most of the varieties exhibit a clear pattern of cropping - alternate or biennial - which indicates an year of optimum or heavy fruiting followed by an year of little or no fruiting (Singh, 1995). The year of optimum or heavy fruiting is called as 'on' year and year of little or no fruiting is called as 'off' year. Irregular or erratic bearing indicates that cropping does not follow a systematic pattern, but an optimum crop is available only once in a number of years.

Different workers suggested remedial measures for this serious problem of bienniality in bearing mango. The suggested measures in nutshell are furnished below.

- Proper care and maintenance of trees in a healthy condition by scientific management practices like judicious manuring, irrigation at fruit set and development stage, plant protection measures, intercultural operations etc.

help in reducing irregular bearing though this will not induce regularity in biennial cultivars.

- Deblossoming in the 'on' year to promote flowering in the 'off' year can be practiced successfully. Spraying NAA (500 ppm) at the full bloom stage in 'on' year results in moderate deblossoming with the least reduction in crop load in 'on' year. This consequently favours moderate flowering and cropping in the following year which otherwise would have been 'off' year (Srihari and Rao, 1996). NAA suppresses vegetative tendency on the deblossomed shoots, favouring the fruit bud differentiation in sub-apical buds that unfurl into flowers in the following year.
- Smudging or smoking to induce flowering
- Bio-regulators are also used to induce flowering and to tackle the problem of biennial bearing. Spraying chemicals like ethrel (Rath and Rajput, 1990), KNO_3 at 1-2% concentration (Anbu *et al.*, 2001a; Sharma *et al.*, 1990), calcium nitrate at 4-18% concentration (Rojas, 1996) etc. during initial period of fruit bud differentiation after harvest of 'on' year resulted in induction of sub apical flowering in fruited shoots is varying degrees in the expected 'off' year. There is an impressive response to soil application of 5 g cultar resulting in induction of regularity of bearing in many varieties. This treatment was reported as most effective to induce more number of flowering shoots and to improve fruit set, retention and quality during 'off' year (Singh and Singh, 2003 and Singh *et al.*, 2005).
- Pruning in 'on' year during July-August to increase emergence of more number of shoot (Swaroop *et al.*, 2001)
- Pruning to open the centre of the trees induces regular flowering in old unproductive trees
- Growing of regular bearing varieties such as Bangalora, Neelum, Rumani, Mallika, Amrapali, Ratna and Sindhu

7. INDUCTION OF OFF SEASON FLOWERING IN MANGO

Induction of flowering out of normal season has been tried in several countries. In some varieties such as Neelum, Bangalora, Beneshan, etc., this occurs

as a natural phenomenon in certain tracts of Tamil Nadu (Sundararaj *et al.*, 1972). Though it is a varietal character, it is also induced by favourable seasonal and climatic conditions.

Adam (1986) observed that KNO_3 when applied as a spray to mango was found to be more reliable than ethrel in inducing off-season flowering. Three sprays of KNO_3 at 4% concentration at 14 days interval was given starting from active developing stage of bud. Fifty to seventy per cent flowering was observed in all the selected cultivars ensuring increased and regular production and also by induction of two mango bearing seasons.

Combined application of paclobutrazol and thiourea is also effective in inducing off-season flowering in mango (Junthasri *et al.*, 2000). The rate of paclobutrazol depended upon size of tree canopy as well as on mango cultivars. For most cultivars, the rate of paclobutrazol is determined by multiplying the diameter of tree canopy (expressed in metres) with 1.0-1.5 g of active ingredient of paclobutrazol. At 120 days after application of paclobutrazol, 0.5% thiourea is usually sprayed for breaking buds. By this method, inflorescence was visible within 2.5 to 4 months after paclobutrazol treatment. Hau *et al.* (2002) also reported that paclobutrazol (5 g/tree) and KNO_3 (15 g/l) are also effective in inducing off-season flowering in 'Cat Hoa Loc' mango cultivar of Vietnam. Among the chemicals, paclobutrazol was more effective and it induced flowering within 85 days after treatment

8. CONCLUSION

To take a balanced crop, every year, various techniques such as cultural methods, canopy management and chemical means are recommended. Efficiency of these methods varies with climatic conditions and crop variety under cultivation. Hence each technique needs to be standardized for each agro-climatic region and variety before they can be exploited commercially.

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DISCUSSION

1. Is there any package for mango for obtaining high yield?

Now techniques have been standardized in various horticultural practices for obtaining a regular and high income from mango. As these vary with agro-climatic conditions, the specific recommendations available to a particular region should be adopted to obtain full efficacy.

2. How can we identify a regular bearing variety of mango?

The known and popular varieties of mango have been classified into regular or irregular bearing based on their bearing habit. If a new variety is introduced into a particular area, it should be grown in that area and its performance evaluated to ascertain its regular/irregular bearing habit.

3. When organic farming is gaining momentum, can we recommend application of different chemicals in mango?

In an absolute organic mango orchard, there are restrictions for use of various chemicals. In such a situation, only those components which are permitted by the certifying agency should be used.

4. Is it feasible/ economical to apply chemicals when only a very few mango trees are grown in homesteads?

Crop regulation through chemicals is mainly recommended for large commercial orchards. In a homestead garden, cultural and canopy management practices can be effectively employed to obtain a profitable crop.

5. When HDP is adopted, whether it will have an adverse effect on yield per tree?

Our ultimate aim of fruit culture is to maximize production and thereby income from unit area. In HDP, eventhough, there may be a slight reduction in individual tree yield, the higher number of trees per unit area will ensure a higher productivity and profit to the farmer.

KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara, Thrissur

Hort. 751 – Seminar

Crop regulation in major tropical tree fruits

Name: P.S.Manoj (2006-22-101)
Time: 9.15 am, 11.05.2007

Venue: Audio Visual Laboratory
Dept. of Pomology and Floriculture

Abstract

India is the leading producer of fruits in the world with an annual production of 43 Mt from an area of 4 Mha contributing 9 – 10 % of world fruit production (Radha and Mathew, 2007). Fruit crops play a significant role in the economic development, nutritional security, employment generation and overall economic growth of our country. As most of the fruit crops are woody perennials having long pre-bearing age, they require intensive cultivation with high investment. The ultimate aim of fruit culture is to increase the production of quality fruits from unit area. This involves regulation of cropping by understanding the physiology of reproduction along with advances in production technology of these crops.

The primary objective of crop regulation is to force a tree for its rest and to produce profuse blossom and fruits during desirable seasons. It also aims to regulate a uniform and good quality fruit to maximise the production as well as profit to the grower (Singh, 2001). Cultural methods, canopy management and chemical means are generally employed to manipulate cropping in tropical tree fruits. Main cultural methods followed include withholding irrigation, root exposure, root pruning, proper nutrition and mulching. Pruning, flower and fruit thinning, use of dwarfing rootstocks etc. are also utilized to regulate cropping. Because of the diverse effects, growth regulating chemicals can beneficially be utilized at various stages of plant growth and development (Kadam *et al*., 2005). Bio-regulators such as NAA, GA, ethephon etc. are utilized to manipulate the growth and developmental process in different fruit plants (Rao, 2001). Use of paclobutrazol in mango mainly to induce regular bearing is an example of such commercial application of bio-regulators in fruit crops (Singh and Ranganath, 2006).

Though many techniques are available to take a balanced crop every year, their efficacy varies with crop variety and climate of a region. Hence these techniques need to be standardized for each agro-climatic condition before they can be exploited commercially.

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FRUITS FOR THE FUTURE

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SEMINAR REPORT

Submitted in partial fulfilment for the requirement of the course

Hort. 752 – Seminar

College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur – 680 656, Kerala

2007

DECLARATION

I hereby declare that the seminar report entitled "Fruits for the future" is a record of the seminar presented by me during the course (20.07.2007) and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
08.08.2007

(P.S.Manoj)
(2006-22-101)

CERTIFICATE

Certified that the seminar report entitled "Fruits for the future" is a record of the seminar presented by Sri.P.S.Manoj (2006-22-101) under my guidance and that this report has been prepared by him independently.

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FRUITS FOR THE FUTURE

1. INTRODUCTION

India is having a rich wealth of different fruit crops and it is estimated that more than 100 economically important fruit species are available in our country. Apart from the major fruit crops, there are other groups of fruit plants which are though domesticated, their full potential has not been exploited. There is another group of fruit plants which is not at all being grown and is utilized only in a very localized manner. Many of these fruit plants are rich in nutrients and have high medicinal value. If properly exploited, they have tremendous potential to evolve as new fruit crops so as to compete with the major fruit in internal and export markets.

The plant species diversity available in earth is detailed in Table 1(Kermali *et al*., 1997)

Table 1. Plant species diversity

Category of plant species	Estimated number of species
Number of described species	17,50,000
Higher plant species	2,50,000
Edible plant species	30,000
Used for food	7,000
Commonly important species	150
Plant species producing 90 % of world's food	103
Plant species producing 60 % of world's food	**3 (Rice, wheat and maize)**

From the above table it is clear that a large number of plant species including many fruit species are available in nature which could be further exploited

2. FRUIT PRODUCTION IN INDIA

India is the leading producer of fruits in the world with an annual production of 49.29 M t from an area of 4.96 M ha contributing 10.3 per cent of world fruit production. The total fruit production in the country is targeted at 150 M t by 2015. As per FAO norms, per capita consumption of fruits should be 85 g per day, but our per capita availability is only 62 g per day. There exists a great scope both for expansion and improvement of fruit culture in the country (Peter *et al.*, 2006). In India we are concentrating on seven major fruit crops and unfortunately no attention is given to the 27 per cent of the fruit production from a large number of minor fruits (Roy, 1997). In order to achieve the targeted production, we need to improve production of these underutilized fruits.

Underutilized fruits are those with underexploited potential for contributing to food security, health, income generation and environmental services (Dawson and Jaenicke, 2006). A large number of edible fruits exists in tropics and subtropics. Many of such fruit species are identified, domesticated and utilized for various purposes, though their full potential has not been exploited. There is yet another group of plants producing edible fruits with great potential in different parts of the world, which are not grown and utilized by people other than in a very localized manner. These are referred to as underutilized or unutilized fruits (Radha and Mathew, 2007). Other terms such as minor, promising, underexploited, neglected, lesser known, traditional, underdeveloped, alternative, local etc. are also used to describe this category of fruit plants.

3. IMPORTANCE OF UNDERUTILIZED FRUITS

Many of the underutilized fruits have high nutritional, therapeutic and medicinal values and possess powerful antioxidant properties. These are rich in vitamins, minerals, proteins, carbohydrates and fats and are less expensive source of these nutrients. They have resistance or tolerance to many abiotic and biotic stresses and can grow well with low inputs in marginal lands. These fruits have unlimited potential in the processed form. Great opportunities exist in these crops for internal and international markets. They hold great promise to evolve as new fruit crops ideally called as Fruits for the Future (Sharma, 2007a; Singh, 2001).

Roy (2000) identified that under utilized tropical fruits have potential in the world market. He further opined that most of the minor fruits like amla, bael, bilimbi, jamun, rose apple, karonda and lovi-lovi are entirely unknown in the world market and need to be popularized. Many of the minor tropical fruits are completely unknown to consumer living in temperate climate. With the interest in exotic and unusual products growing in the more developed countries, real potential exists to utilize an export market, based on the production of tropical fruits (Nichols and Christie, 1993). Many under explored fruits were considered as marginal crops in the past in many countries like Turkey, Egypt, Israel and Cyprus, as reported by Mason (1995), Gregoriou (1995) and Menson (1995).

Because of the crisis within agriculture, there is increasing interest in the commercial production of underutilized fruit crops (Romero *et al*. 1995). There is increasing interest in development production of some minor fruits, but at the same time production of some minor fruits is declining due to limited domestic and international

market (Blumenfeld, 1995). Factors influencing the demand for tropical fruit include the growth of market share in retail distribution by multiples, consumer purchasing power, product promotion, consumer education and above all the increased availability of and access to well presented quality fruit (Proctor, 1990).

4. LIMITATIONS OF UNDERUTILIZED FRUITS

Most of these underutilized fruits have only local or regional importance and many are unknown to the world market. Their yield is also low and they are usually not available in bulk. Market value is also less for majority of these crops. They also do not have specified or HY varieties. Some of them are having high degree of acerbity, astringency or bitterness so that they can not be consumed in the fresh form. For most underutilized fruits, there is no recognized orcharding, their nutritional and plant protection management is nobody's business, owners if any, go only to collect the harvest and little is known about their utilization or value addition (Singh, 2002).

5. NUTRITIONAL SECURITY BY UNDERUTILIZED FRUITS

A number of underutilized fruits are rich in vitamins, minerals, protein, carbohydrates and fats and provide an appropriate solution to the serious problems like malnutrition and related health disorders such as blindness, nutritional marasmus, osteoporosis etc. in the developing and underdeveloped countries.

Underutilized fruits not only provide nutrition, strength and vigour to our body and restore the loss of minerals and amino acids and thus protect against deficiencies and certain chronic diseases (Singh *et al*, 1998). Minor fruits are not only rich source of minerals and vitamins, but can also contribute in a big way in maintaining national health and to overcome hunger and malnutrition (Misra and Rai 2000). Apart from traditional fruits, there are numerous non traditional fruits such as

jack fruit, phalsa, pummelo, wild apricot, amla, bael, jamun which can supplement carotene, vitamin C, riboflavin, calcium and iron which would help to satisfy our dietary needs of nutrition (Rathore, 2001). Sethi (1987) reported that about 15 g of amla consumed daily could help to meet the dietary need of vitamin C.

Demand for West Indian cherry has increased in the Brazilian and external market mainly due to its high vitamin C content (Lopes *et al.*, 2000).Maini and Kaur (2001) identified that bael fruit is a rich source of riboflavin and ascorbic acid. Jujube fruit is highly nutritious and rich in vitamin A, B complex and C whereas phalsa fruit is rich in vitamin A and C (Chadha, 1990).

Ascorbic acid, carotene and carbohydrate are found to be present in abundance in passion fruit (Khurdiya, 1994). Kaushal *et al.* (1999) reported that kiwi fruit is a good source of vitamin C and B. Besides a good source of ascorbic acid, acerola cherry is also known as an excellent source of bioflavanoids. Acerola is also a good source of retinol, iron, calcium and phosphorus and can be processed easily into jelly, puree, confectionary, liquors and sauces (Mezquita and Vigoa, 2000). Jamun is a good source of iron (Pal *et al.*, 1999). Rathore (2001) advocated that minor fruits like phalsa, bael and wood apple are excellent sources of calcium and phosphorus whereas custard apple, ber and karonda are rich sources of iron. Sadashivan and Neelakantan (1976) had found that ripe cocl. fruit bulbs are rich in sugar and contain fair amounts of carotene, protein and mineral.

More than 95 per cent of the volatile compounds in one of the underexploited fruit *Mahpighia glabra* were identified by Casadabang *et al.* (2000). Pino *et al.* (2000) identified various volatile components in annona which include

alpha-thujene and alpha-pinene as major components, but it is not utilized properly so far. Vaidehi *et al.* (1977) suggested that, if we make several uses of our underutilized fruits, we may overcome the problem of deficiencies due to lack of fruit and vegetables in our diet and these fruits could be processed and utilized for domestic and export markets.

Some of the underutilized fruit crops which are rich source of nutrients are furnished in Tables 2, 3, 4 and 5 (Pareek *et al.*, 1998).

Table 2. Rich sources of vitamin A

Source	Vitamin A (IU/100 g)*
Eugenia stipitata	12917
Barbados gooseberry	3215
Persimmon	2710
Egg fruit	2000
Lucuma obovata	1500
Cape gooseberry	1000 - 5000

*Daily requirement 1000 – 4000 IU

Table 3 Rich sources of vitamin C

Source	Vitamin C (mg/100 g)*
West Indian cherry	1500 - 5600
Jaboticaba	700 - 2900
Seabuckthorn	600 - 2500
Eugenia tomentosa	931
Indian gooseberry	625
Jujube	188 - 544

*Daily requirement 20 - 27 mg

Table 4. Rich sources of protein

Source	Protein (%)
Breadnut seed	13.3
Macadamia nut	9.2
Akee	8.8
Tamarind	3.1
Egg fruit	2.5
Durian	2.5
Karonda (dry)	2.3
Jack	1.9
Avocado	1.7
Custard apple	1.6
Bread fruit	1.5

Table 5. Rich sources of calcium

Source	Calcium (%)*
Tamarind	0.74
Barbados gooseberry	0.17
Karonda	0.16
Wood apple	0.13
Bael	0.10
Date	0.07
Indian gooseberry	0.05

*Daily requirement 0.6 – 1.0 g

6. MEDICINAL VALUE OF UNDERUTILIZED FRUITS

Value of underutilized fruits in traditional medicine is well known. The underutilized fruits have a close association with local beliefs and are used in health care of the people (Singh *et al.* 1998). George *et al.* (2000) have explained that most of these minor fruits are blessed with immense medicinal values and are extensively

used in Indian system of medicine. These have been a major source of raw materials for drugs since antiquity and have provided bulk of products used in the traditional system of medicine. In India, the fruits of Indian gooseberry, *Terminalia chebula and T. bellerica* are the most common entering in to 219 patented drugs. Similarly bael is used in 60 patented drugs (Sharma, 2007b).

Minor fruit like jamun has stomachic, carminative and diuretic properties apart from its cooling and digestive properties (Pal *et al.*, 1999). It is also valued for its medicinal and therapeutic properties with the use of its volatile components (Vijayanand *et al.*, 2001). It has been successfully identified that jack fruit could be very useful in the treatment of the dreaded disease of human beings - AIDS (Chadha, 1999). In ayurveda, it has been used for curing inflammation, constipation, skin disease and wound healing (George *et al.*, 2000). Bael fruits are mildly laxative and the slices of the unripe fruits in the form of murabba are used in chronic cases of diarrhoea and dysentery (Singh, 1963).

Many of the minor tropical fruits are completely unknown to consumer living in temperate climate. With the interest in exotic and unusual products growing in the more developed countries, real potential exists to utilize an export market, based on the production of tropical fruits (Nichols and Christie, 1993).

7. PHYTOCHEMICALS IN FRUITS

The discovery of phytochemicals in fruits and vegetables and their strong antioxidant potential in scavenging free radicals has generated tremendous attention among scientists. Many phytochemicals found in fruits are powerful antioxidants and they neutralize free radicals thereby protecting cells and organs from damage. Thus they effectively impart protection from cancer and degenerative

diseases. In addition, these compounds also impart colour, flavour and odour to the produce.

Some of the promising phytochemicals which act as antioxidants are Bioflavanoids (vitamin P), phenolics, carotenoids, antioxidant vitamins (vitamin C and E) and glucosinolates (Roy, 2001).

7.1. MODE OF ACTION OF ANTIOXIDANTS

Direct antioxidants such as vitamin C and E neutralize dangerous free radicals to which cells are exposed or that are generated by cells themselves before free radical can harm cell. In this process, a direct antioxidant molecule binds to a free radical molecule rendering them harmless thus protecting cells from damage. Once the antioxidant reacts with a radical, the antioxidant is destroyed and can not be used again

The indirect antioxidant works as a catalyst. It does not neutralize free radicals directly but rather boost the body's own elaborate antioxidant systems that exert ongoing and prolonged antioxidant activity. This is a broad spectrum of activity, cycling over and over again, that removes many free radicals

8. AGENCIES PROMOTING UNDERUTILIZED FRUITS

There are several international and national agencies working for the promotion of underutilized fruits. The important agencies are

i) International Plant Genetic Resources Institute (IPGRI), Rome, Italy

It is redesignated as Bioversity International from December 2006

ii) International Centre for Underutilized Crops (ICUC)

It was established in 1992 at University of Southampton, UK and shifted to Battaramulla, Sri Lanka in 2005. It also operates a network project named Underutilized Tropical Fruits in Asia Network (UTFANET).

iii) Indian Council of Agricultural Research

Under ICAR two projects are implemented

a) **All India Network Project on Underutilized Crops**, NBPGR, Pusa, New Delhi

b) **National Network Project on Underutilized Fruits** with Central Institute for Sub-Tropical Horticulture, Lucknow as lead centre. It is having four collaborating centres at Chethalli (IIHR), Godhra (CIAH), Port Blair (CARI) and New Delhi (NBPGR).

iv) **Other agencies** are State Agricultural Universities, Tropical Botanic Garden and Research Institute, Agricultural/ Horticultural Departments and Non Governmental Organizations

8.1 PRIORITY UNDERUTILIZED FRUITS FOR SOUTH EAST ASIA

IPGRI and ICUC have prioritized several underutilized fruits for promotion in South East Asia (William and Haq, 2002). They are bael, jack, mangosteen, rambutan, carambola, durian, ber, tamarind, Indian gooseberry, passion fruit, custard apple, litchi and longan

9. UNDERUTILIZED FRUITS- INDIGENOUS AND INTRODUCED

9.1 MANGOSTEEN: *Garcinia mangostana* (Clusiaceae)

It is referred to as Queen of tropical fruits due to its appealing taste and pleasant aroma. The crop originated in Malay Archipelago, Moluccas and Sunde

Islands and the main growing countries are Thailand, Indonesia, Malaysia, Philippines, Sri Lanka and Vietnam It was introduced to India in 1887.

Fruit is sub-globose with dark purple coloured rind. White soft flesh (pulp/aril) covering seed is the edible portion. Fruits weigh about 55-75 g. The number of seeds ranges from 0 to 4. Seed is parthenogenetic in nature.

The crop is adapted to humid tropical climate. It is having a long juvenile period of about 10 – 15 years. Propagation is through seeds as well as by softwood grafting (George *et al.*, 1994) Flowering season is January - February. Fruit set to harvest takes about 90-105 days. The main harvesting season is April-June in plains and August – September in hilly areas. Yield ranges from 500-1500 fruits/tree/year (25-75 kg/tree/year)

Fruits are rich in reducing sugar (10.27-13.8 %) and are considered as 'Energy tablet' (Nakasone and Paull, 1998) About 52 compounds are found to contribute to flavour of the fruit, the major being hexyl acetate (Kanchanapom and Kanchanapom, 1998). Fruit is used as a dessert fruit and also processed into jam, preserve, jelly, juice, squash etc

Fruit, rind, leaves and bark are used in traditional medicine for the treatment of dysentery, diarrhoea, arthritis, liver complaints, respiratory disorders, skin infection etc. Rind is the richest source of natural xanthone compounds. Five poly oxygenated xanthones having antioxidant property viz. mangostin, β-mangostin nor-mangostin, gartanin and 8-desoxy gartanin have been isolated from the rind. They have antibacterial and antifungal properties. Rind also contains 7-15 per cent tannin

which is used in leather industry. Red anthocyanin pigment of the rind is used as a natural food colour.

9.2 MALABAR TAMARIND: ***Garcinia gummigutta*** **(Clusiaceae)**

The crop originated in Western ghats. Fruits are edible, but raw consumption is difficult due to high acidity. Dried rind is used as a spice. Rind contains hydroxy citric acid (14 - 18.9 %) which is a potent metabolic regulator of obesity (George *et al*., 1999). Rind is having antiseptic properties and decoction of fruit is used against rheumatism, enlargement of spleen and bowel complaints. It is also used in veterinary medicine. Seed contains about 31 per cent edible fat.

Propagation is through seeds and softwood grafting (George *et al*., 1994). Flowering is during January to April and harvesting from May to July. Yield is about 25-100 kg/plant. Rind is smoke or sun dried, mixed with salt and stored.

9.3 KOKAM: ***Garcinia indica*** **(Clusiaceae)**

It is an important oil seed tree. Rind constitutes 50-60 per cent of fruit and contains malic, tartaric and citric acids. A refreshing drink called 'Amrit kokam' is prepared from ripe rind. Dried rind - 'amsole' – is used in curries. Seed yields kokam butter (25-30 %) which contains fatty acids like myristic, palmitic, stearic, oleic and linoleic acids. Kokam butter is used in cosmetics, confectionaries, chocolate preparations, pharmaceuticals, soap industry and also as cocoa butter substitute.

Other *Garcinia* sp. yielding edible fruits are *Imbe* (*G. livingstonei*), Sour mangosteen (*G. hombroniana*), *Rajapuli* (*G. xanthochymus*), *G. cowa*, *G. dulcis* etc.

9.4 JACK: *Artocarpus heterophyllus* (Moraceae)

It is a native of India and our country is having a rich diversity in this crop. Even though the crop is grown widely in Kerala, its full potential is not exploited presently. It is estimated that that about one third of production is wasted and only 20 per cent is marketed in our state.

Wide variability in vegetative, flowering and fruit characters was observed by Muthulakshmi (2003). There are two groups namely firm flesh and soft flesh types. PLR-1, Burliar-1, Peechiparai-1 are few improved selections. Propagation is through seed and epicotyl grafting technique was also standardised (Jose, 1989)

Fruit is a rich source of vitamins (vitamin A and thiamine) and minerals like iron (500 mg), phosphorus (30-40 mg) and calcium (20-30 mg) per 100 g. It is a good source of pectin also (1.5 to 6 %). Immature fruit is used as vegetable and ripe as table fruit. The fruit can be processed in to jam, nectar, preserve, squash, candy, jelly, chips etc. Seeds contain high starch (25.8 %) and protein (6.6%). Seed flour can be mixed with wheat flour up to 20 per cent for *chapatti* making and it can also be mixed with cattle feed up to 40 per cent without adversely affecting milk yield (Srikrishna, 2005)

A natural protein obtained from fruit - lectin- is used in cancer treatment. A seed protein - jacalin - is useful for evaluation of immune status of patients infected with HIV.

9.5 BREAD FRUIT : *Artocarpus altilis* (Moraceae)

The crop originated in Malaya and introduced to India about 100 years ago. It is grown as a homestead crop in Kerala and West Coast of India. The crop is

adapted to warm humid climate with high rainfall. Propagation is through root suckers, root cuttings and layers It is rich in carbohydrate (20-30 %), protein, calcium, vitamin A and B. Fruit is used as a vegetable and as a substitute for bread when baked. It is also boiled, roasted, steamed or made into soups or dough. Yield is about 50-150 fruits/tree/year. Fruit is having a short shelf life and storage life could be extended up to 9 days under refrigerated condition in PE pouches or in cling film (Pillai, 2001).

Other moraceous fruits of economic importance are mulberry (*Morus sp.*), aini (*Artocarpus hirsuta*), Breadnut (*Artocarpus camansi*) etc. Seeds of breadnut is highly nutritive and contains 13.3 to 19.9 g protein/ 100 g. It is also a promising rootstock for breadfruit (George *et al*. 1997)

9.6 INDIAN GOOSEBERRY /AONLA: *Emblica officinalis* (Euphorbiaceae)

It is a native of tropical South East Asia and in India it is mainly grown in UP, Maharashtra, Gujarat, Tamil Nadu etc. The important cultivars are Banarasi, Krishna, Kanjan, Neelam, Amrit etc. It is adapted to subtropical climate but performs well under tropical climate also. The crop is suitable for arid and semi-arid regions. It is a hardy crop and is utilized for cultivation in wasteland and marginal lands. Propagation is through seeds and vegetative methods like inarching and patch budding. Yield is about 100-150 kg/tree/year.

It is a rich source of vitamin C and contains about 700-900 mg/100 g of edible portion. Vitamin C is stable due to the presence of polyphenols and leucoanthocyanins. Fruits are having hard pulp and astringency and hence not normally consumed in fresh form. It is processed in to products like pickles, preserves, dried fruit, candy, foods, jellies etc.

Fruit is widely used in ayurvedic medicine. It is antiscorbutic, diuretic, laxative, antibiotic and is used in the treatment of chronic dysentery, bronchitis, diabetes, fever etc. Fruits are extensively used in the preparation of *chyavanaprasa*. Fruits contain folic acid having high antioxidant property. Dried fruits are used in the preparation of herbal oil, dye, shampoo, face cream, tooth powder etc.

9.7 RAMBUTAN: *Nephelium lappaceum* (Sapindaceae)

It originated in Malaysian - Indonesian region and is grown in South East Asian countries (Indonesia, Malaysia, Thailand, India), Central America, China, Africa etc. The crop is adapted to tropical climate with an annual rainfall of 200 cm. Fruits are produced in bunches with long thick soft hairs. Fruit colour is either red or yellow. Edible portion is pearly white, gelatinous, juicy aril surrounding the seed. It is propagated by seeds and vegetative means such as air layering, budding and approach grafting. Yield is about 150 kg/tree/year.

Fruit is high in TSS (18° brix) and vitamin C content (185-225 mg/100 g). It is used as fresh fruit, canned or preserved in syrup and also processed into jam, jelly, etc.

9.8 ANNONACEOUS FRUITS (Annonaceae)

The important annonaceous fruits are

a) *Annona squamosa* - Custard apple/sugar apple/sweetsop Sitaphal

b) *A. reticulata* Bullocks heart/Ramphal

c) *A. cherimola* - Cherimoya /Cherimoyer/ Hanumanphal

d) *A. atemoya* Atemoya - It is a natural hybrid of *A. squamosa* x *A. cherimola*

e) *A. montana* Mountain soursop

f) *A. muricata*: Soursop/Pickling custard apple

g) *A. glabra*: Pond apple

h) *Rollinia deliciosa*: Wild sweetsop

i) *R. mucosa*: Biriba

CUSTARD APPLE: originated in West Indies and South America and is widely grown in Australia, Brazil, Chile, Egypt, Israel, Philippines etc. In India it is cultivated in south India, Assam, Bihar, Madhya Pradesh, Maharashtra, U.P. etc. The main cultivars are Balanagar, Mammoth, Arka Sahan and Red sitaphal. The crop is adapted to tropical climate and moderate winter and high humidity during flowering improves fruit set. It can also withstand moisture stress. Propagation is through seeds and grafting. Yield is about 100-150 fruits/tree and economic life is upto 25 years. Edible portion is pulp which constitutes 45 per cent of fruit. Pulp is cream coloured, granular and custard like. Fruit is used for fresh consumption and pulp used in ice-cream and puddings. It is also processed into jam, jelly, squash, syrup, nectar, RTS beverage, wine etc. Seed contains 30 per cent oil and is used in soap and paint industry. Oil is having insecticidal property. Fruits, seeds, leaves and roots are used in ayurvedic and Yunani systems of medicine. Root is used as a drastic purgative.

9.9 LITCHI: *Litchi chinensis* (Sapindaceae)

The crop originated in Southern China or Northern Vietnam or Malaysia and introduced to India in 17th century. It is cultivated in China, Taiwan, Vietnam, Thailand, India, Madagascar, South Africa and Reunion islands. In India, it is grown in Bihar, UP, Punjab, Haryana, West Bengal etc.

Fruit is a one seeded nut produced in bunches. Pericarp is papillate and ırn pinkish red on ripening. Aril is the edible portion which is an outgrowth of outer ell layers of seed coat. It is fleshy, succulent, translucent, white and soft in texture engal, Bombai, China, Deshi, Shahi, Mazaffarpur, Rose scented are some of the rominent cultivars. Crop is adapted to warm subtropical climate. Air layering is the ommercial method of propagation. Yield is about 80-150 kg/tree/year and life span is ound 100 years.

It is used as a dessert fruit, canned and frozen fruit, dried fruit (litchi nut), uash, jelly, preserve, pickle, wine etc.

10 DURIAN: ***Durio zibethinus*** **(Bombacaceae)**

It is a native of South Asia and is grown widely in Thailand, Malaysia, Indonesia, Philippines, India and Sri Lanka. It is propagated by seeds and grafts. Fruit is with thick fibrous rind and greenish sharp pointed spines. Edible portion is the white yellowish buttery aril with strong odour due to the presence of hydrogen sulphide and ethyl hydrosulphide. Fruit weight is about 0.5-1.5 kg. It is eaten fresh or processed in to products such as durian paste, candy, sauce etc. Fruit is used in the treatment of infertility.

9.11 PUMMELO: ***Citrus grandis*** **(Rutaceae)**

It is the largest fruit in the citrus family. It is a bushy tree originated in Thailand and Malaysia. The tree is adapted to tropical climate. Fruit is rich in vitamin C. Both white and pink fleshed types are available. Fruits are used for fresh consumption and extraction of juice as a drink. Fruit is a source of pectin and the inner pith is processed into candies. Fruits have long shelf life. Pummelo peel is having anti

inflammatory effect. Decoction from all plant parts and fruit juice are used against fever and gastric disorders.

Wide variability is observed in fruit characters, chemical characters and sensory attributes. Cutting and layering was found to be successful propagation techniques among which layering was found to be superior to cuttings (Anupama, 2006).

9.12 MACADAMIA NUT: *Macadamia integrifolia* (Proteaceae)

The crop originated in Australia and is adapted to subtropical climate. Both raw and roasted nuts are edible and is used in confectionaries, ice creams etc. Kernel contains 75 to 80 per cent oil. Nut is rich in protein (9.2 g/100 g). Yield is about 40 – 50 kg/tree.

9.13 MAHUA: *Bassia latifolia* (Sapotaceae)

It is a native of India and is grown in Central and North India. The crop prefers tropical and subtropical climate. It is a hardy crop and is suitable for waste land cultivation. Flowers, fruits and seed oil are edible. Flowers and fruits are used for fresh consumption and also processed into bakery products, syrup, vinegar, alcohol etc. Seed oil is edible and oil cake is used as an insect repellent and manure.

9.14 LEMON: *Citrus limon* (Rutaceae)

Lemon is an early plant relatively free from die-back and canker. It can be propagated easily through cuttings. It also produces fruits round the year. Fruits can be used for extraction of juice as well as for pickling.

9.15 *BADHAPULI: Citrus pennivesiculata* (Rutaceae)

It is a pickling type of citrus usually referred as poor man's pickling citrus. It is a native species of Kerala. The crop is adapted to warm humid tropical climate. It is more

confined to the homesteads of Kerala. It is propagated through seeds. Acid lime which is commonly used for pickling shows stray fruiting under warm humid tropical climate of our state with undersized small juicy vesicles. But *Baduvapuli* flowers and fruits luxuriantly and is well adapted to warm humid tropical climate.

Cuttings, layering and softwood grafting are successful propagation methods. Five node hardwood and terminal soft wood leafy cuttings are best and optimum time for rooting is August -September. For layering, hard wood shoots are the best and optimum time is March to October. Soft wood grafting is successful with Rangpur lime as rootstocks (Sereena, 1996).

9.16 AVOCADO / BUTTER FRUIT: *Persea americana* (Lauraceae)

It is a native of Central America and introduced to India about 100 years ago. It is mainly cultivated in Mexico, USA, Dominican Republic, Brazil and Columbia. In India it is grown in the hill slopes of Tamil Nadu, Kerala and Karnataka. The crop is adapted to subtropical climate. Fruit is a berry which is pyriform or globose in shape. Edible portion is the thick fleshy mesocarp surrounding a single large seed. Fruits are having yellow-green to maroon or purple skin colour which are either smooth or warty. There are three ecological races namely Mexican, Guatemalan and West Indian. Fuerte, Hass, Lula, Pollock, TDK-1 are some of the popular cultivars. Propagation is through grafting (cleft, whip and approach grafting methods) or air layering. The crop exhibits synchronous dichogamy in which each flower opens twice and first it functions as female and then as male. Flower opening and closing is also uniform on a tree. Yield is about 100-500 fruits/tree (20 t/ha).

Fruits are rich in fat (15-23 %), protein (1.8-2 %) and minerals (K, P, Ca, Mg) and low in carbohydrate and hence recommended as high energy food for diabetics. It is used as fresh fruit, pulp preserved by freezing and also used in ice-cream, milk shakes etc. Fruit contains 5 to 30 per cent oil rich in vitamins and amino acids and this is used in pharmaceutical and cosmetic industries.

9.17 JAMUN / BLACK PLUM: *Syzygium cuminii* (Myrtaceae)

It is a native of India and the main growing countries are Thailand, Philippines, Madagascar, West Indies, East and West Africa and Israel. It is adapted to tropical and subtropical climate. It is a hardy crop suitable for neglected and marshy areas. Propagation is by seeds, budding and inarching. Yield is around 80-100 kg/tree/year.

Fruit is rich in iron (0.1%) and the astringency is due to high tannin content (386-428 mg/100 g). Purple colour of the fruit is due to anthocyanin pigments (210-242 mg/100 g). It is used as a dessert fruit and also processed into products such as beverage, jelly, jam, squash, wine, pickle, vinegar, pickle etc.

Fruit is used in the treatment of diabetes, dysentery and liver complaints and vinegar is having carminative, diuretic and digestive properties. Fruit syrup has the ability to control diarrhoea and blood pressure. Seed powder is used in the treatment of diabetes.

9.18 ROSE APPLE: *Syzygium jambos* (Myrtaceae)

The crop originated in Malay Archipelago and Upper Burma. It is cultivated in humid and arid tracts in South India. Propagation is by seeds or an

layering. Fruits are rose scented, creamy white or light pink, ovoid or globose. They are eaten fresh or processed into candy, sauce, jelly etc.

9. 19 WATERY ROSE APPLE: *Syzygium aqueum* (Myrtaceae)

It prefers tropical climate and propagation is by seeds, layering and budding. Fruits are white, rose or red in colour which are juicy, spongy and watery. Fruits are eaten fresh or made into syrup and beverages.

9.20 SURINAM CHERRY: *Syzygium uniflora* (Myrtaceae)

It is a native of South America and grows in tropical and subtropical climates. Propagation is by seeds or layering. Fruits are ribbed, deep crimson red and are eaten fresh or processed into jellies, jams and pickles. Leaf extract is used against stomach disorders.

9.21 MALAYAN APPLE/ MALAY ROSE APPLE: *Syzygium malaccense* (Myrtaceae)

The crop originated in Malay Archipelago. It is a tropical plant and propagation is by seeds and budding. Fruits are oblong or pyriform, reddish pink or white striped crimson pink, with white flesh. Fruits are eaten fresh or made to jam, pickles etc. Leaf extract is used in the treatment of high blood pressure and roots have diuretic property.

9.22 TAMARIND: *Tamarindus indica* (Fabaceae)

The crop is originated in tropical East Africa and is grown in Tamil Nadu, Andhra Pradesh, Maharashtra, Karnataka, Kerala etc. Important cultivars are Pratisthan (sweet types), Urigam, Cumbum, PKM-1 (sour types) etc. It is adapted to humid to dry hot tropics and is an ideal tree for arid and semi arid zones. Propagation

is by seed, approach grafting and softwood grafting. Yield is about 200 kg fruit/tree/year.

Fruits are rich in carbohydrates (67.4 g/100 g), protein (3.1 g/100 g), minerals (2.9 g/100 g), tartaric acid (6-10 %) and pectin (4.4 %). Sour type is used in curries, chutney, sauces, soups etc. and exported in fresh, dry and paste form. Sweet type is used as a table fruit and in canned form. Pulp is a source of pectin. Tamarind Kernel Powder (TKP) is used in textile and leather industry. Seed oil is utilized in paint and varnish industry. Wood is used for the preparation of implements, tool handles, wheels etc. Pulp has carminative, laxative, antiscorbutic, antiseptic and refrigerant properties

9.23 PASSION FRUIT : *Passiflora* spp. (Passifloraceae)

It is of two types

Yellow or golden passion fruit - *Passiflora edulis* f *flavicarpa*

Purple passion fruit - *P. edulis*

The crop originated in tropical America and is cultivated in Brazil, Fiji, South Africa, Venezuela, Australia, Hawaii and Japan. Important cultivars are Kavery (Purple x Yellow), Purple Gold and Black Beauty. Purple type is adapted to cooler subtropics or at high altitudes in the tropics and yellow type is suited to tropical low lands. Yield is about [illegible] t/ha. The fruits are rich in vitamin A, C and K and minerals like P and Ca. Pulp is eaten fresh and added to fruit salad or ice-cream or processed into products such as squash, syrup, jam, jelly, fruit nectar etc. Dried flowers contain an alkaloid passiflorine which is used for relieving pain and inducing sleepiness

Other *Passiflora* spp. used as edible fruit are giant granadilla (*P. quadrangularis)* and water lemon (*P. laurifolia*).

9.24 LOVI-LOVI: *Flacourtia* sp. (Flacourtiaceae)

It is a native of Malaysia. There are two types:

Flacourtia cataphracta - sweet fruits, dioecious with spines on trunk

F. inermis - sour fruits, bisexual and spineless

The crop is adapted to tropical climate. Propagation is by seeds, air layering and softwood grafting. Fruits are small, round, turn dark red on ripening. Yield is about 80-100 kg/tree/year. Fruits are used for the preparation of jam, jelly, syrup and preserves. Leaves and young shoots are astringent and stomachic and are used against bleeding gum and toothache.

9.25 WEST INDIAN CHERRY/ BARBADOS CHERRY / ACEROLA: *Malpighia punicifolia* (Malpighiaceae)

The crop originated in Caribbean Island, Central and South America and is adapted to tropical and subtropical climates. It is propagated by seeds, cuttings or layers. It is the richest natural source of ascorbic acid and contains 1000 to 4000 mg/100 g edible pulp. Fully mature berries are superior to red ripe berries in vitamin C, minerals and fibre content while TSS and β - carotene was found maximum in red ripe fruits (Jyothi, 2006). Fruits are consumed as table fruit, jelly, juice, jam, preserves and sherbets. Fruits are useful against diarrhoea, dysentery and liver disorders.

9.26 BILIMBI / CUCUMBER TREE: *Averrhoa bilimbi* (Oxalidaceae)

It originated in Moluccas and is grown mainly in Malaya, India, Indonesia, Philippines and Sri Lanka. The plant produces flowers and fruits on main trunk. Fruits are available throughout the year. Fruits are used for pickling and culinary purposes.

Juice is a cooling beverage. Fruit contains high amount (6%) of oxalic acid. Fruits are used against internal haemorrhoids.

9.27 CARAMBOLA / STAR FRUIT: *Averrhoa carambola* (Oxalidaceae)

It is a native of Indonesia and is grown in SE Asian countries. There are two types - sweet and sour. Propagation is by seeds, approach grafting and shield budding. Yield is about 50-100 kg/tree. Fruits are rich source of vitamin A (570-700 IU) and minerals (0.3-0.5%). Acidity is due to oxalic and malic acids. Fruits are used as preserve, candy, jam, chutney, pickle and RTS beverage. Juice is a cooling drink. Root extract is used against poisoning and crushed leaves against ring worm and scabies. Fruits are diuretic and powdered seeds act as sedative in asthma and colic pains.

9.28 KARONDA / CHRIST'S THORN: *Carissa carandas* (Apocyanaceae)

The crop originated in India and grows wild in South India, Maharashtra, UP, Rajasthan and Bihar. It is a hardy crop suitable for thorny hedging in orchards, waste and arid land utilization. Propagation is through seeds, hard wood cutting and air layers. There are two types - dark purple to black and pink and white fruited. Important varieties are Pant Sudarhan, Pant Manohar and Pant Suvarna (Kumar *et al*., 2007). Yield is about 5 to 6 kg/bush. Fruit is processed into pickles, chutney, preserve, candy, [illegible] jelly, [illegible]. It is rich in iron (3.9 % on dry weight basis) and is used for cure of anaemia. Fruits have antiscorbutic property also.

9.29 LANGSAT: *Lansium domesticum* (Meliaceae)

It is a native of Malaysia and is adapted to tropical and subtropical climates. Propagation is by seeds, grafting and budding. Fruits are round, oblong

ovoid or ellipsoid in shape, pubescent with white juicy translucent pulp and are produced in bunches. Yield is about 15-20 kg/plant. Fruits are eaten raw or preserved in syrup or candied. Dried peel is used in the treatment of diarrhoea and intestinal spasm. Seed is used as vermifuge and febrifuge.

9.30 LONGAN: *Dimocarpus longan* (Sapindaceae)

The crop originated in Southern China and is adapted to subtropical climate. Propagation is by seed, air layering and grafting. Fruits are produced in clusters and are eaten fresh, dried or canned. Fleshy, translucent, sweet aril is the edible portion. Yield is about 50-250 kg/tree/year. Flesh of fruit is stomachic, febrifuge, vermifuge and is an antidote for poison. Decoction of dried fruit is a tonic in treating insomnia and neurotic complaints.

9.31 LOQUAT: *Eriobotrya japonica* (Rosaceae)

It is a native of China and is grown in Spain, Algeria, Italy, Brazil, Turkey etc. In India, UP, Uttaranchal, Delhi, Punjab, Himachal Pradesh, Assam, Maharashtra are major producers. It is adapted to mild subtropical climate. Propagation is by seed and vegetative methods such as air layering, shield budding and inarching. Pith and cortical areas constitute the edible portion. Yield is about 15-20 kg/tree/year. It is used as a dessert fruit and processed into jam, jelly, preserves, juice, wine etc. Fruit is used against vomiting, [illegible] pirators disease.

9.32 EGG FRUIT / CANISTEL: *Pouteria campechiana* (Sapotaceae)

The crop originated in tropical America and is grown in South Indian states. Propagation is by seeds, grafting and budding. Fruits are yellow or orange on ripening with yellow flesh having a strong musky odour. It is also rich in carotene

(2000 IU/100 g) and protein (2.5 %). Fruits are consumed fresh and used in milk shakes, custards, juice, jams etc. Yield is about 300-400 fruits/tree/year. Leaf infusion is used against abdominal disorders.

9.33 WHITE SAPOTE: *Casimiroa edulis* (Rutaceae)

It is a native of Mexico and Central America and is adapted to tropical and subtropical climates. Propagation is by seeds, grafting and budding. Fruits are spherical or oblong, produced in clusters. Skin is yellowish green and slightly ribbed. Flesh is creamy white or yellowish and is eaten fresh or processed into jelly. Fruits are used as a soothening agent for rheumatic pains.

9. 34 NONI / INDIAN MULBERRY: *Morinda citrifolia* (Rubiaceae)

The crop originated in South East Asia. It is a small evergreen tree adapted to tropical climate. Propagation is by seeds and cuttings. It flowers throughout the year and start yielding from 2nd year onwards. Fruit is greenish white to pale yellow, fleshy, ovoid or globose. Yield ranges from 90 to 100 kg/tree and life span is about 40-50 years.

Noni juice is a rich source of vitamins and minerals and is having high antioxidant content. It stimulates immune system and is used to manage tuberculosis, heart diseases, cholesterol, blood pressure, kidney disorders, anaemia, diabetes, digestive disorders, rheumatic etc. (Sabharwal, 2007).

9.35 INDIAN HOG PLUM / AMBARELLA: *Spondias pinnata* and *S. mombin* (Anacardiaceae)

It is a medium to large tree and fruits are pear shaped and produced in bunches. There are sweet, sour and bitter types. Sour types are propagated by

hardwood cuttings and sweet types by seeds and softwood grafting (George *et al.*, 2005). It is used as table fruit, pickle, jam, juice, preserve etc.

9.36 STAR APPLE: *Chrysophyllum cainito* (Sapotaceae)

The crop originated in Tropical America and is adapted to tropical climate. Propagation is by seeds and various vegetative methods such as cuttings, layering, budding and grafting. Fruits are smooth, globose, green, purple or copper coloured. Edible portion is pulp which is eaten fresh or processed. Yield is about 65-70 kg/tree/year.

9.37 CAPE GOOSEBERRY: *Physalis peruvia* (Solanaceae)

It is a native of tropical America and in India, it is mainly grown in UP, Punjab and Rajasthan. It is adapted to tropical climate. Propagation is by seeds and cuttings. Edible part is a berry which is enclosed in a papery husk developed from calyx. Yield is around 3-5 kg/plant and life span is 3-4 years.

9.38 BAEL / BENGAL QUINCE: *Aegle marmelos* (Rutaceae)

It is a native of India and is grown in Sri Lanka, Pakistan, Bangladesh, Myanmar, Thailand and South East Asian countries. In India, it is most common in UP, Bihar, West Bengal and Orissa. Some of the popular cultivars are Mirzapuri, Narendra Bael 5 and Narendra Bael 9. The crop is adapted to sub tropical climate and it is a deciduous tree. It is a hardy plant and thrives well in swampy, alkaline and stony soils. Propagation is by seeds, root suckers and patch budding. Yield is about 300-400 fruits/tree. Fruits are used for fresh consumption and processed into preserve, candy, squash, nectar, RTS beverage, jam etc. Fruits contain 'marmelosin' having laxative properties. It is also used as a restorative tonic.

9.39 AKEE: *Blighia sapida* (Sapindaceae)

The crop originated in West Africa. It is a medium to large tree adapted to tropical climate. Fruits are pear shaped and lobed with red-yellow waxy skin. Edible portion is yellow fleshy aril which is rich in protein. It is eaten in fried form or after boiling in salt water or milk.

9.40 BARBADOS GOOSEBERRY/LEMON VINE: *Pereskia aculeata* (Cactaceae)

It is a native of West Indies. The plant is a climbing leafy cactus with spines on trunk. It is adapted to tropical climate. Fruits are round, lemon or orange yellow or reddish in colour. Fruits are eaten fresh or stewed, preserved with sugar or made into jam. It is rich in vitamin A (3215 IU/100 g) and calcium (174 mg/100 g).

9.41 WATER CHESTNUT: *Trapa natans* (Onagraceae)

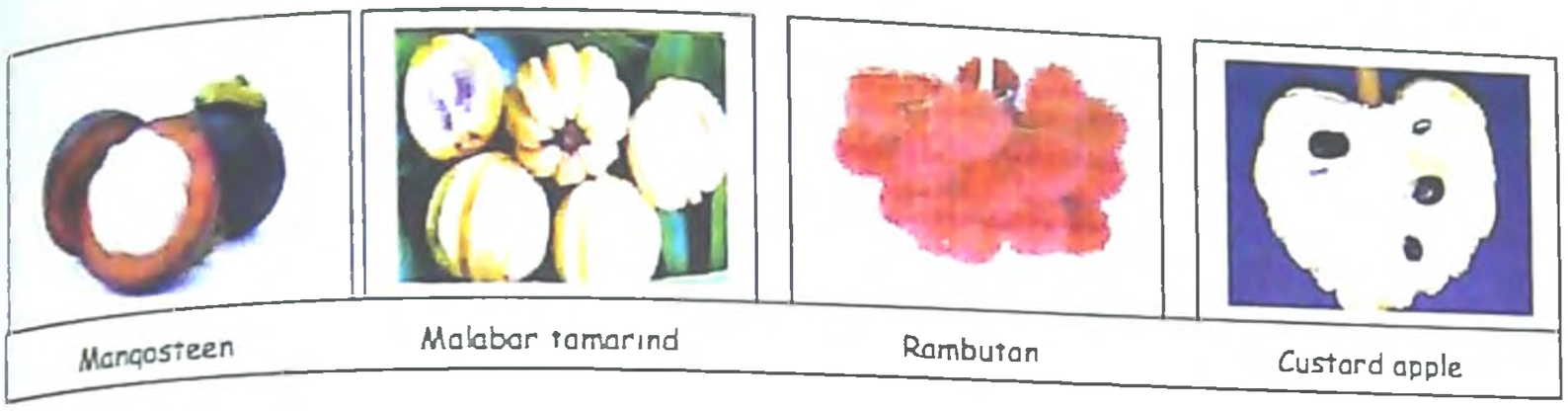
It is an aquatic floating annual adapted to tropical and subtropical climate. Fruit is a one seeded top shaped drupe and kernel is the edible portion. It is eaten fresh or boiled or dried. Yield is 2.5 – 3.8 t/ha of pond (Mazumdar, 2004).

9.42 MANILA TAMARIND: *Pithecellobium dulce* (Fabaceae)

The crop originated in South Mexico and is adapted to tropical climate. It is a medium sized tree tolerant to drought. Edible portion is the acidic sweet pulp. It is eaten in fresh form and also used for the preparation of beverages.

9.43 *Mootippacham*: *Baccaurea courtallensis* (Euphorbiaceae)

It is a wild fruit and a medium sized tree having cauliflorous nature. Flowers are bright crimson coloured. Fruits are edible and sweet - acidic in nature. Ripe fruits are crimson red and used as a table fruit and pickle.



Underutilized fruits

10. PROMISING FRUITS IN OTHER COUNTRIES

10.1 DRAGON FRUIT: *Hylocereus undatus* (Cactaceae)

Origin of the crop is Southern Mexico and it is adapted to tropical climate. It is a vining, terrestrial or epiphytic cactus. Fruits are round, red with prominent scales. Rind is thin enclosing a large mass of sweetly flavoured white or red pulp-containing small black seeds. It is often eaten chilled. Unopened flower buds are used as vegetable.

10.2 CACTUS PEAR: *Opuntia ficus - indica* (Cactaceae)

It originated in Mexico and is adapted to semi arid climate. Fruit is pear shaped with small spines. Pulp is soft and juicy. It is consumed fresh or processed into paste, jam, drinks. Fruits have anti-diabetic property.

10.3 MIRACLE FRUIT: *Synsepalum dulcificum* (Sapotaceae)

This is a native of West Africa and is a bush or small tree. It is called miracle fruit since it has the capacity to make sour food to taste sweet after eating one fruit of this plant. The fruit contains a protein, miraculin, which binds to sweet receptors on the tongue and modifies response to the food. The effect lasts for about one to two hours. It is also used as a natural sweetener.

10.4 MAMEY SAPOTE: *Pouteria sapota* (Sapotaceae)

The plant originated in Southern Mexico and Southern America and is adapted to tropical climate. Propagation is by grafting. Fruits are large with orange flesh and are consumed as a dessert fruit or beverage.

10.5 BALI SALAK/ SNAKE SKIN FRUIT: *Salacca edulis* (Arecaceae)

It is a native of Indonesia. It is a thorny palm and produce fruits in large clusters. Fruits have brown reptile like skin and hence the name snake skin fruit.

Edible portion is the fleshy pulp which is eaten fresh or candied. Another related species producing edible fruit is Thai salak (*Salacca wallichiana*).

10.6 STRAWBERRY TREE: *Arbutus unedo* (Ericaceae)

It originated in Southern Europe and is adapted to tropical climate. It is a medium bushy tree having ornamental value also. Fruits are rough skinned and red coloured. Dry and powdery pulp is the edible portion which is eaten fresh or used for the preparation of alcoholic beverages.

10.7 KEI APPLE : *Dovyalis caffra* (Flacourtiaceae)

It is a native of South Africa and is a shrub or small tree. Fruits are thin skinned, bright yellow or orange coloured. Pulp is juicy and acidic. It is eaten fresh or processed into jellies and preserves.

10.8 BLACKBERRY JAM FRUIT: *Randia formosa* (Rubiaceae)

It originated in central America and is a small bushy shrub. Fruits are hard shelled and edible portion is the black pulp which tastes like blackberry jam. It is eaten fresh or processed into jam, jellies and preserves.

10.9 ABIU /YELLOW STAR APPLE: *Pouteria caimito* (Sapotaceae)

It is a medium sized tree common in Brazil. Fruits are round or oval with creamy jelly like flesh which is eaten fresh.

10.10 PEPINO: *Solanum muricatum* (Solanaceae)

It is a small bush or shrub and is adapted to subtropical climate. It is popular in New Zealand and USA. Fruits are medium sized with juicy soft flesh which is eaten fresh.

10.11 MAMEY APPLE: *Mammea americana* (Clusiaceae)

It is a native of central America. Fruits are round with brown skin. Edible portion is orange coloured flesh which is eaten fresh, used in salad, ice creams and preserves.

10.12 PEANUT BUTTER FRUIT: *Bunchosia argentia* (Malpighiaceae)

It originated in central America. It is a small bushy tree adapted to tropical climate. Fruits are orange or red coloured and the sticky dense pulp is eaten fresh.

10.13 JOBOTICABA: *Myrciaria cauliflora* (Myrtaceae)

It is a native of Brazil. Fruits are black grape like berries and are cauliflorous. Purple black translucent pulp is the edible portion. Skin has high tannin content. Fruit is eaten fresh or processed into jam, jellies and wines.

10.14 RUM BERRY: *Myrciaria floribunda* (Myrtaceae)

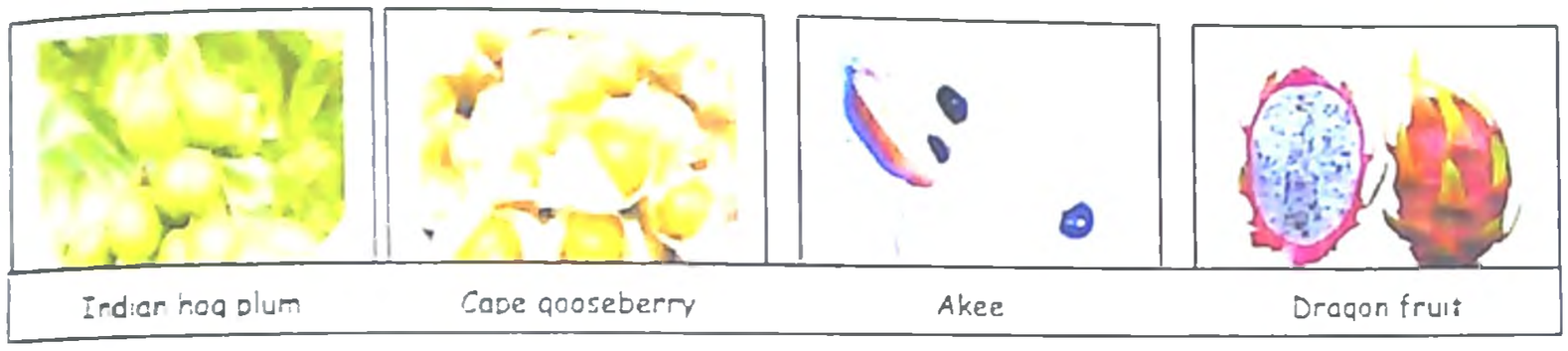
The crop originated in Southern America. It is a small tree adapted to tropical climate. Fruits are dark red to purple with excellent acidic flavour. It is eaten fresh and used for the preparation of alcoholic beverages.

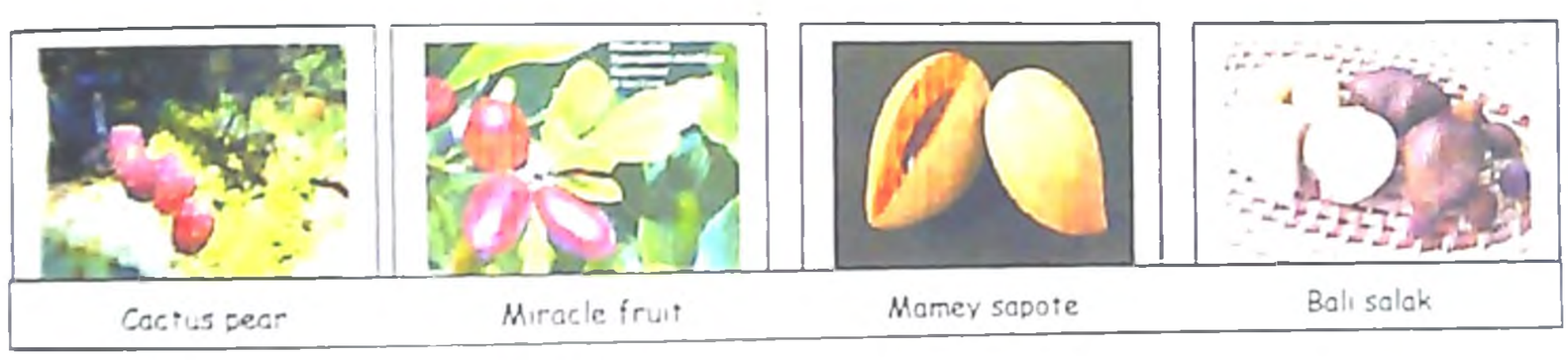
10.15 BLUE GRAPE: *Myrciaria vexator* (Myrtaceae)

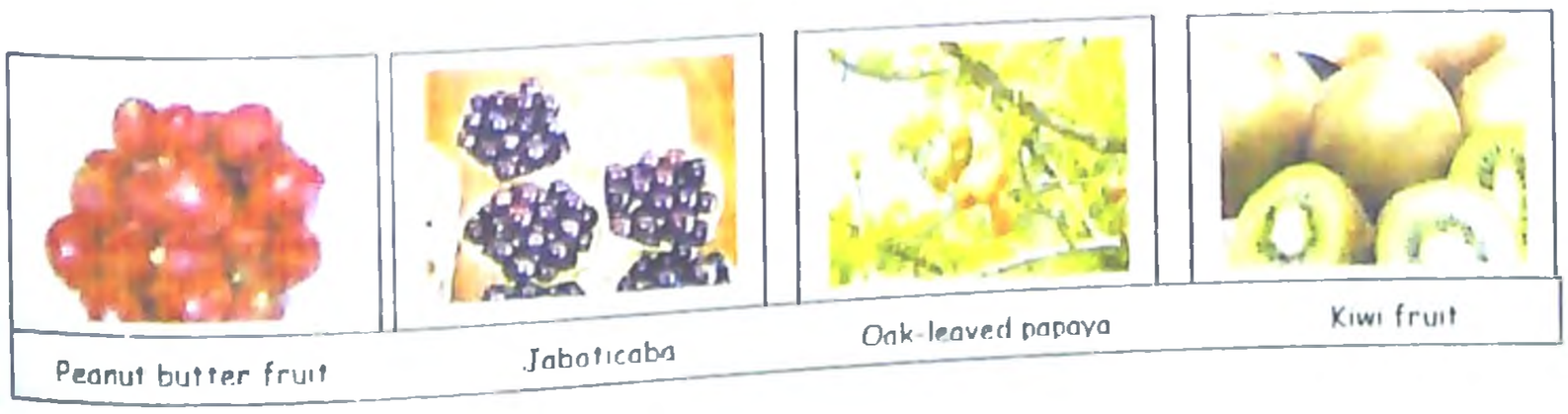
It is a native of America and is a small tree adapted to tropical climate. Fruits are dark purple with good flavoured pulp which is eaten fresh.

10.16 OAK-LEAVED PAPAYA: *Carica quercifolia* (Caricaceae)

It originated in Andes Mountains. It is a fast growing herbaceous plant. Fruits are small, long and bright orange coloured. Pulp is sweet and eaten fresh.







Underutilized fruits

10.17 KIWI FRUIT/CHINESE GOOSEBERRY: *Actinidia deliciosa* (Actinidiaceae)

It is a native of China and popularized by New Zealand and hence the name Kiwi which is the national bird as well as symbol of New Zealand. It is a deciduous vine, trained on support. Fruit is rich in minerals and vitamin C (1370 mg/100 g). Yield is about 25 t/ha.

11. SUPERFRUITS

Based on antioxidant strength, nutrient density and potential health benefits, ten fruits of the world have been classified as superfruits. They are:

i) BLUEBERRY *Vaccinium angustifolium* (Ericaceae)

ii) WOLFBERRY *Lycium barbarum* (Solanaceae)

iii) ACAI *Euterpe oleracea* (Arecaceae)

iv) CRANBERRY *Vaccinium macrocarpon* (Ericaceae)

v) SEABUCKTHORN *Hippophae rhamnoides* (Elaeagnaceae)

vi) GUARANA *Paullinia cupana* (Sapindaceae)

vii) GRAPE *Vitis vinifera* (Vitaceae)

viii) MANGOSTEEN *Garcinia mangostana* (Clusiaceae)

ix) NONI *Morinda citrifolia* (Rubiaceae)

x) POMEGRANATE *Punica granatum* (Punicaceae)

The classification is not widely accepted all over the world and more fruits are being evaluated to include under superfruits category.

12. PRIORITY UNDERUTILIZED FRUITS FOR KERALA

Based on adaptation to humid tropics and commercial importance several fruit crops have been identified as priority fruit crops for Kerala. They are

a) Jack
b) Mangosteen
c) Rambutan
d) Tamarind
e) Malabar Tamarind
f) Passion fruit
g) Custard apple
h) *Citrus* sp. – *Baduvapuli*, Lemon and Pummelo
i) Breadfruit

These fruits can be successfully incorporated into the homestead system of cultivation practiced in the state or as intercrop in the predominant coconut based cropping system

13. RESEARCH ACTIVITIES AT KERALA AGRICULTURAL UNIVERSITY

In addition to the various research programmes carried out as detailed in the foregoing discussion under individual crops, the department of Pomology and Floriculture, College of Horticulture, Vellanikkara is holding a large germplasm collection of many of the underutilized fruit crops. Over 100 accessions are maintained and being evaluated. All these accessions have been given accession number by NBPGR, New Delhi

14. CONCLUSION

[illegible] for the rich wealth of underexploited fruits which has to be collected, documented, evaluated and conserved for further utilization. In the case of most of these crops, production technologies have not been standardized. So development of suitable technologies for sustained production needs to be given importance. Many of the underexploited fruit crops have high nutrient content and medicinal value which need to be [illegible]. They have tremendous potential for

processing and hence viable technology is to be evolved to utilize value added products from these crops. There are many underexploited fruit crops having high demand in international market. Steps should be initiated to introduce such crops so as to exploit the export market. Domestication of wild edible fruits such as *Baccaurea courtallensis* having high processing potential can also be attempted. Many of these wild fruits are being used in traditional medicine and such traditional knowledge need to be documented for future use. Now there is tremendous interest worldwide in antioxidant compounds and health promoting substances of natural origin. So there is urgent need to document our indigenous wealth of fruits with respect to these properties Even though most of these underexploited fruit crops are having tremendous potential people are not aware about its credentials and hence efforts should be taken to popularize its cultivation in homesteads as well as on a commercial scale

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1. How underexploited fruits provide environmental services?

Most of these underexploited fruits are generally not overly competitive and often fit well into particular niches in highly diverse farming ecosystems, thereby contributing to overall farm production stability. This makes farmers and their environment less sensitive to rapid external changes. In addition, some species can grow well with low inputs in marginal lands that are often difficult to place under alternative production system.

2. Which are the main underexploited fruits having export potential?

Mangosteen, rambutan, durian, litchi, kiwi fruit etc. are some examples of underexploited fruits having great export potential.

3. When the land availability in our state is limited to accommodate newer crops, how is it possible to expand the area under underexploited fruits?

In our state, we are following a unique homestead system of cultivation in which crops, livestock etc. are integrated. This is a flexible system which can accommodate newer crops without adversely affecting the production of existing crops.

4. What do you mean by nutrient density?

Nutrient density is defined as a ratio of nutrient content (in grams) to the total energy content (in kilocalories or joules). Nutrient-dense food is opposite to energy-dense food. According to the dietary guidelines, nutrient-dense foods are those foods that provide substantial amounts of vitamins and minerals and relatively few calories.

5. Why many of the underexploited fruits have a higher price in market?

Now, there is tremendous interest worldwide in antioxidant compounds and health promoting substances of natural origin. Most of the underexploited fruits are rich source of these compounds. Compared to their demand, the supply is limited due to less area under cultivation, lack of suitable technologies for sustained production, unorganized harvesting and marketing etc. This greater demand than the supply fetches them a good price in market. Research efforts should be strengthened to improve their production and productivity.

KERALA AGRICULTURAL UNIVERSITY
College of Horticulture, Vellanikkara, Thrissur
Hort. 752 – Seminar

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Fruits for the Future

Name: P.S.Manoj (2006-22-101)
Time: 9.15 am. 20.07.2007

Venue: Conference Hall
College Library

Abstract

Underutilized plant species are those with underexploited potential for contributing to food security, health, income generation and environmental services. A large number of edible fruits exists in tropics and subtropics. Many of such fruit species are identified, domesticated and utilized for various purposes, though their full potential has not been exploited. There is yet another group of plants producing edible fruits with great potential in different parts of the world, which are not grown and utilized by people other than in a very localized manner. These are referred to as underutilized or unutilized fruits (Radha and Mathew, 2007).

India is the leading producer of fruits in the world with an annual production of 49.29 Mt from an area of 4.96 M ha contributing 10.3 per cent of world fruit production. The total fruit production in the country is targeted at 150 Mt by 2015. As per FAO norms, per capita consumption of fruits should be 85 g per day, but our per capita availability is only 62 g per day. There exists a great scope both for expansion and improvement of fruit culture in the country (Peter *et al.*, 2006). In India we are concentrating on seven major fruit crops and unfortunately no attention is given to the 27 per cent of the fruit production from a large number of minor fruits (Roy, 1997).

Many of the underexploited fruits have high nutritional, therapeutic and medicinal values and possess powerful antioxidant properties. These are rich in vitamins, minerals, proteins, carbohydrates and fats and are less expensive sources of these nutrients. They have resistance or tolerance to many abiotic and biotic stresses and can grow well with low inputs in marginal lands. These fruits have unlimited potential in the processed form. Great opportunities exist in these crops for internal and international markets. They hold great promise to evolve as new fruit crops ideally called as Fruits for the Future (Sharma, 2007, Singh, 2001).

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CONVENIENCE FOODS: GROWTH AND PROSPECTS

By

Bijila.P.V.
(2006-22-102)

SEMINAR REPORT

Submitted in partial fulfillment for the requirement of the
Course No. Proc. 751, Seminar (0+1)

DEPARTMENT OF PROCESSING TECHNOLOGY
COLLEGE OF HORTICULTURE
KERALA AGRICULTURAL UNIVERSITY
VELLANIKKARA-680656
THRISSUR, KERALA, INDIA
2007

DECLARATION

I hereby declare that the seminar report entitled **"Convenience foods: Growth and prospects"** is a record of the seminar presented by me during the course (05.05.2007) and that this report has been prepared by me independently after going through the references cited herein.

Vellanikkara
6.06.07

Bijila.P.V.
(2006-22-102)

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Certificate

Certified that the seminar report entitled **"Convenience foods: Growth and prospects"** is a record of seminar presented by **Ms. Bijila.P.V. (2006-22-102)** under my guidance and that this report has been prepared by her independently.

06.06.2007

Vellanikkara

Dr. K.B. Sheela

Major advisor

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Introduction

In modern living food is no longer consumed for satisfaction of hunger alone. but for promoting nutrition and health, coupled with enjoyment. The present trend in the market indicates that consumers need to obtain food that is ready to eat along with variety, high quality, taste, nutrition and safety. Convenience foods are those foods that have undergone major processing by manufacturer such that they require little or no secondary processing and cooking before consumption (Arya, 1992)

The convenience foods consist of heterogeneous group of foods which vary in size, shape, method of preparation and processing, even with respect to their functions in the diet This literally ranges from dehydrated products to ready mixes. canned and frozen foods, fresh cut fruits and vegetables and sophisticated warm and serve type dinners Convenience foods can be defined as those products in which all or a significant portion of their preparation has been transferred from the consumers kitchen to the processing plant

All over the world during the last two decades, the convenience food market has witnessed breath taking changes in quality and quantity of products available and the packaging and technology employed for their processing

Definition

Convenience foods can be broadly defined as "foods that have undergone major processing by the manufacturer such that they require little or no secondary processing and cooking before consumption" This means, apart from warming, thawing, cooking, frying, diluting and reconstitution, the food is ready to eat

A food may be classified as convenience food if it meets the criterias like:

i) The food must have undergone considerable amount of food preparation by the manufacturer before it reaches the retailer

ii)It must require minimal cooking or processing before consumption by the consumer

iii) The preparation time before consumption should be minimal.

Scope and importance of convenience foods

Convenience food is a relatively recent term in food science. It can be defined as those products in which all or a significant portion of their preparation has been transformed from consumers kitchen to processing plant.

The dry mixes of several Indian dishes such as idli mix, vada mix, sambar mix, payasam mix are very popular not only in India but also in other countries. These product has export potential to USA, UK, Canada and Gulf countries. All over the world, the convenience food market has witnessed breath taking changes in quality of products available. In India too many of the products which were once marketed by food service establishment or small scale artisans are now marketed by multinational companies (Arya, 1992). Till the early 1960's convenience foods were rare at sight and reported to be consumed only by the higher strata of the society. Today they have made successful inroads into the rural households, though not on a large scale

Convenience foods are gaining popularity in recent years as they are very easy to handle, require minimum storage space and are attractive. Nowadays convenience foods have emerged as a new set of products in the international market

Instant mixes are high in nutritive value especially those needed for armed forces, light in weight, easy to carry and prepare. Rapid industrialization and urbanization and change in eating habits of Indian people led to very high demand for ready to eat foods.

Changing life styles and values are at the root of considerable changes in eating habits over the last few years. Hand in hand with a strong demand for fast and take away foods, there has been marked upturn in popularity for convenience foods in India (Manohar *et al*., 2005)

The major reasons for this trend are:

i) Decline of the family meal time

ii) Growth in income

iii) Desire for more leisure time and demand for foreign dishes

Advantages of convenience foods

i) Reliable and consistent in quality

ii) Available at all season

iii) Ethnic and exotic meals are readily available

iv)Saving in space and labour

v) Have nutritive value equal to or higher that of fresh foods

vi) Extra cost is offset by the reduction in preparation time and no wastage

vii)Allows greater variety and quality

Limitations of convenience foods

i)High initial investment

ii)High cost as compared to fresh foods

iii)Improper handling will leads to microbial contamination and spoilage

Factors promoting the demand for convenience foods are

i) Increased education and employment opportunity for women

ii) Large number of employed couples

iii) Increased industrialization and urbanization

iv) Large floating population due to promotion of tourism

v) Increased number of nuclear families

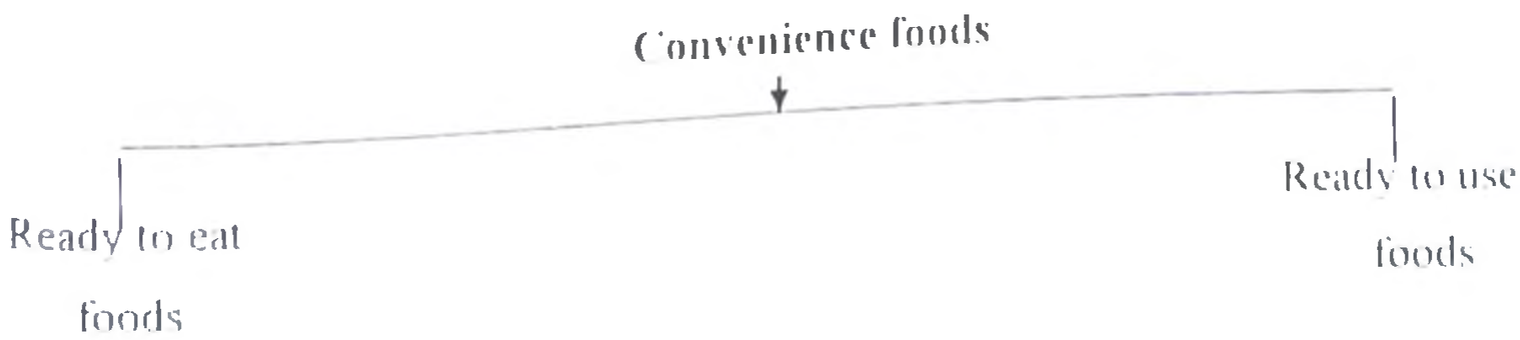
vi) Changing life style and food habits of middle income people

vii) Better awareness about processed foods

viii) Better availability of convenience foods

Classification of convenience foods

According to Manohar *et al* (2005), convenience foods can be classified in to four major categories



1.Ready to eat foods

The foods which can be directly consumed from the package with or without warming or thawing and without preparation

Eg Processed cheese,Retort processed foods,Gulab jamun, Rasagola

Ready to drink beverages such as sweet lassi and cold coffee in tetrapack, Horlicks malt shake and boost in tetrapak, fruit juice concentrates etc.

2. Ready to use foods

Foods which need some preparation like cooking, frying, reconstitution, dilution etc before consumption The four categories of ready to use foods are

i) Ready to cook foods (eg. Noodles, Instant Rava Idli mix)

ii) Ready to reconstitute foods (eg. Instant ice cream mix, instant gulab jamun mix)

iii) Ready to use spice mixes (eg. Vegetable khorma gravy, Garam masala, Ginger garlic paste)

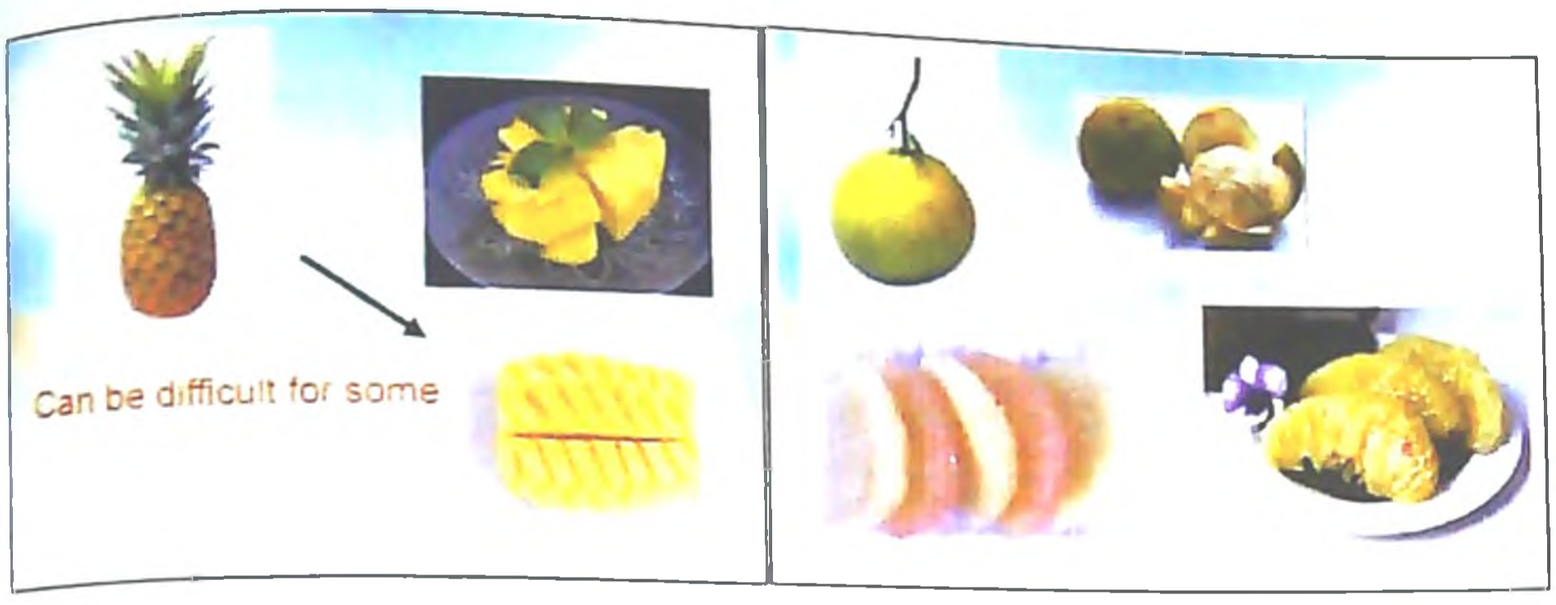
iv) Minimally processed fresh cut fruits and vegetables

Minimally processed fresh cut fruits and vegetables

Minimal processing of fruit and vegetables is a relatively new and developing part of the fresh produce industry. Growing evidence suggests that increasing dietary consumption of vegetables and fruits has long-term health benefits and may prevent or reduce the risk of some chronic diseases. Since vegetables and fruits are the important source of some nutrients, maximum utilization through proper fresh cut processing is highly desirable. The major thrust area of minimal processing of vegetables and fruits is in terms of their packaging and storage and irradiation as well as the treatments used to maintain fresh cut vegetable and fruit quality

Minimally processed fresh cut fruits and vegetables are ready to use products which include fresh, washed and chopped fruits and vegetables, ready for use and packaged in sealed polymeric films or trays (Fig 1) The key criteria for a vegetable or fruit, product to be considered a fresh cut product is that it must consist of 100 per cent usable material and that the tissue is in living, respiring, physiological state The trade of fresh cut products started in 1980's as a solution to an emerging

Fig 1. Minimally processed fruits available in the market



consumer demand for convenience and for high quality and preservative free products with the appearance of fresh products through less severe processing.

Table 1 Commonly marketed minimally processed fruits and vegetables

Commodity	Minimally processed form
Fruits	
Apple	Peeled, cored, sliced
Oranges	Peeled sections
Pineapple	Peeled slices, chunk, peeled cylinders
Grapes	Washed and destemmed
Melon	Slices with and without rinds
Oranges	Peeled sections
Vegetables	
Carrot	Peeled slices and sticks, shredded
Onion	Sliced, rings
Tomato	Sliced
Broccoli	Individual floret with or without stalk

(Donald, 1995)

Advantages of minimal processing

i) Minimally processed products retain flavour, aroma and nutrition better than conventionally processed products

ii) Prepackaging allows for more efficient portion control

iii) It reduces the post harvest loss and increase the consumption of fruits and vegetables

iv) Labor cost for preparation are reduced

v) A wide variety of menu items, including unusual or unique salad combinations can be available throughout the year

Problems in minimal processing

i) Increased transpiration and respiration leads to water loss and finally causes wilting

ii) Accumulation of secondary metabolites like poly phenoloxidase will leads to browning

iii) Susceptibility to microorganisms

Shelf life of minimally processed fruits can be enhanced by:

1 Modified Atmospheric storage
2 Refrigerated storage
3 Chemical treatments
4 Ionizing Radiation
5 Mild Heat treatments
6 Reduction of water activity
7 High pressure processing

1. Modified Atmospheric Storage (MAP)

Modified atmosphere packaging delays postharvest spoilage resulting in enhanced quality attributes MAP relies on the integrity between the natural processes of respiration and gas exchanger through the package The increase of CO_2 and depletion of O_2 due to respiration and regulation of the gas exchange through the polymeric film contribute to an adequate O_2 and CO_2 concentration at the equilibrium MAP having various advantages such as, which reduces the product respiration and transpiration rate, reduce the ethylene synthesis and also slow down the browning reactions

Consumer demand for fresh and convenient foods has led to the growth of modified atmosphere packaging (MAP) as a technique to improve product image MAP has become a widely used food preservation technique in which the food is not kept under normal air but under a different gas mixture atmosphere (Handenburg, 1971) A concentration of 5-10% CO_2 and 2-5% O_2 has been suggested for modified atmospheric packaging of minimally processed products (Kader *et al*., 1989) O_2 was

effective in preventing browning and softening in sliced pear whereas 12%. CO_2 atmosphere prevented softening in strawberry (Rosen and Kader, 1989).

Low O_2 and or elevated CO_2 environments in the package of fresh cut produce can extend shelf life of minimally processed fruits and vegetables (Day, 1996). High CO_2 (15%) improved firmness in cut cantaloupe (Madrid and Cantwell, 1993) but caused tissue softening in cut peppers (Galvez *et al.*, 1997). Oxygen enriched atmosphere have been tested for packaging of iceburg lettuce, orange and potato tubers (Amanatidou *et al.*, 2000). They reported that a combination of 50% O_2 + 30% CO_2 prolonged the shell life of sliced carrot compared to storage in air by 2-3 days at 80°C. But high CO_2 and low O_2 concentration may adversely affect the product quality and commodities differ in their response to storage atmosphere (Toivonen and De Ell, 2002).

2. Refrigerated storage

Minimally processed fruits and vegetables are perishable and demonstrate fast degradation of quality when stored at inadequate temperatures as a consequences of damage caused to tissues during peeling, cutting, shredding and packaging.

Storage of minimally processed foods at low temperature (<4°C) decreases enzymatic activity. Therefore refrigerated storage results in less biochemical and microbiological changes (Elliot and Michenoer, 1995). John and Narasimham (1998) reported that minimally processed bread fruit stored at 8°C had a maximum shelf life of 10 days and 13 days respectively and fruits stored at 0°C exhibited chilling injury and loss of sensory qualities.

3. Chemical treatments

Many physical and chemical methods have been employed to control the undesirable physiological and microbiological spoilage changes (King and Bolin, 1989). Chemical additives are used primarily as an aid to preservation and not as primary mode of preservation (Huxsoll *et al.*, 1989).

i) Preservatives

There are few antimicrobial chemicals that may be applied directly to fresh cut products. Chief among these are weak acids, sorbic acid or potassium sorbate and benzoic or sodium benzoate. These compounds are most effective against yeast and

molds but also act against many decay and human pathogenic bacteria. Other organic acids such as citric acid may also be considered antimicrobial food additive but they act mainly to acidify the surface of fruit or vegetable tissue.

ii) Texture improvers

Calcium is involved in maintaining the textural quality of fruits and vegetables. Calcium ions form cross links or bridges between free carboxyl groups of pectin chains which strengthen cell wall (Rosen and Kader, 198; Dong *et al.*, 2000) Calcium chloride is the most frequently used firming agent. In fresh cut melons dipped in calcium chloride solution at different temperatures, texture was firmer in samples treated at 60°C than at 40°C or 20°C (Guzman *et al.*, 1999). Dong *et al.* (2000) have also reported that pear slices dipped in 1.0% calcium lactate solution had better texture as compared to control.

iii) Antibrowning agents

Several chemicals have been used in the control of browning. Some of them act directly as inhibitor of polyphenoloxidase, others render the medium inadequate for the development of browning reactions (Garcia and Barrett, 2002)

iv) Acidulants

Reduction of pH below 4.5 reduces PPO activity and irreversible inactivation of PPO can be achieved below pH 3.0 (Richardson and Hyslop, 1994) Effectiveness of acidulants in inhibiting browning increases when used in combination

4. Ionizing Radiation

Food irradiation is an important application of nuclear energy for the benefit of food preservation, which can be used to extend the shelf life of fresh food and to improve microbiological safety by eliminating several pathogens from it. Irradiation delays ripening, inhibits growth and sprouting and disinfects fresh produce (Kader, 1986)

Chuanyo, *et al.* (1993) irradiated Golden Delicious apple and found that firmness of apple irradiated with 0.3 to 0.9 K Gy was higher than that of non-irradiated apples. Irradiated fruits softened at a much lower rate than did non-

irradiated fruit during storage Irradiation dose of 1KGy resulted in acceptable apple quality for marketing after 11-month storage.

Minimally processed melons were treated with gamma irradiation (0.1,0.2,0.3,0.4 or 0.5 KGy), those irradiated with 0.1 and 0.2 KGy gave best results. Irradiation with and 0.5 KGy was not considered suitable for minimally processed melon In cucumber gradual decrease in firmness was observed with increasing radiation rate. whereas the texture remained within acceptable limits up to a radiation rate of 2.5KGy The appearance and flavour scores of cucumber decreased with increasing radiation rate and the overall acceptability was better after radiation at 2.5 and 3.0 KGy (Khattak *et al*., 2005).

5. Mild heat treatment

Heated water would also be useful alone or as a supplement to sanitizer treatment in reducing microbial population on fresh cut products Three log reduction in microbial population on fresh cut lettuce washed in chlorinated water at 47°C for three minutes was observed compared to one log reduction using 4°C chlorinated water (Delaquis *et al*., 2002)

6. Reduction of water activity

Reduction of water activity may be activated by removing moisture by partial dehydration or by addition of water soluble ingredient with high osmotic pressure. such as sugar or salt. which binds with the product water (Huxsoll and Bolin. 1989) Osmotic dehydration at mild temperatures preserves fresh like characteristics of fruits and vegetables and reduces the water activity Vaccum impregnation increases the rates of water and solid gain and these processes result in low reduction in weight (Bharat *et al*., 2002)

7. High pressure processing

Enzymes like polyphenoloxidase have been inactivated by application of high pressure at low temperature (Hendricks *et al*., 1998) There was either no adverse effect or minimal effects on flavour and nutritional value of foods Hong and Kim (2001) studied the storage quality of chopped garlic treated with organic acids given at high pressure treatment conferred best storage stabilization to chopped garlic

Emerging technologies in production of convenience foods

1 Retort processing

2 High pressure processing

1. Retort processing

It is based on thermal processing. Retort is an autoclave which is used to sterilize the packaged foods.

i) Retort pouch

Retort pouch is a promising packaging technology. It is a flexible laminated package that can withstand thermal processing temperatures and combines the advantages of both metal cans and plastic packages (Fig 2) (Soojin *et al* . 2006)

According to Griffin (1987), retort pouches are constructed with four plylaminate consists of

Polyester	Outside layer
Nylon	Second layer
Aluminium foil	Third layer
Poly propylene	Inner layer

Advantages of retort pouch over metal cans

i) Reduced working time

ii) Better taste and nutritive value

iii) High heat penetration

iv) High package durability

v) No need for refrigeration

vi) No need for addition of preservatives

vii) No over cooking of the products

viii) Easy to handle

ix)Require only less storage space

Fig 2. Retort Processing

a) A high speed retort pouch filling machine

b) Retort pouches

Chia *et al.* (1983) reported that when compared to canning, retort processing requires 34% less time for processing. Gopal *et al.* (1986), reported that retort processed foods showed firm texture and better organoleptic quality than conventional processing. According to Dileep and Sudhakara (2007), there was not much variation in retort prcessed and raw shrimp in quality characters , but increasing trend of protein, ash and fat was observed and it may be due to the addition of ingredients such as coconut gratings and also due to some other ingredients added during curry preparation(Table 2)

Table 2 Proximate composition of raw shrimp and retort pouched shrimp product

Parameters	Raw shrimp (%)	Product (%)
Moisture	81 2	71 8
Protein	17 1	21 0
Fat	0 77	3 28
Ash	0 90	4 26

(Dileep and Sudhakara 2007)

ii) Self heating containers

Self heating food containers, popularly known as "Kitchen in carton" are designed to heat and eat on the move (Sajeevkumar *et al*., 2005). The system design depends on whether it is used for heating canned foods or ready to eat food products in flexible pouches. Heating of food products such as soups, beverages etc. in tin or plastic containers are a complicated process. In general it is a 'can in can' type system with one can containing the food product and other containing the chemicals undergoing exothermic reactions

Once the reactions started in the reaction chamber, the heat is transferred to the product in the food chamber through conduction and thus food is warmed to the consumption temperature. The system may have an insulator to minimize the heat loss to the surroundings (Fig. 3).

Chemicals used for creating exothermic reactions

1. Hydration of anhydrous calcium oxide
2. Electrochemical reaction of an alloy of Mg and Fe in an electrolytic solution
3. Oxidation of poly hydroxy alcohol in the presence of oxidizing agents.

Self heating containers consists of:

i Chemicals which can undergo exothermic reaction

ii A reaction chamber

iii Ready to eat retort processed foods in a flexible pouch kept in contact with the chemical pad

Working principle of Self heating containers

It consists of precooked meals in a flexible polymeric pouch, a Flameless Ration Heater (FRH), 40 ml sachet of water to activate the heater, a dish, knife, fork, salt and pepper. All the items are put together in a compact outer box. The heater consists of magnesium iron alloy, sodium chloride, silica and wetting agent inside a fiber pouch, which is further sealed in a strong outer sleeve

The heater sleeve and food pouch are placed inside the polystyrene tray provided, and 40 ml of water is added to activate the heater. The tray is placed inside the box, which act as an oven during heating. A string is attached to the bottom of the box which when pulled release water to the heater and generate exothermic reactions. Once heated, the heater will remain hot for 45 minutes (Mohan, 2000)

Characteristics of good self heating chemicals

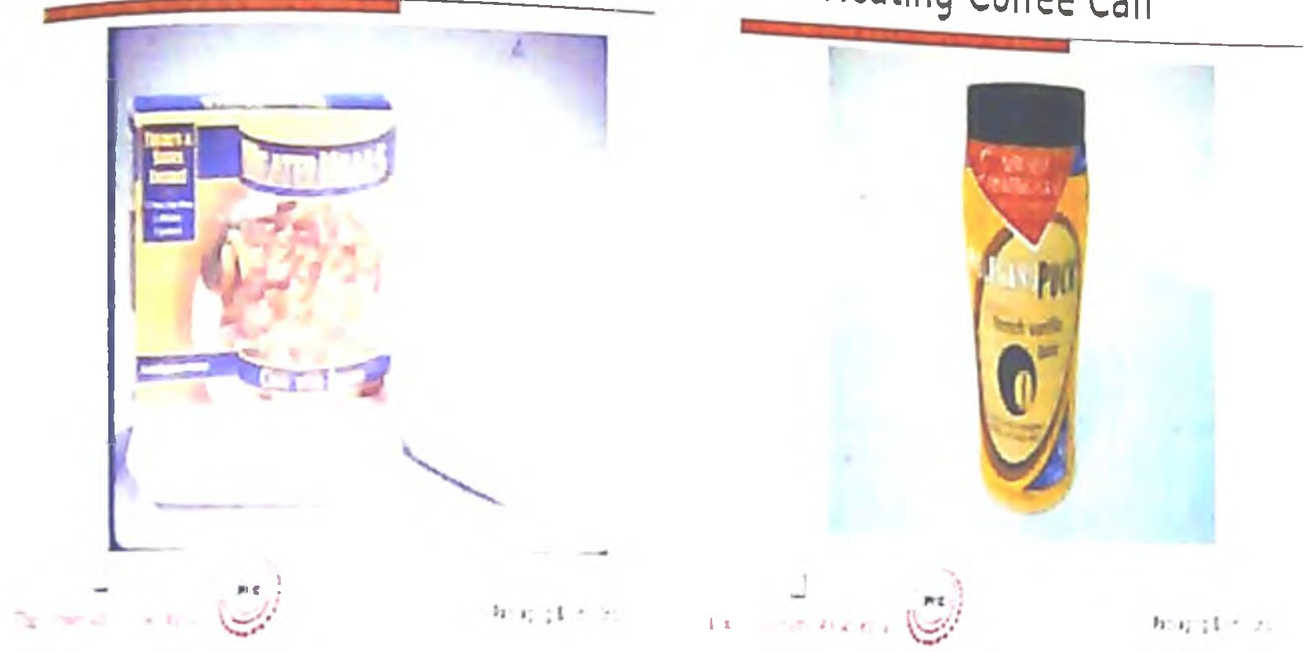
a) It should release large amount of heat during reaction

b) No production of hazardous by products

c) Produce exothermic reaction with water

d) Being cost effective

Fig. 3 Self heating containers available in the market



Advantages of self heating containers

1. Shelf stable even up to 5 years
2. The heater residue i.e., magnesium hydroxide or calcium hydroxide is non toxic.
3. People are able to get hot and tasty meals any time, any where without use of any external energy source such as electricity.

Commercially available self heating food containers

i Hot can

ii Zestro Therm's Flameless Ration Heater (USA)

iii Heater meals (USA)

iv Alpine Aire Foods

v Ontro Inc Poway, California

2. High Pressure Processing (HPP)

High pressure processing is a novel non thermal food processing technique Food preservation using high pressure is a promising technique in food industry as it offers numerous opportunities for developing new foods with extended shelf life, high nutritional value and excellent organoleptic quality (Messens *et al*., 1997)

HPP uses an isostatic pressure at room temperature, between 100-600 mpa and induces special effect on food products It is an alternative to thermal processing (Ramaswamy and Balasubramaniam, 2003)

Exposure of food to high pressure causes inactivation of microorganisms, denaturation association and gel formation of biopolymers resulting in modification of food quality attributes such as appearance, texture, susceptibility to enzymes etc The treatments can helps in shelf life extension of foods (Farr, 1990) Preservation of fruit and vegetable juices through HPP conserve sensory characteristics such as flavour and taste at the same time it ensures microbial safety

Advantages of High Pressure Processing

i) Operation at low or ambient temperature

ii) Helps in retain the food quality

iii) Maintains the freshness

iv) Helps in extending the shelf life

v) Suitable for heat sensitive products

vi) No addition of preservatives

vii) Uniformity in application

viii)Hygienic and ecofriendly process

Limitations of High Pressure Processing

i) HPP cannot be used to make shelf stable version of low acid foods

ii) Some spores of microorganisms are resistant to HPP

iii) Food must contain water and not have internal air pockets.

iv) Food enzymes generally requires very high pressure for inactivation.

v) Most pressure processed foods need low temperature during storage and distribution

HPP works based on Pascal's law. Pascal's isostatic principle indicates that pressure applied to a sample including biological products is transmitted in a uniform and instantaneous manner. In a typical HPP process, the product is packaged in a flexible container and is loaded in to a high pressure chamber filled with a pressure transmitting hydraulic fluid. The hydraulic fluid normally water in the chamber is pressurized with a pump, and this pressure is transmitted through the package into the food itself. Pressure is applied for a specific time i.e., 3-5 minutes. The processed product is then removed and stored in the conventional manner. Pressure is transmitted uniformly, food retains its shape, even at extreme pressures and no heat is needed, the sensory characteristics of the foods are retained without compromising microbial safety (Fig 4)

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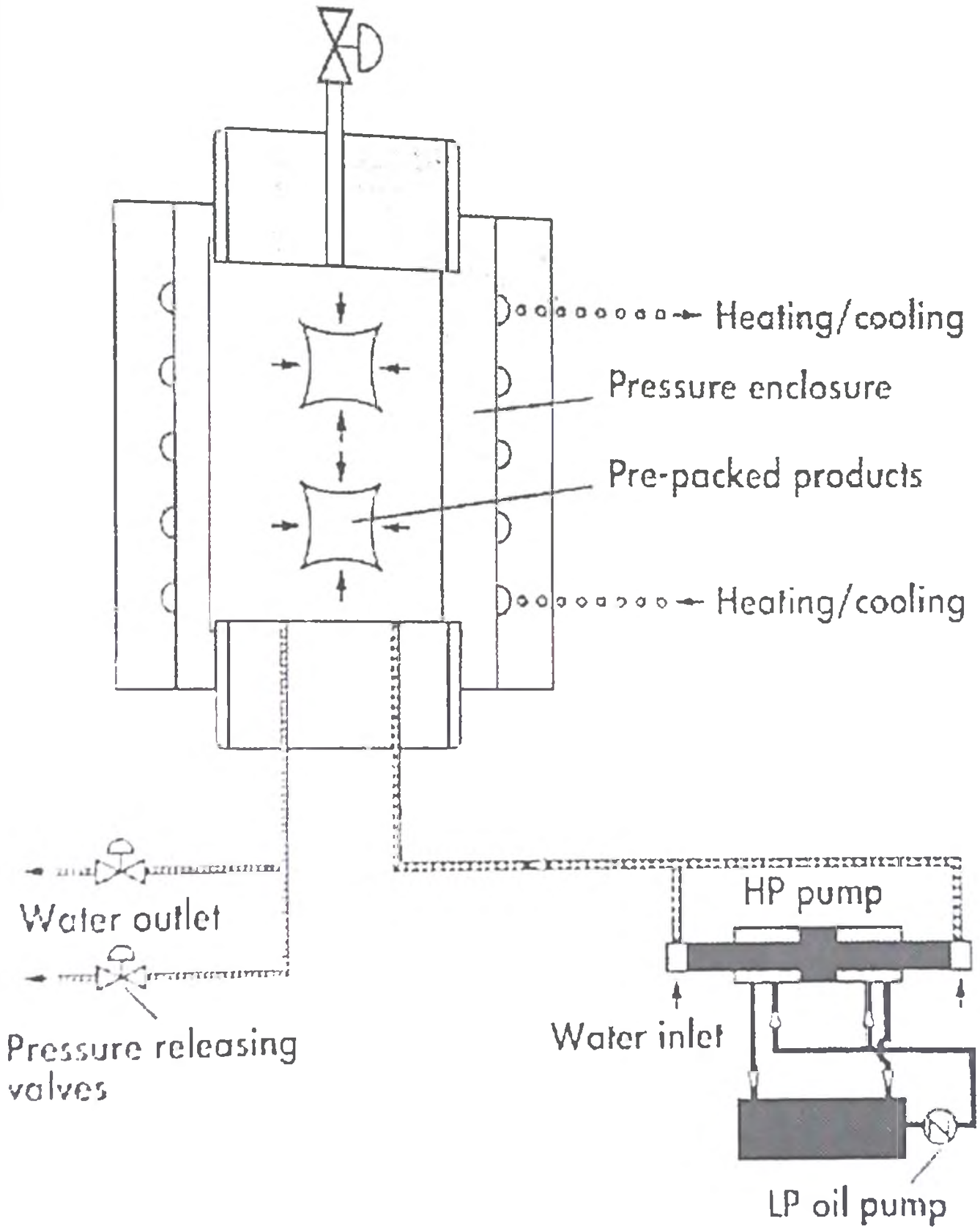
Fig. 4 High pressure processing



Venugopal *et al.*(2001) observed that when different types of meats were subjected to high pressure processing , quality was maintained without causing any deteriorative changes (Table 3).

Table 3 Effect of high pressure on some individual products

Food Item	Pressure MPa	Quality
Beef	200-350	Pink meat
Buffalo meat	400	No change in colour
Pork	400-500	High digestibility
Rabit meat	350	Soft smooth and glossy

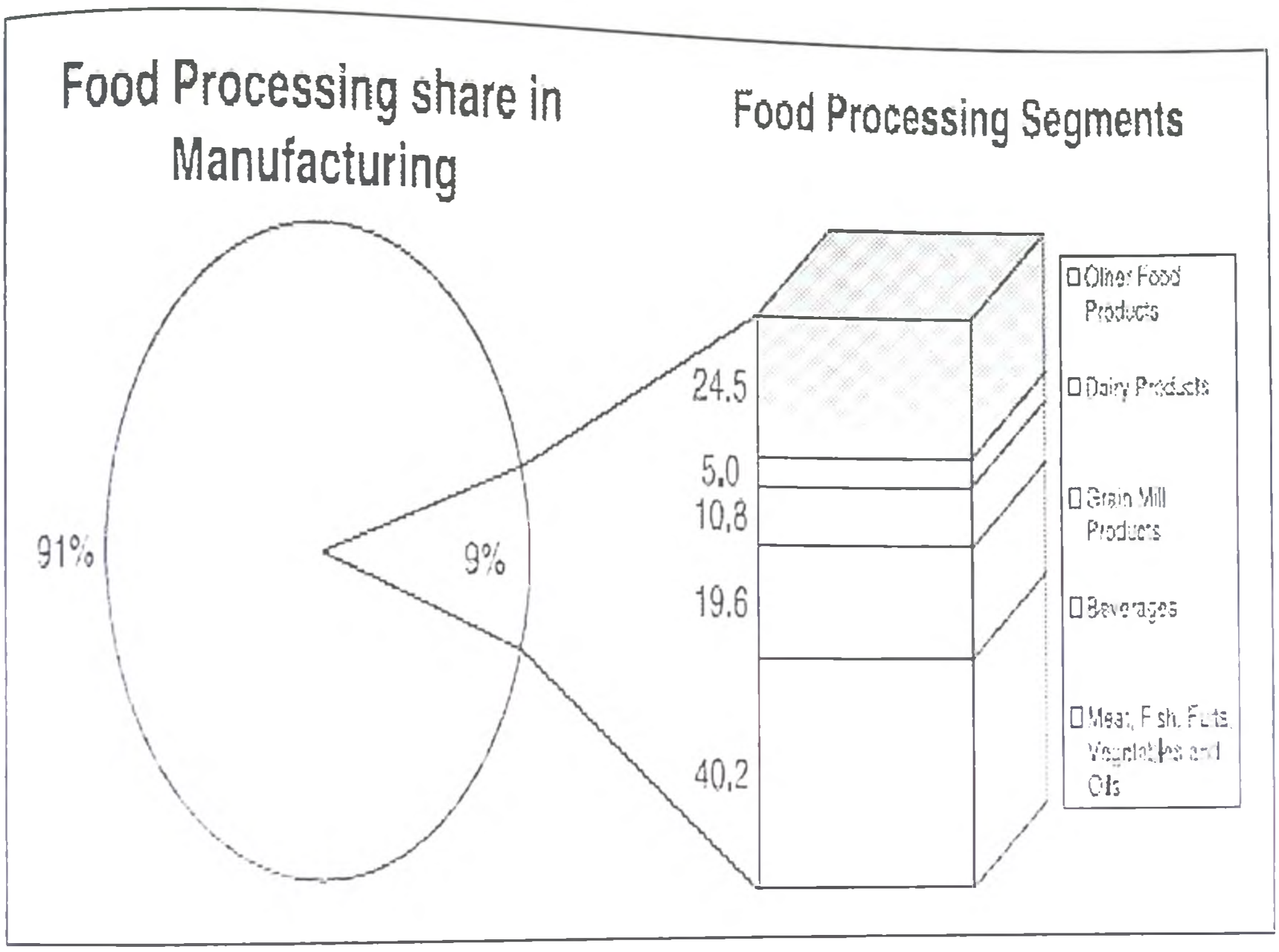
Current status and future prospects of convenience foods

India is one of the world's top producers of food. It is first in milk and cereal production and second in fruits and vegetables. With an estimated food production of about 600 million tones of food and a US $ 70 billion industry including US $22 billion of value added products. India is stated to become a global outsourcing hub.

Compared to clothing, transportation and communication, medical care, education etc. an average Indian household is reported to be spending 48 % of their expenditure on food items of which 5% on branded products. Size of semi processed and ready to eat foods in India is estimated as US $ 22 billion which is growing at a rate of 20 % per year.

Today the food processing industry is growing rapidly driven by changing consumer trends. Over 266 milk product units,171 milk producing units,418 fish processing units,171 meat processing units,609 sweetened and soft drinks,820 flour mills and many other food processing units are existing in the organized sector of our country. The food processing industry in India has a share of 1.5% in the total GDP of the country, and as a part of total manufacturing accounts for 9% India's share in world trade in respect of processed food is about 1.6% (Fig. 5).

Fig 5. Overview of Indian food industry



The four fundamental shifts which further emphasize growth in the convenience foods are

- Rapid growth in organized retail, a catalyst for the processed food development
- Convenience and enjoying life driven by demographic trends in age , income levels and more women in the work force
- Global shift to outsourcing from India across products or services including foods
- Deregularization and liberalization of the Indian economy since from 1991

Table 4 . Major food processing companies

Major MNC	Major Indian companies	Indian companies likely to enter
Nestle	ITC	Reliance
Kellogs	Dabur	Bharti group
Unilever	Britannia	Tata
Glaxo Smithkline	Parie	Wipro
Heinz	MTR	

Conclusion

In a busy and fast moving life with the changing socio economic factors, the demand for convenience foods is increasing steadily. India being one of the largest producers in the world is stated to become a hub for production of convenience foods Therefore in India the food processing is identified as a sunrise industry The de regularization and liberalization of Indian economy has boosted the food processing industry which is mainly engaged in production of convenience foods Understanding the scope and opportunities in convenience foods, more and more industries are venturing to the food industry Today more than 30 companies are processing and marketing convenience foods in the country

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Discussion

1 What is the major factor that governs the shelf life of retort and high pressure processed foods?

pH of the food is the major factor which helps to enhance the shelf life of food products Most of the microorganisms are very susceptible to high acid content ie pH of <3 7. so it requires only less temperature for processing In the case of low acid food, most of the spore forming microorganisms will survive, so it requires high temperature for processing

2 Can we store the convenience foods in ambient condition?

We can store all the thermally processed food items under ambient condition. but incase of high pressure processed food items we have to store it under refrigerated condition. because here we are not involving any temperature treatment for controlling spore forming microorganisms

3 What is the storage life of ready to eat foods?

In case of canned foods. we can store it even upto 5 years. but generally it ranges from 1-2 years depending up on the method used for processing and type of food materials For example in case of retort processed foods we can expect shelf life upto two years

4 How will you pack minimally processed fresh cut fruits and vegetables?

Modified atmosphere packaging is used for enhancing the shelf life of minimally processed fresh cut fruits and vegetables The most commonly used packaging materials are poly propylene covers. poly ethylene covers and poly styrene trays

5 Is. bread and milk are coming under convenience food?

Yes. it is also coming under a convenient food because it is available in a convenient form

6 What are all the pre treatments done for improving the shelf life of minimally processed fresh cut fruits and vegetables?

i) use of sanitizing agent such as sodium hypochlorite(30 ppm) and glacial acetic acid (2%).

ii) Treating with antibrowning agents like ascorbic acid, potassium meta bisulphate, citric acid and cysteine

iiii) Firmness retention agents like, calcium chloride (1%) and calcium lactate(0.5 %)

iv) preservatives such as sorbic acid, sodium benzoate, benzoic acid

7 Is there any research work underwent in KAU with regarding to minimal processing of fresh cut fruits and vegetables?

Research work on standardization of minimal processing of vegetables was done under KSCSTE project at Dept of Processing Technology during the year 2005 and research work on minimal processing of jackfruit is also going on here

8 At what situation we can choose convenience foods?

At any where at any time we cannot expect the fresh foods, at that condition we can go for convenience foods. Increasing demand of convenience food may be due to the increasing number of working couples and changing life style and food habits of middle income people

9 What is the reason behind, in increasing the protein content of retort processed shrimp curry?

It may be due to addition of coconut gratings and some other ingredient added during curry preparation

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KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE
Proc. 751 – Seminar

Topic: CONVENIENCE FOODS: GROWTH AND PROSPECTS

Name: Bijila, P.V. (06- 22-102)
Time: 10. 15 am., 05-05-07

Venue: Audio Visual Laboratory,
Dept. of Pomology & Floriculture

ABSTRACT

In modern living, food is no longer consumed for satisfaction of hunger alone, but for promoting nutrition and health, coupled with enjoyment. The present trend in the market indicates that consumers need to obtain food that is ready to eat along with variety, high quality, taste, nutrition and safety. Convenience foods are those foods that have undergone major processing by manufacturer such that they require little or no secondary processing and cooking before consumption (Arya, 1992).

The convenience foods consist of heterogeneous group of foods which vary in size, shape, method of preparation and processing, even with respect to their functions in the diet. This literally ranges from dehydrated products to ready mixes, canned and frozen foods, fresh cut fruits and vegetables and sophisticated warm and serve type dinners. Fresh cut fruits and vegetables are ready to eat which include fresh, washed and chopped vegetables, ready for use and packaged in sealed polymeric films or trays (Narendranath and Nirankar, 2006). However they deteriorate faster than intact products due to the wounding associated with processing, which leads to a number of physical and physiological changes affecting the shelf life and quality of the produce (Brecht,1995).Convenience foods are very handy, hygienic, reliable and consistent in quality, available at all season and moreover it requires minimum time for preparation. The technologies commonly adopted in the production of convenience foods are dehydration, freezing, canning, retort processing and high pressure processing (Ramaswamy and Balasubramaniam, 2003).

The major drawbacks of convenience foods are high initial investment, high cost as compared to fresh foods and chances for microbial contamination and spoilage, if not properly handled. In spite of the limitations, the demand for convenience food is growing at a faster pace due to change in socio economic factors as well as increase in urbanization, buying power, lesser time for food preparation and also the desire to taste new products (Potty, 2003). In a busy and fast moving life with rapidly increasing standard of living , these ready to use products will definitely find a place in our day to day life.

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NATURAL FOOD FLAVOURS: PROBLEMS AND PROSPECTS

By

Bijila P.V.
(2006-22-102)

SEMINAR REPORT

Submitted in partial fulfillment for the requirement of the
Course No. Proc. 752, Seminar (0+1)

DEPARTMENT OF PROCESSING TECHNOLOGY
COLLEGE OF HORTICULTURE
KERALA AGRICULTURAL UNIVERSITY
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THRISSUR, KERALA, INDIA
2007

DECLARATION

I hereby declare that the seminar report entitled "**Natural Food Flavours: Problems and Prospects**" is a record of the seminar presented by me during the course (20.07.2007) and that this report has been prepared by me independently after going through the references cited herein.

Bijila 30/8/07

Vellanikkara
30.08.07

Bijila.P.V.
(2006-22-102)

Certificate

Certified that the seminar report entitled **"Natural Food Flavours : Problems and Prospects "** is a record of seminar presented by **Ms. Bijila,P.V. (2006-22-102)** under my guidance and that this report has been prepared by her independently.

30..08.2007

Vellanikkara

Dr. K.B. Sheela

Major advisor

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Introduction

Flavour is a combination of taste, aroma and perception. Appearance of food is important but it is the flavour that ultimately determines its quality and acceptability (Sharma *et al.*, 1999). It is important to note that without desirable flavour, many essential foods containing high quality proteins and other vital nutrients would not be eaten due to their insipid and unpalatable nature (Sethi and Shruti, 2005).

Abundant varieties of flavouring substances have been provided by nature and the chemists have attempted to duplicate their properties (Khare *et al.*, 2006). Three classes of flavouring substances are available and they are natural, nature identical and synthetic. Natural flavouring substances such as spices, essential oils and fruit juice concentrates have been used since long back in food preparations but as their supply has not kept up with demand due to rise in their cost, natural flavouring agents have been largely substituted by synthetic ones (Hall,1990). However this can be overcome by adopting advanced extraction methods and encapsulation techniques. In India, use of food additives like flavouring substances are regulated by Prevention of Food Adulteration Act (1954) and its subsequent amendments.

Nowadays consumers are more health conscious and prefer food free of chemical additives. Synthetic food flavours often causes health hazards like hyperactivity, brain damage, cancer etc. In the recent years, consumer demands for natural food flavours which are safe and have no side effects have increased considerably. As the world today is searching for natural products, there exists an urgent need to produce more natural flavours.

Definition

"Flavour is a sensory phenomenon which is a combination of sensations of taste, odour, aroma, heat, cold and texture or mouth feel" (Srivastava and Kumar, 2002).

Fig 1. Flavour perception in human being

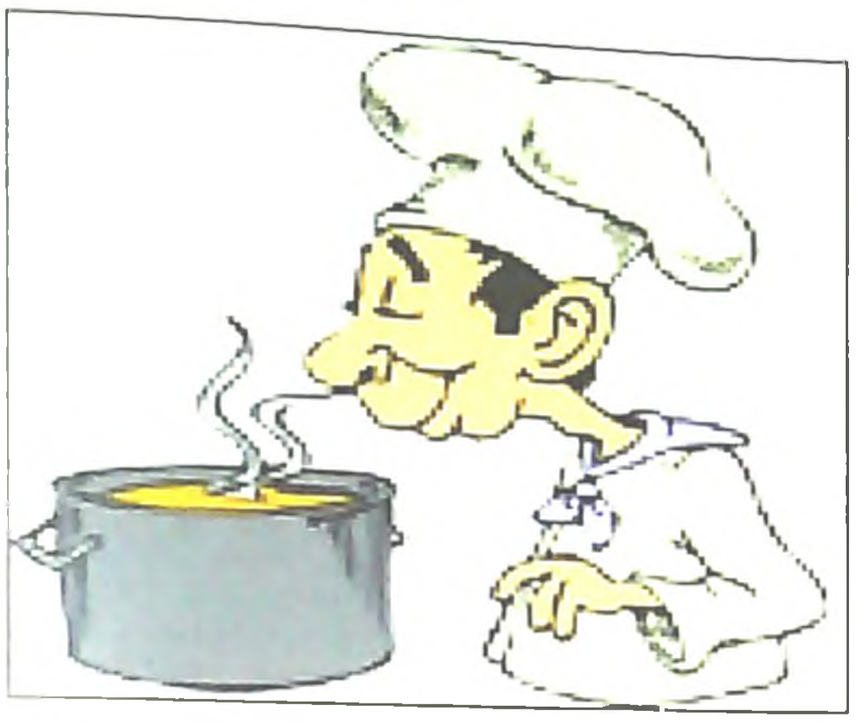


Fig 2.Flavour perception by tongue

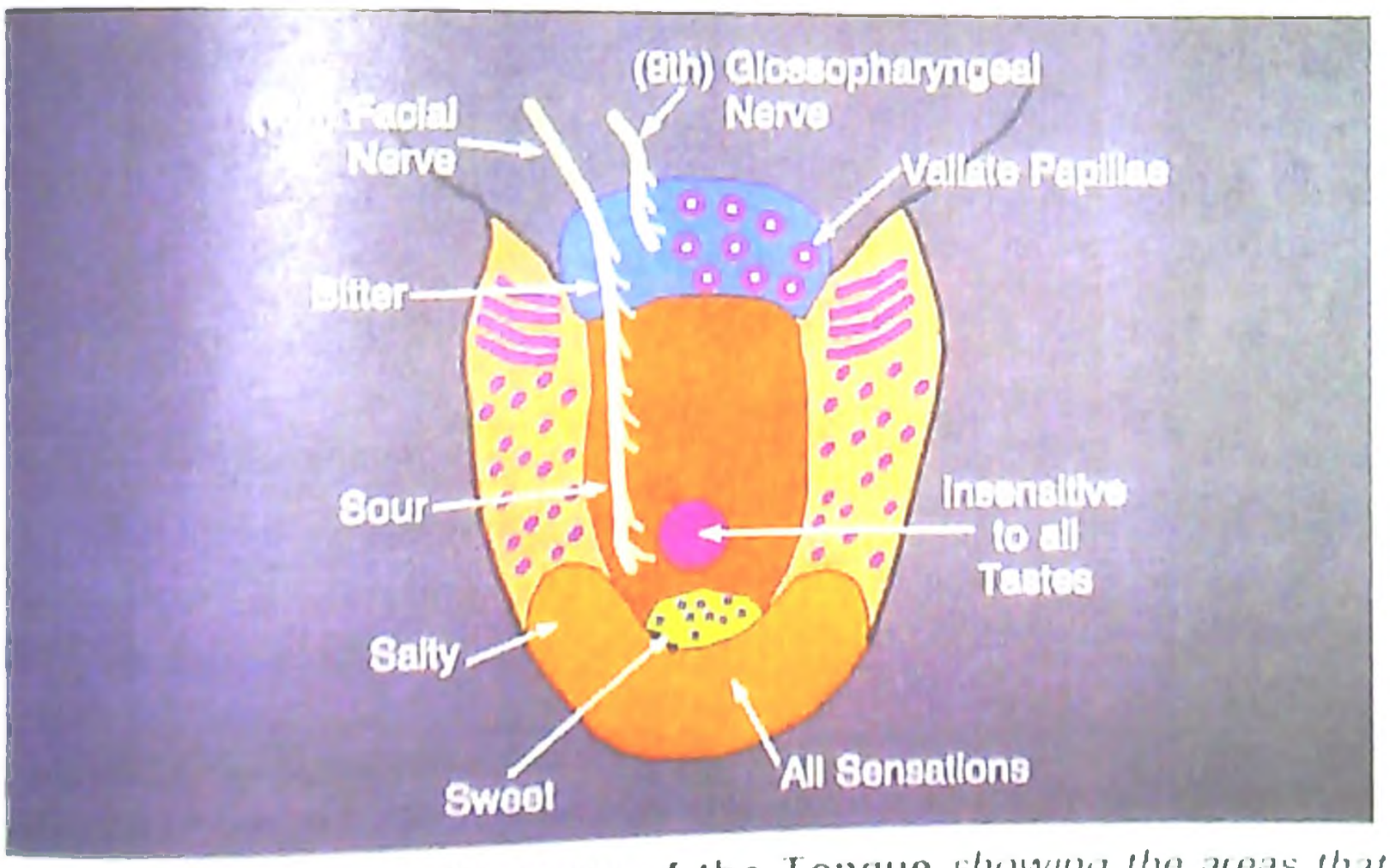


Fig 1 A Schematic Diagram of the Tongue *showing the areas that seem to have the maximum sensitivity to the four basic tastes: sweet, sour, salty and butter*

(Nagodawithana *et al.*, 1994)

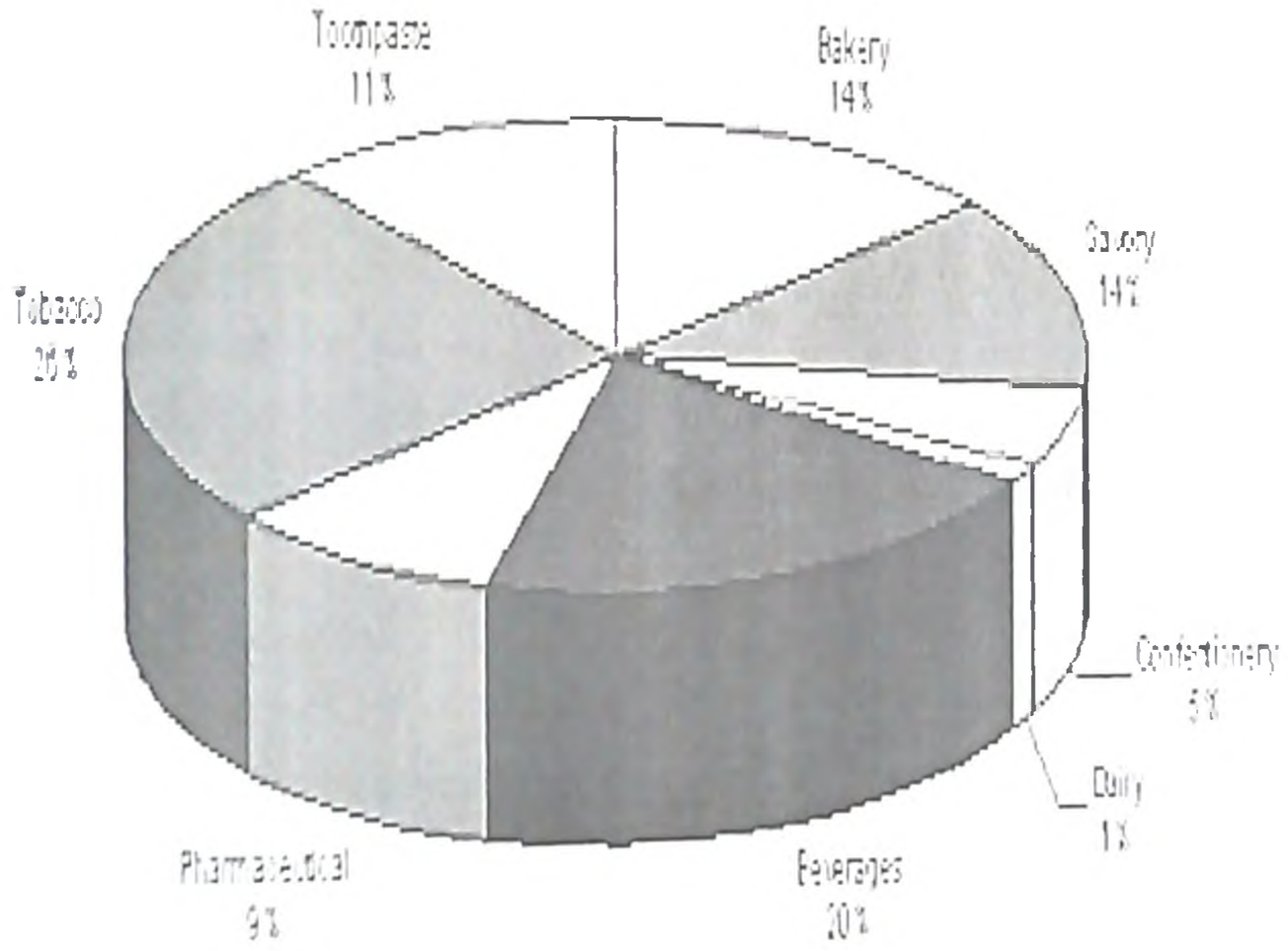
Application of flavours

Flvours have various applications in food, pharmaceuticals, perfumery and other industries such as masticatory, cosmetics, tooth pastes, paints, varnishes etc.

Fig 3. An overview of Indian flavour industry

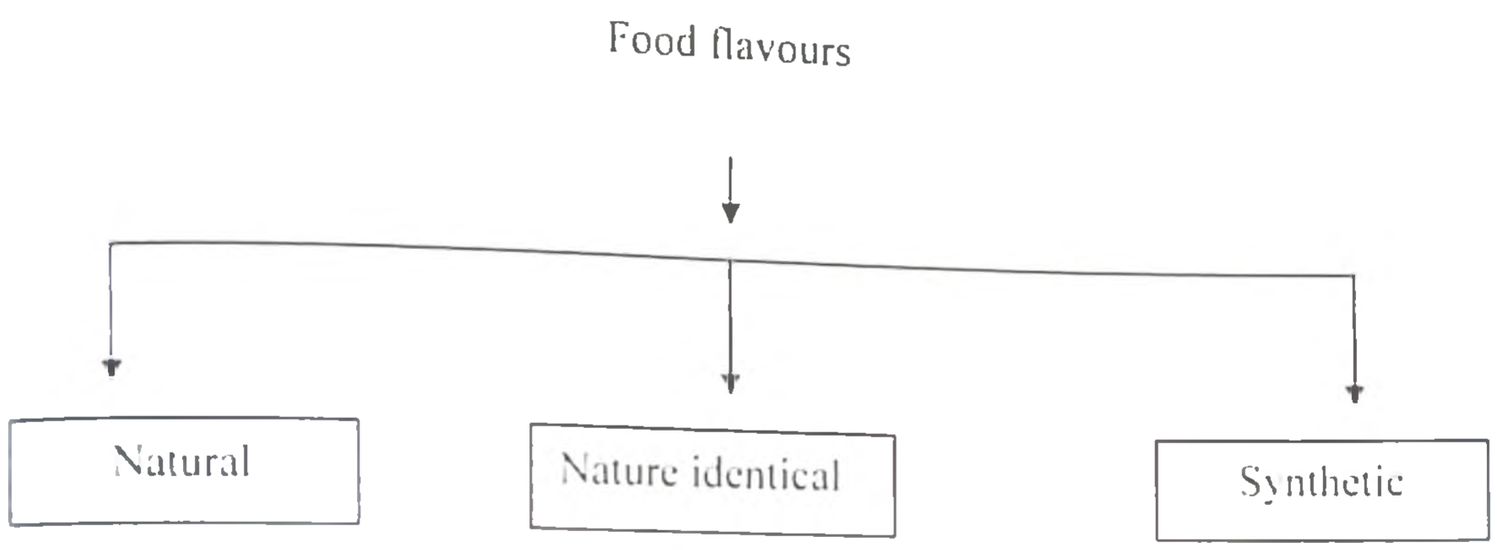
Chart 3

Segment-wise sales of the Indian flavors market: Break-up by revenues for the year 2004



Note: All figures are rounded. Source: Frost & Sullivan

Classification of food flavours



i) Natural Flavours

"Natural flavours are the essential oils, oleoresin, essence or extractive which contains the flavour constituents that are derived from natural sources".

eg: spices, herbs.

ii) Nature identical flavours

These are substances chemically isolated from aromatic raw materials or obtain synthetically. But they are chemically identical to substances present in natural products

eg: vanillin

iii) Synthetic flavours

Substances which have not yet been identified in natural products for human consumption, they are produced synthetically in laboratory.

eg: Allyl caproate

Inspite of the various merits of natural flavours, our food industry is still depending on synthetic flavours due to the following reasons.

i) They are stable and have a very long shelf life

ii) Cost effective

iv) Readily available

v) They are independent of natural cropping

vi) They can be made to give the optimum desired flavour effect

Drawbacks of synthetic flavours

i) Give unpleasant after taste

ii) Often leads to health hazards such as carcinogenicity, hyperactivity, renal problems etc.

iii) Synthetic flavours do not possess reservoir of flavour precursors

iv)Imitation flavours require either solvent or carrier. This may cause problems in texture of end product.

Table1. Synthetic flavours used in food industry

Chemical compounds	Fruity odour
Allyl hexanoate. butyl butyrate	Pineapple
Benzyl acetate. Ethyl formate	Strawberry
Ehtyl butyrate. amyl acetate	Banana
Linalyl formate. ethyl valerate	Apple
Methyl anthranilate. ethyl lactate	Grapes
Ethyl formate	Lemon
Nonyl caprylate. octyl acetate	Orange
Linalyl acetate	Lavender,Sage
Methyl acetate	Pepper mint
Ethyl vanillin	Vanilla

(Menon and Mulky, 1995)

Processed flavours

Flavours are produced only after processing (Patva and Pai,2004)

eg: Cocoa flavour, vanilla flavour

Flavour enhancers

They do not possess any flavour themselves but they intensify the flavours of other substances by synergistic effect" (Nagodawithana, 1994).

Eg. Monosodium glutamate, common salt

Scope for Natural flavours

More than 95% of our food and other allied industries are depending up on synthetic flavours. Due to increasing demand for healthy and natural food there is a growing interest to produce more natural flavours. Moreover synthetic food flavours often leads to health hazards such as carcinogenicity, hyperactivity, renal problems etc.

(Smith *et al.*, 2003)

Sources of Natural flavours

- Spices
- Aromatic plants
- Fruits
- Flowers

Spices

- Seed spices (Fig 4)
- Tree spices (Fig 5)
- Other spices (Fig 5)
- Herbal spices

Aromatic plants

Eg: mint, patchouli

Fruits

Eg: Citrus peel oil, fruit juice extracts, spray dried fruit powders

Flowers

Eg: Rose, Jasmine, Lotus

Constraints in using natural flavours

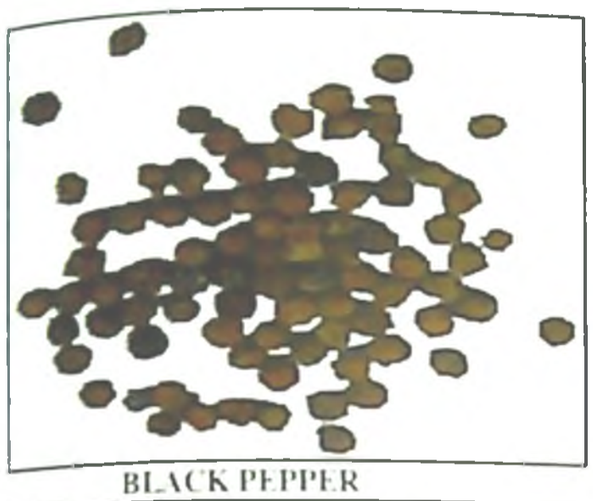
i) Many natural flavours have a low in flavour intensity and in consequence have to be used at high concentration / dosage rate in order to produce the desired effect in the end product.

ii) More Expensive

iii) Concentration of natural flavours needs extraction or evaporation which leads to changes in flavour profile

iv) Supply of natural flavours is becoming more uncertain and the quantity available now falls far short of demand

Fig.4 Important seed spices



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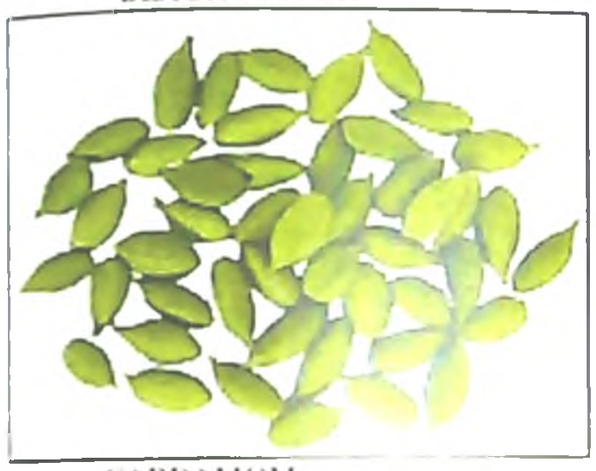
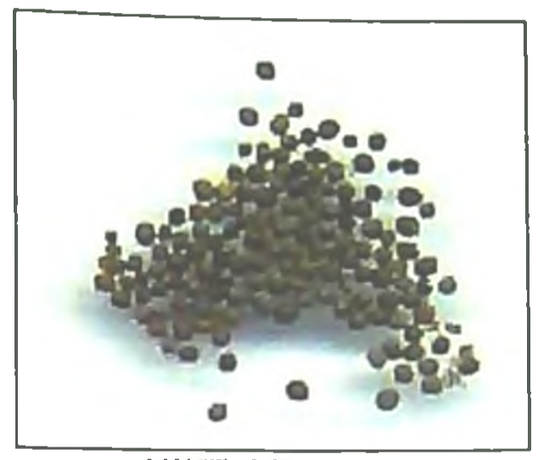


CARDAMOM

CARAWAY

DILL



MUSTARD

FENNEL

ANISE

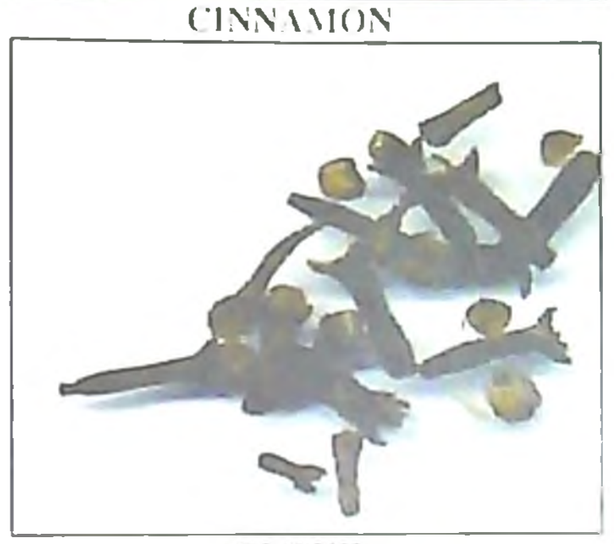
CORIANDER

Fig 5 Tree spices and other spices

ALL SPICE

CINNAMON

NUTMEG



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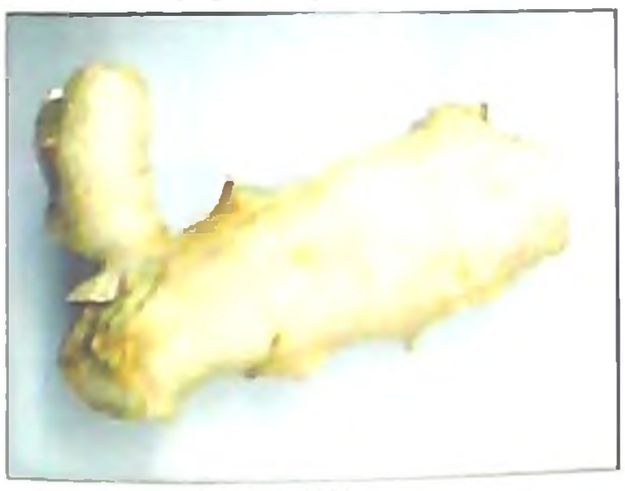
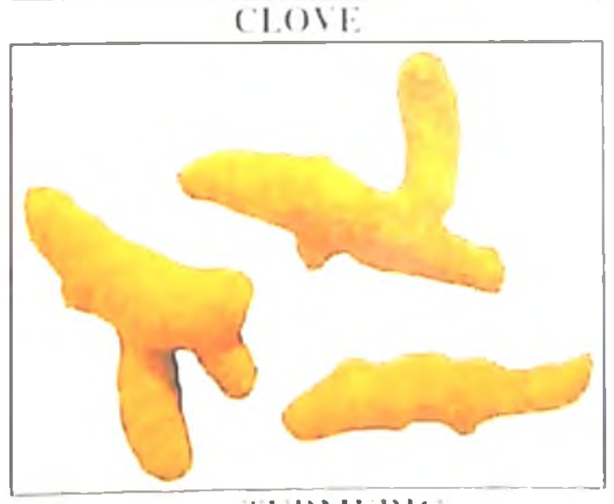


GINGER



TURMERIC

SAFFRON

VANILLA

v) Natural flavours exhibit variation in strength and quality which depends on the ripeness at harvest and its subsequent handling

vi) Most natural flavours are unstable and undergo changes during post harvest handling, processing and storage

Table 2. Important flavour compounds in spices

Spice	Flavour compounds
All spice	Eugenol, beta caryophyllene
Anise	E-anethole, methyl chavicol
Black pepper	Piperine, beta caryophyllene,beta pinene
Caraway	d-carvone,carvone derivatives
Cardamom	Alfa terpinyl acetate, linalool
Cinnamon	Cinnamaldehyde, eugenol
Chilli	Capsaicin ,di hydro capsaicin
Mustard	Allyl isothiocyanate
Nutmeg	Sabinine, alfa pinene, myrsticin
Parsley	Apiol
Saffron	Safranol
Turmeric	Turmerone , 1-8 cineole
Vanilla	Vanillin, para hydroxy benzaldehyde

(Nybe *et al.* 2007)

Table 3. Important flavour compounds in culinary herbal spices

Spices	Flavour compounds
Sweet basil	Methyl chavicol, linalool
Bay laurel	1,8 cineole
Marjoram	Sabinene
Rosemary	Verbenone, linalool, camphor
Sage	Salvial , linalool, thujone, camphor
Savory	Carvacrol
Tarragon	Methyl chavicol, anethole
Thyme	Thymol, carvacrol
Pepper mint	l-menthol, menthone
Spear mint	l-carvone,carvone derivatives
Tarragon	Methyl chavicol, anethole

(Pruthi, 2001)

Table 4. Important flavour compounds in fruits

Fruits	Flavour compounds
Apple	2 hexanol ,n butyl acetate
Banana	Isoamyl acetate, 2 heptyl acetate
Cherry	Methyl anthranilate,methyl salicylate
Custard apple	Beta pinene, germacrene
Citrus	Terpenes, citral, neral
Mango	Methyl hexanoate, geranyl acetate
Pineapple	Methyl β methyl thiopropionate
Peaches	Lactones, monoterpenes
Plum	benzaldehyde
Strawberry	Ethyl hexanoate, ethyl butanoate

(Bose *et al*., 2001)

Flavourings prepared from natural raw materials

With the exception of ground herbs and spices, few natural flavouring materials can be used directly in food processing. For this reason, numerous processes have been devised to separate out the flavouring components.

This includes

i) essential oil

ii) oleoresin

iii) extracts

iv) fruit juice concentrates

i) Essential oil

An essential oil is the mixture of organic compounds derived by some physical process, usually steam or water distillation from odorous plant materials. They generally constitute the odorous principle by which material is recognized, the amount present being an index of the flavorings strength. Essential oil may be further processed to give concentrated terpeneless and sesquiterpeneless oils which have specific uses in flavouring of food products.

ii) Oleoresin

Oleoresins are prepared from comminuted herbs and spices by extraction with a suitable solvent. The solvent used in the extraction is completely removed from the product by distillation under vaccum. These products are difficult to handle directly in food as flavouring agents, so they are usually diluted to an acceptable level as emulsions, encapsulated in gelatin or edible gum.

iii) Extracts

They are obtained by maceraton or percolation with a solvent usually ethanol. The solution of flavouring constituents may be used directly or subsequently concentrated by wholly or partially removing the solvent under vacuum. Great care is necessary to ensure the minimum damage to heat labile flavour components.

iv)Fruit juice concentrates

For use as a flavouring agent, it is necessary to process the fruit to separate juice from non-flavourful cellular pulp. Such juices must be preserved and are usually held in cold storage to ensure retention of their fresh character. The following concentrated products are available to the flavourists: a) Concentrated juices b) De pectinised juice c) Spray dried fruit powders It is difficult to extract the flavour compounds from fruits and the only commercially exploited flavour compound from fruit is citrus peel oil.

Extraction Methods

i) Water distillation (Fig 6)

ii) Steam distillation (Fig 7)

iii) Solvent extraction (Fig 8)

iv) Super critical carbon dioxide extraction (Fig 9)

v) Cold pressing

i) Water distillation

It is simplest and cheapest method of extraction of essential oil from plant materials. In this method, plant material is mixed with water in still pot. A perforated grid may be inserted above the base of the still pot to prevent the plant material settling on the bottom and coming in direct contact with the heated base of the still and charring. (Fig 6).

Disadvantges

- Get only poor quality oil because of over heating and direct water contact
- Slower extraction process
- Water distilled oil is darker in colour and having stronger off- note

(Krishnamoorthy, 1999)

ii) Steam distillation

It is the most common method of extracting essential oils from plant materials. In this method, fresh or dried botanical material is placed in the plant chamber of still, and pressurized steam is generated in a separate chamber and circulated through the plant material. The heat of the steam forces the tiny intercellular pockets that hold the essential oil to open and release them. The temperature of the steam must be high enough to open the pouches, yet not so high that it destroys the plants the essential oil (Fig 7).

As they are released, the tiny droplets of essential oil evaporate and together with the steam molecules travel through a tube into the still's condensation chamber. As the steam cools it condenses into water. The essential oil forms a film on the surface of the water. To separate the essential oil from the water, the film is then decanted or skimmed off the top. The remaining water a by product of distillation is called floral water, distillate or hydrosols. It retains many of the therapeutic properties of the plant, making it valuable in skin care for facial mists and toners (Meyer and Albert, 1998).

iii) Solvent extraction

Another method of extraction used on delicate plant and also for the extraction of oleoresin from spices (Fig 8). This method yields a higher amount of oleoresin at lower cost. In this process, chemical solvent such as petroleum ether, ethanol, propylene glycol, ethyl acetone and hexane are used to saturate the plant material and pull out the aromatic compounds. This renders a substance called as concrete. The concrete can then be dissolved in alcohol to remove the solvent. When the alcohol evaporates, an absolute

Fig.6 Water distillation

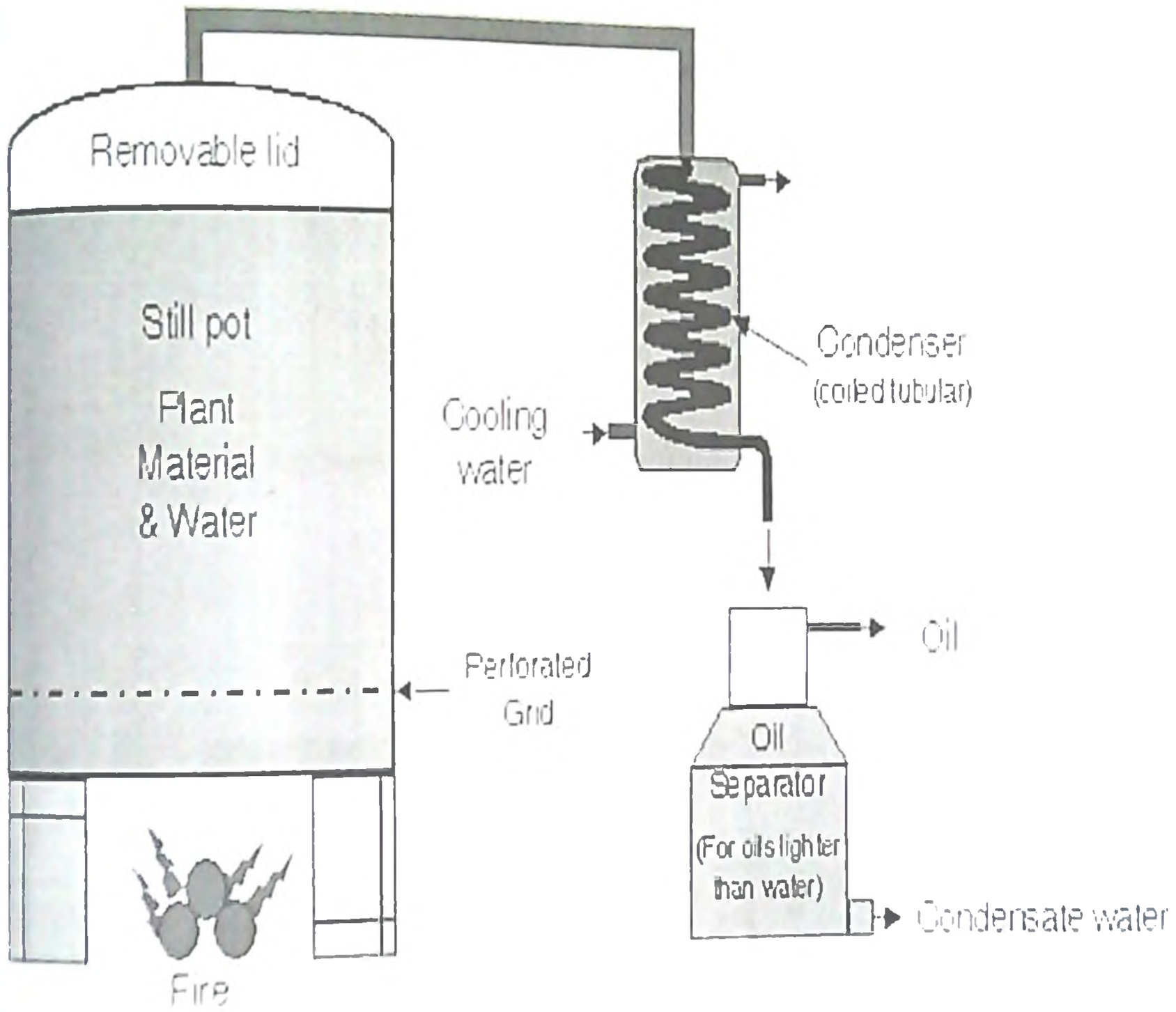
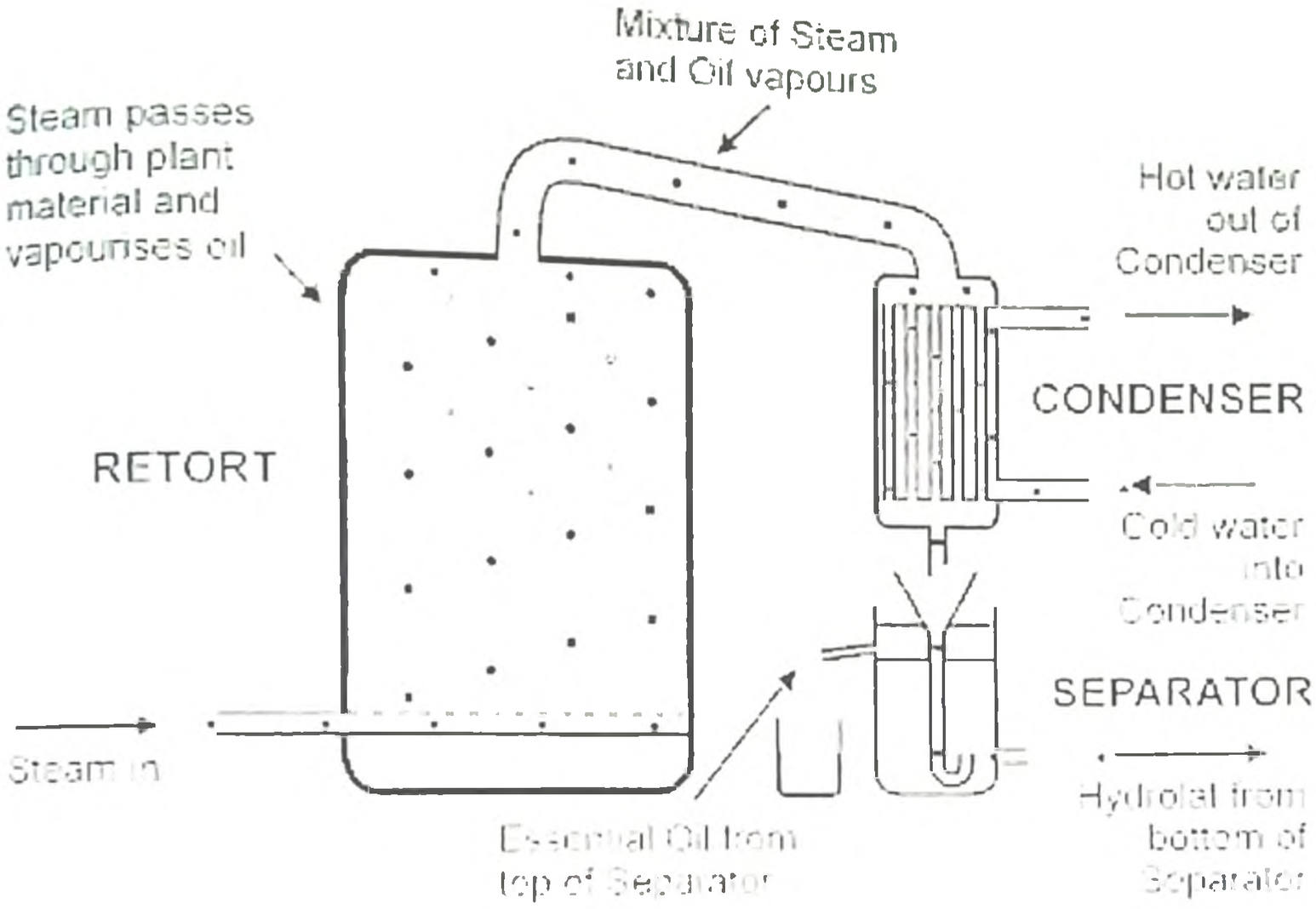


Fig.7 Steam distillation



Steam Distillation Unit

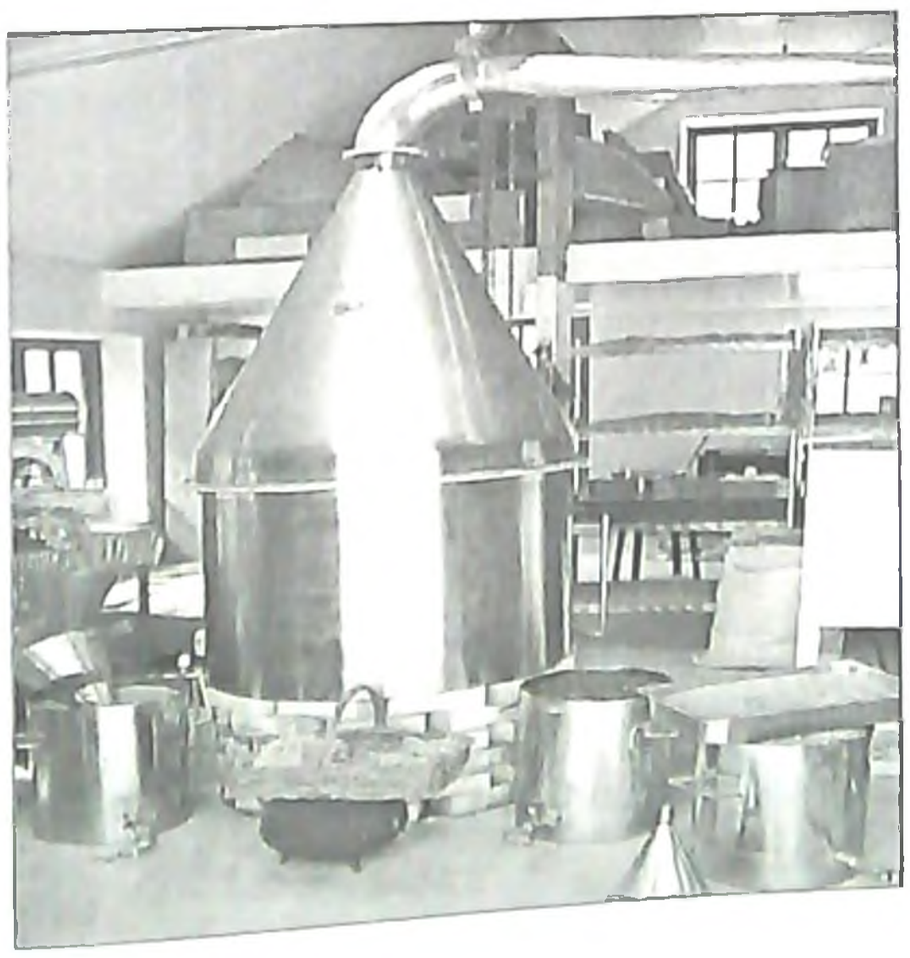
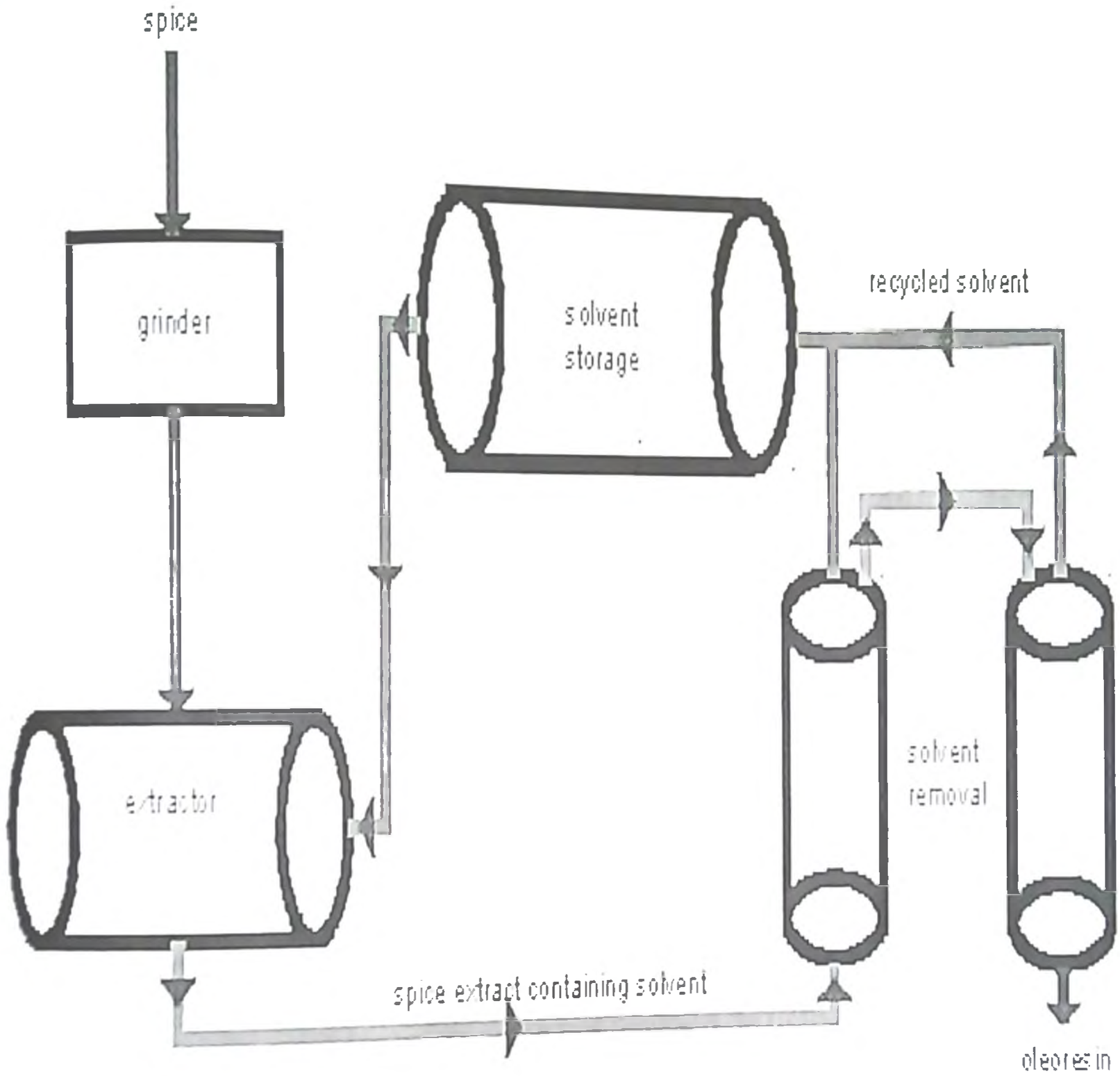


Fig.8 Solvent extraction



only remains. Although more cost efficient than other methods it have some disadvantages. Residues of solvents may remain in the products and often leads to health hazards (Thyagarajan and Kumar. 1995).

Advantages

- Suitable for heat sensitive plant materials
- Solvent extracted oleoresins are very concentrated and are very close to natural fragrance

Disadvantages

- Solvent residue of 6-20 % still present in finished product

iv) Super critical carbon dioxide extraction

Super critical carbon dioxide extraction method uses carbon dioxide under extremely high pressure to extract essential oils. Supercritical properties of carbon dioxide occurs at relatively mild conditions, temperature 31.2° C and pressure 73.8 bar which is attainable without affecting thermo labile components.

Advantages

- No solvent residue
- High recovery of oil
- Good solvating power
- Prevent oxidation of essential oils
- Suitable for heat sensitive materials
- Solvent used is odourless and inert
- Easy to separate and recover from solute mixtures due to low boiling point and heat of evaporation
- Neither harmful to man nor inflammable
- Free of explosion hazards and toxicity

Working of SCCE plant

Essentially, SCCE unit consists of four components

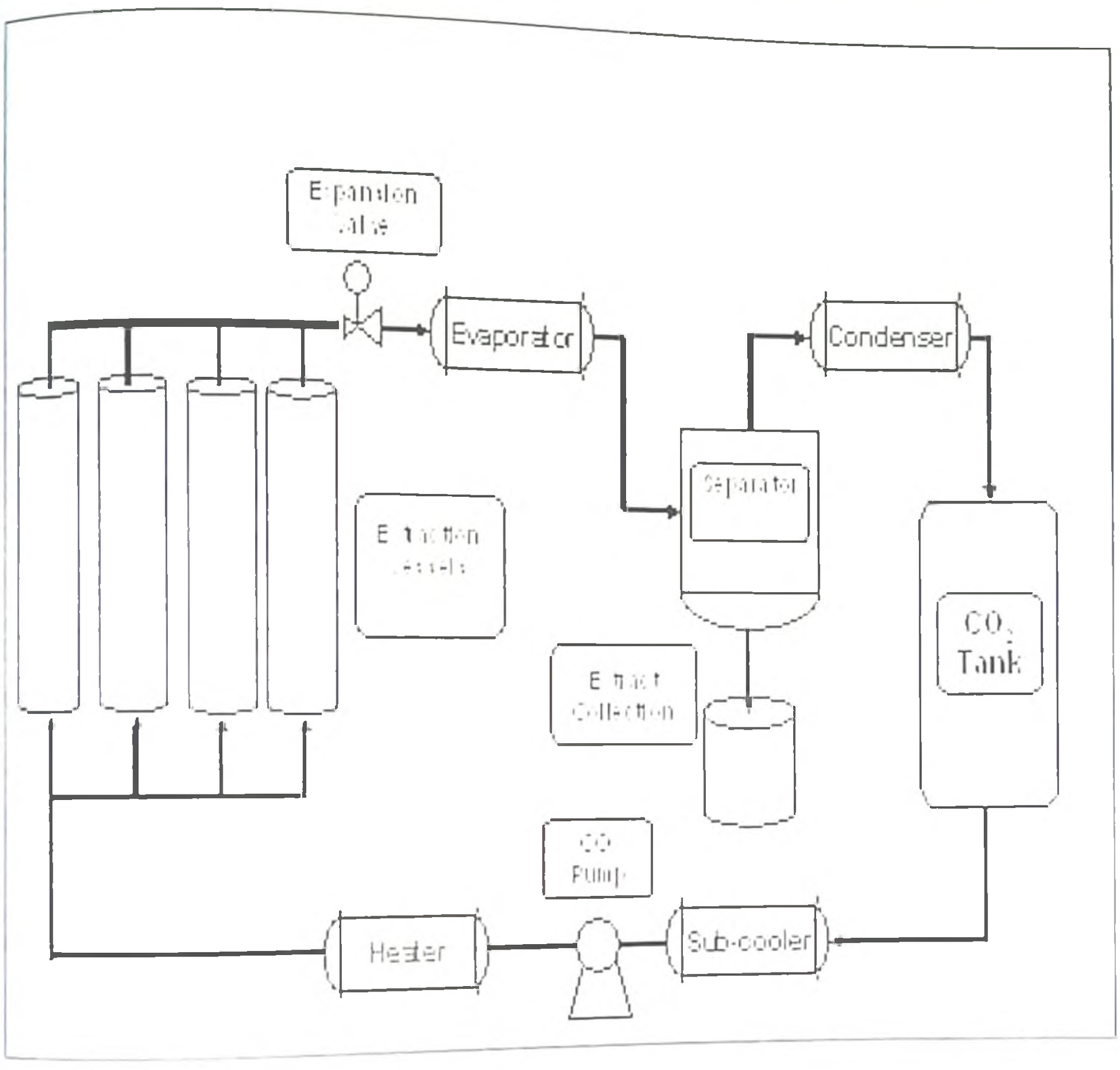
a). solvent compressor or high pressure pump

b). temperature pressure control system

c). extractor or pressurized vessel

d). separator or adsorber

Fig.9 Super Critical Carbon Dioxide Extraction Method



The material containing product of interest is fed into the extractor.Carbon dioxide gas is brought to the extraction pressure by the use of high pressure pump. It is then heated to the required extraction temperatures and passed to the extraction vessel. Under high pressure, carbon dioxide turns into a liquid and act as a solvent to extract the essential oil from the plants. The solvent coming out of the extractor will be laden with extracted solute. Separation can be done by reducing the pressure. When pressure is reduced, carbon dioxide returns to gaseous state, leaving no residues behind (Fig 9).

COLD PRESSING

It is used for obtaining essential oil generally from citrus peel (Fig10&11). Rinds are separated from fruit and they are ground, chopped and pressed at low temperature (40° C).

Advantages

- Suitable for heat sensitive plant material and it is the only method extraction of flavour compounds from fruits

Fig 10 & 11 Cold pressing machine

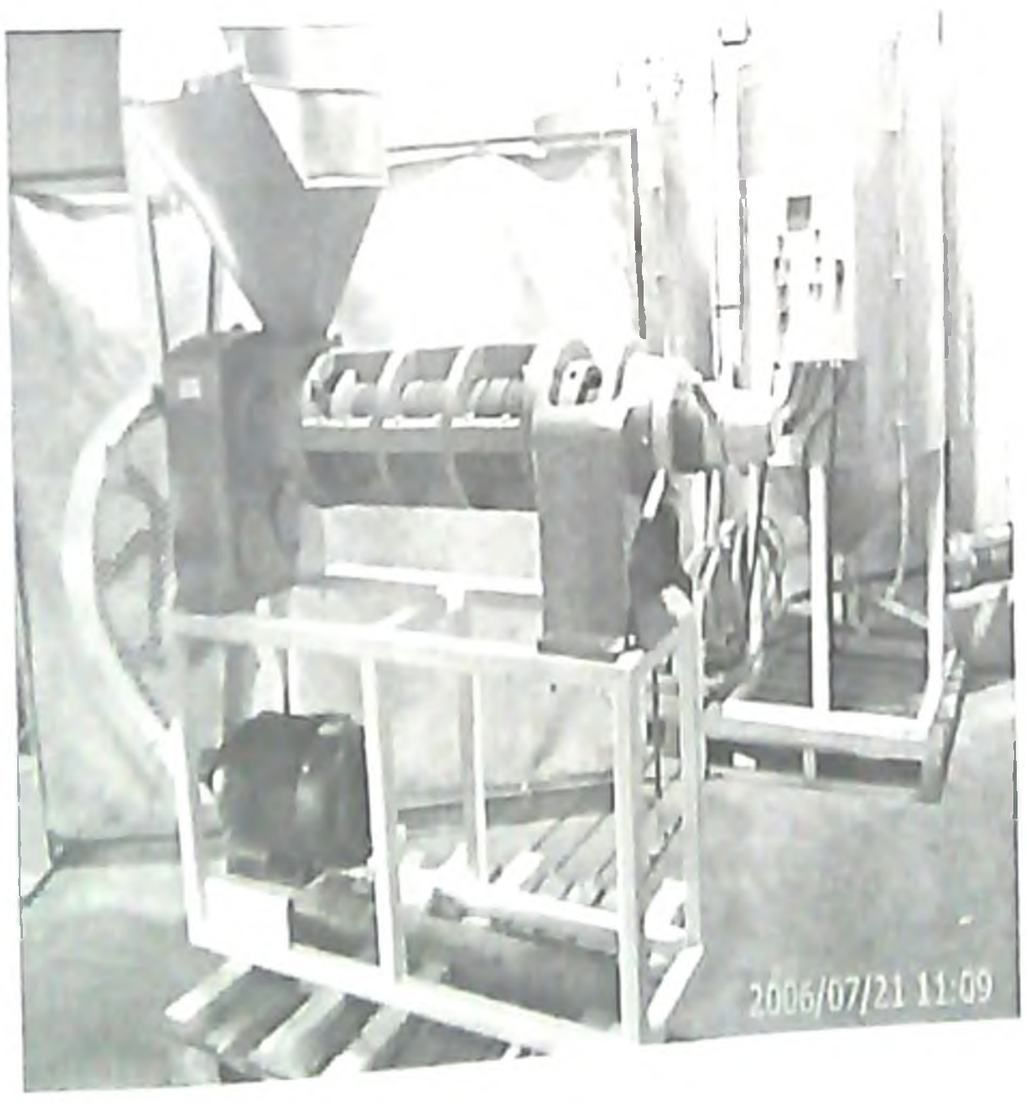
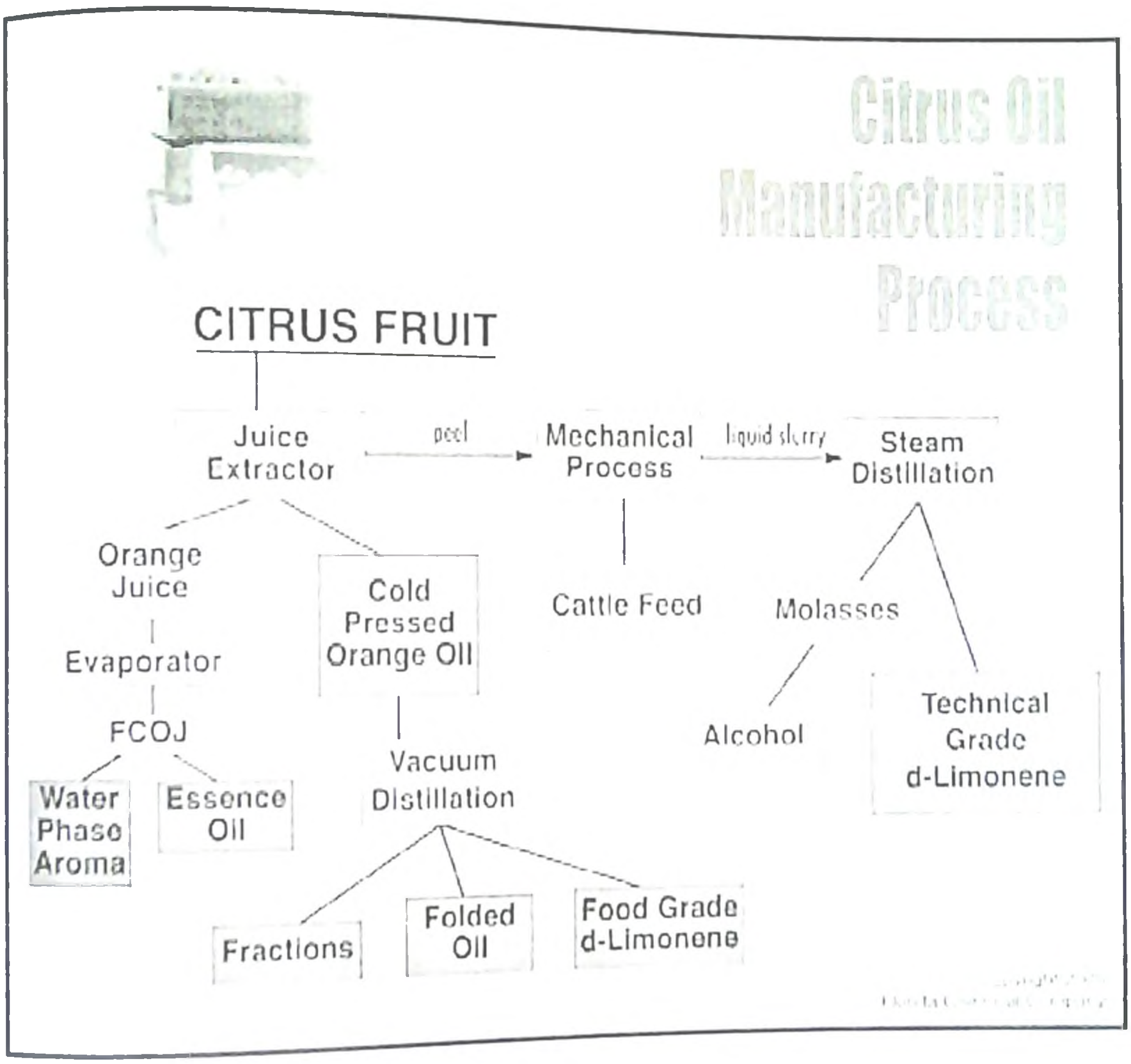


Fig.11 Citrus Oil Manufacturing



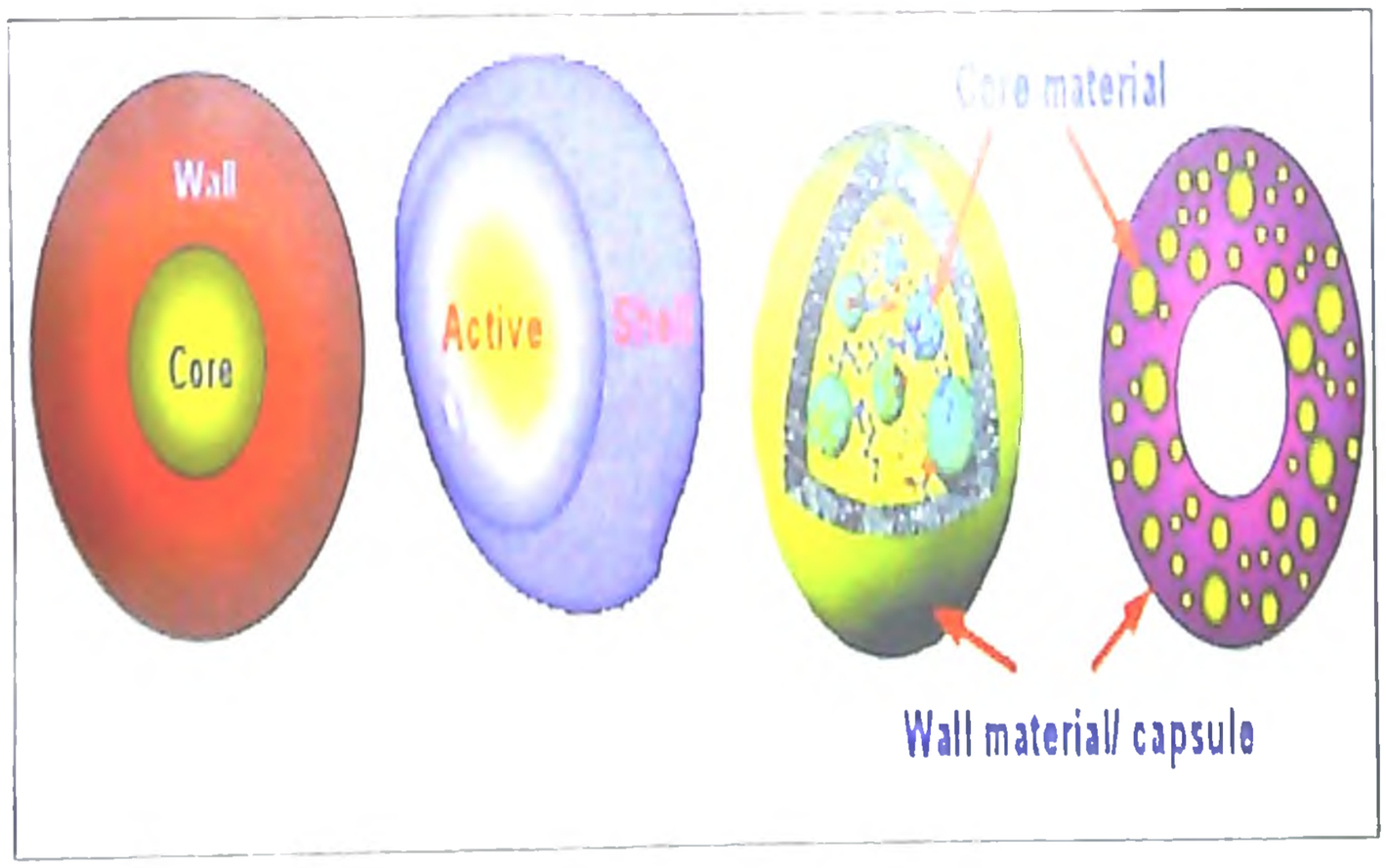
Flavour protection

Flavours can be among the most valuable ingredients in any food. Even small amount of some aroma substances can be expensive, and they are usually delicate and volatile. So preserving the flavour components is often a top concern of food manufacturers. Encapsulation describes different process to cover an active compound with a protective wall material and it can be employed to treat flavours so as to impart some degree of protection against evaporation, reaction or migration in a food (Prakash, 2004).

Micro encapsulation

It is the technique of packaging minute particles of solids, liquids or gas within continuous individual shells designed to release their contents in a predictable manner under predetermined conditions (Fig 12) (Jain *et al.*, 1997).

Fig.12 Micro encapsulation



(Khatkar, B.S. 2007)

Scope for micro encapsulation

- It helps to retain the flavours in food products during storage
- Protect the flavour from undesirable interaction with food
- To minimize the flavour - flavour interaction
- To guard against light induced reaction or oxidation
- Helps in control release of flavours

(Reineccicus, 1991)

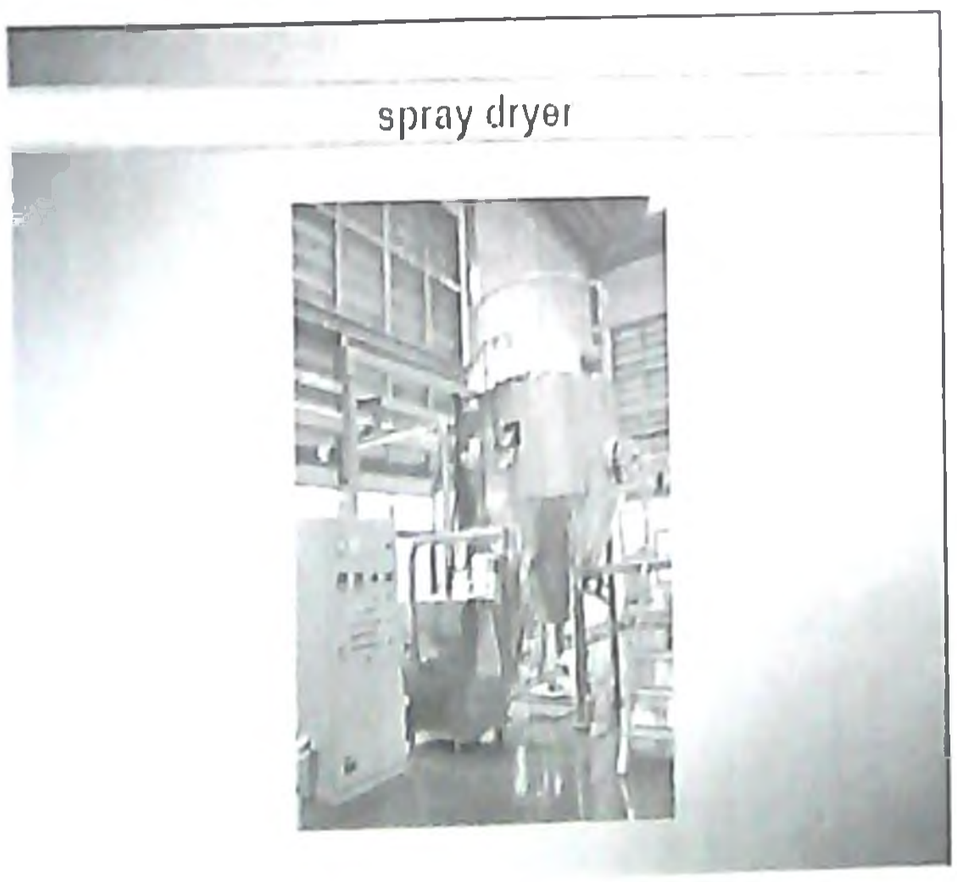
Spray drying

One of the most widely used method for micro encapsulation is spray drying (Fig 13). The selection of spray dried encapsulated flavours consists of selection of suitable carrier material. In the preparation of flavour microcapsules, the wall material is first dissolved or dispersed in water at a temperature and concentration which largely dependant upon the choice of wall materials (Sharma and Tiwari, 2005).

Generally used wall materials are:

- Gum arabica
- Modified starches
- Malto dextrins
- Hydrolyzed starch

Fig 13 Spray drier



Flavour analysis

The methods used for identification of flavour components are

i) Gas Chromatography

ii) Gas Chromatography Mass Spectrometry

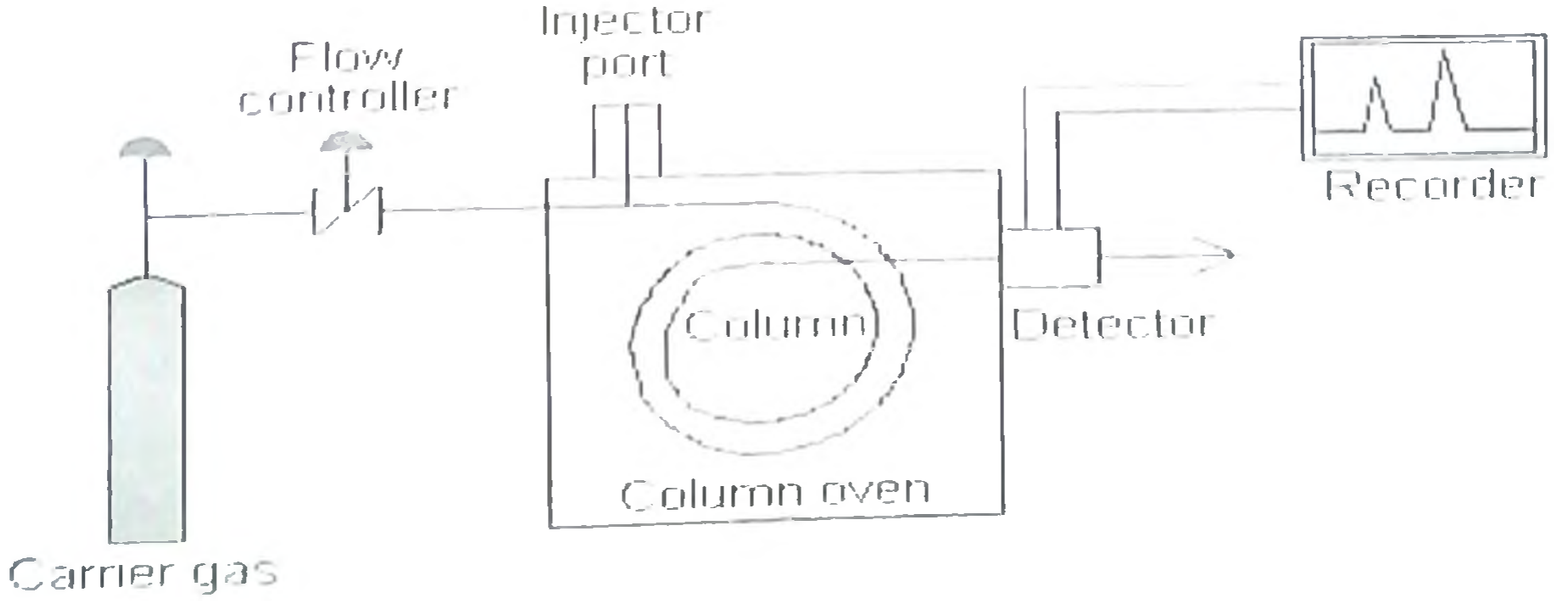
i) Gas Chromatography

Gas chromatography involves a sample being vapourised and injected on to the head of chromatographic column. The sample is transported through the column by the flow of inert gaseous mobile phase.The column itself contains a liquid stationary phase which is adsorbed on to the surface of an inert solid (Fig. 14).Commonly used gases include nitrogen, helium, argon and carbon dioxide.Sample should be introduced on to the column as a plug of vapour. Mycro syringe are used for injecting the sample. There are many detectors which can be used in gas chromatography. A non selective detector respond to all type of compounds except the carrier gas, a selective detector respond to range of compounds with a common physical or chemical property and a specific detector respond to a single chemical compound (Charles and Mankoo, 2004).

ii) Gas Chromatography Mass Spectrometry

The GCMS instrument has two parts.GC portion separates the chemical mixture into pulses of pure chemicals .Mass spectrometer (MS) identifies and quantifies the chemicals. The GC separates chemicals based on their volatility and MS is used to identify chemicals based on their structure

Fig.14 Gas Chromatography



(Charles and Mankoo, 2004)

Regulation of flavouring substances

The present legislation on food called the prevention of food adulteration (PFA act, 1954) as amended up to date and prevention of food adulteration rules, 1955. The legislation is more directed towards prevention of food adulteration. India following WHO/ FAO recommendation for food additives. The list of permitted chemical is not based on our own toxicological studies.

The regulations for flavouring substances under PFA rules are

- Individual flavouring agents should not exceed 300 ppm
- Yeast extract and protein hydrolysates maybe added up to 5 % in foods
- Ethyl maltol and maltol are allowed in biscuits and baked foods within a prescribed limit
- Where an extraneous flavouring agent has been added to any article of food it should be written just beneath the list of ingredients on the label attached to any food package. The statement should bein capital letters "CONTAINS ADDED FLAVOUR".

Because of number of flavouring agents used in food is so vast, most countries find it impossible to enumerate, classify and establish the permissible levels of their usage based on toxicological data. Such data are not available for most of those compounds. Most countries therefore have a list of prohibited flavouring agents, rather than a list of permitted ones.

Restriction on use of flavour enhancers

- Mono sodium glutamate is the most widely used flavour enhancers in Chinese food preparations.
- MSG may be added to any article of food under proper label declarations as provided in sub rule of Rule 142
- Total glutamate content in any ready to serve foods shall not exceed 1 %
- It shall not be added to any food for use by infants below 12 months
- Monosodium glutamate is allowed in meat products to a maximum of 500 ppm

(Sanganeria, 2004)

Table 5. Some of the Generally Recognised As Safe (GRAS) flavouring substances

FEMA No.	Substance name
3909	Cyclohexanone
3912	9-decenal
3923	3-hexenal
3941	Maltol propionate
3958	Phenyl acetate
3961	2-propyl pyrazine
3963	Iso amyl acetate
3964	Allyl caproate
3965	Carvyl acetate
3966	3-Decanone
3988	Vanillic acid
4015	Pyrazine

(Smith *et al.*, 2003)

Restriction on use of flavouring agents in India

- Coumarin
- Dihydro coumarin
- Cinnamyl anthracilate
- β-asarone
- Estragole
- Ethyl methyl ketone
- Ethyl 3 phenyl glycidate
- Eugenyl methyl ether
- Methyl ß naphthyl ketone
- Para propyl anisole
- Saffrole and isosaffrole
- Thujone and isothujone
- α and ß thujone

(Strong, 1988)

Solvents prohibited in flavours

- Di ethylene glycol
- Mono ethyl ether
- Diethyl ether
- 1.2 di chloro ethane
- 1.1.2 tri chloro ethylene
- Hexylene glycol

(Sinha,1999)

Permitted solvents in flavours

- Ethyl alcohol
- Propylene glycol
- Glycerol
- Iso propyl alcohol
- Liquid paraffin
- Propane diol

(Acharya, 2000)

Conclusion

Consumers all over the world are now more health conscious, they prefer food free of chemical additives.Inspite of various merits cited for natural flavours, our food industry is still depending on synthetic flavours for meeting 95 % of its requirement. India is blessed with rich sources of natural flavourings in spices and aromatic plants. Fruits and vegetables contain many aromatic compounds but we have yet to develop a technology for extraction of flavour components from fruits. Inorder to increase the use of natural flavours, we have to develop a cheap and viable technology for extraction of natural flavourings. Since there is an increased awareness among the consumers about health hazards associated with synthetic additives like flavourings, we can expect a great demand for natural flavours in near future.

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Discussion

1. What is the difference between aroma and flavour?

Aroma indicates the pleasant smell only but flavour is the combination of sensations of taste, odour, aroma, heat, cold and texture or mouth feel.

2. Mention the major advantage of cold pressing over other extraction methods?

It is suitable for extraction of flavour components from heat sensitive plant materials because it involves only a low temperature (40 ° C).

3. What are the main criteria for choosing different extraction methods for extraction of natural flavours?

Major criteria for selecting a particular extraction method for extraction of natural flavour is :

i) volatile nature of flavour compounds.

ii) heat sensitivity of plant material

iii) cost effectiveness and also product of our interest.

4. Is there any company in Kerala producing natural flavours?

Synthite Industrial Chemicals, Cochin

Arpuna Natural Extracts Ltd., Aluva

A.V. Thomas and Co Ltd., Cochin

5. Why cold pressing is more advantageous than steam and water distillation?

Because cold pressing involves only a low temperature (40 ° C), so it is suitable for extraction of heat labile components from plant material. In the case of water and steam distillation we are involving a high temperature for extracton so there is a chance for flavour loss through volatilization and some cases off note of essential oils also occur.

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KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE

Proc. 752 – Seminar

Topic: NATURAL FOOD FLAVOURS: PROBLEMS AND PROSPECTS

Name: Bijila, P.V. (06- 22-102)
Time: 10.15 am.. 20-07-07
Venue: Conference Hall

ABSTRACT

Flavour is a sensory phenomenon, which is a combination of sensations of taste, odour, aroma, heat, cold and texture or mouthfeel (Srivastava and Kumar, 2002). Appearance of food is important but it is the flavour that ultimately determines its quality and acceptability (Sharma *et al.*, 1999). It is important to note that without desirable flavour, many essential foods containing high quality proteins and other vital nutrients would not be eaten due to their insipid and unpalatable nature (Sethi and Shruti, 2005).

Abundant varieties of flavouring substances have been provided by nature and the chemists have attempted to duplicate their properties (Khare *et al.*, 2006). Three classes of flavouring substances are available and they are natural, nature identical and synthetic. Natural flavouring substances such as spices, essential oils and fruit juice concentrates have been used since long back in food preparations but as their supply has not kept up with demand due to rise in their cost, natural flavouring agents have been largely substituted by synthetic ones (Hall,1990). However this can be overcome by adopting advanced extraction methods and encapsulation techniques. In India, use of food additives like flavouring substances are regulated by Prevention of Food Adulteration Act (1954) and its subsequent amendments.

Nowadays, consumers are more health conscious and prefer food free of chemical additives. Synthetic food flavours often causes health hazards like hyperactivity, brain damage, cancer etc. In the recent years consumer demands for natural food flavours which are safe and have no side effects have increased considerably. As the world today is searching for natural products, there exists an urgent need to produce more natural flavours.

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