

**MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR
CHARACTERIZATION IN LANDRACES OF MELON
(*Cucumis melo* L.)**

R. KANDASAMY

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for the degree of**

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**Department of Olericulture
COLLEGE OF AGRICULTURE
Vellayani, Thiruvananthapuram - 695522**

**Dedicated
To
My Beloved Parents**

DECLARATION

I hereby declare that this thesis entitled “**Morphological, biochemical and molecular characterization in landraces of melon (*Cucumis melo* L.)**” is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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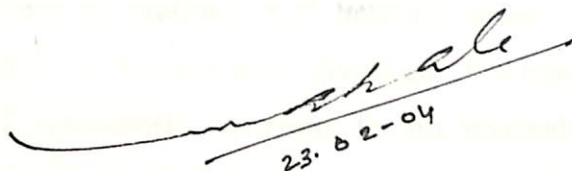
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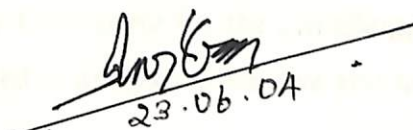
Dr.M.ABDUL WAHAB
(Chairman, Advisory Committee)
Associate Professor,
Department of Olericulture
College of Agriculture, Vellayani
Thiruvananthapuram-695522

APPROVED BY:**CHAIRMAN**

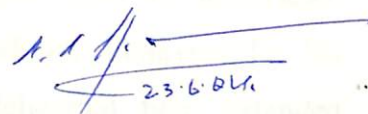
Dr. M. ABDUL WAHAB,
Associate Professor,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.


23.02.04
MEMBERS


Dr. L. RAJAMONY,
Associate Professor and Head,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.


23.06.04

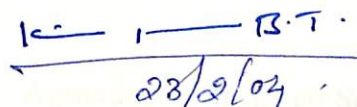
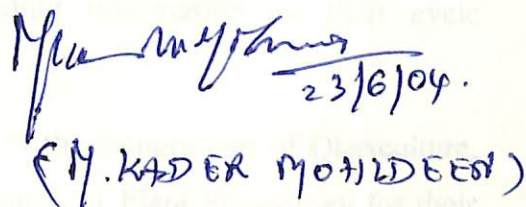
Dr. K. RAJMOHAN
Associate Professor and Head,
Plant Molecular Biology and
Biotechnology Centre,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.


23.6.04

Dr. D. WILSON
Associate Professor,
Department of Plant Breeding and Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.


23/6/04

Dr. B.T. KRISHNAPRASAD
Assistant Professor,
Department of Plant Physiology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.


28/2/04
EXTERNAL EXAMINER

23/6/04.
(M. KADER MOHIDEEN)

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LIST OF ABBREVIATIONS

SE	Standard Error
CD	Critical difference
g	Gram(s)
ml	Millilitre
kg	Kilogram(s)
μl	Microlitre
%	Per cent
mM	Milli mole
N	Normality
μg	Microgram(s)
pM	Pico mole
KAU	Kerala Agricultural University
cm	Centimetre(s)
Fig.	Figure
nm	Nanometre
<i>viz.</i>	Namely
<i>et al.</i>	And others
mg	Milligram(s)
DMC	Dry Matter Content
M	Mole
ng	nanogram(s)
CM	<i>Cucumis melo</i>
μ	Micron(s)
GA	Genetic Advance
SDS-PAGE	Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis
RAPD	Random Amplified Polymorphic DNA
sec.	Second(s)
r_p	Phenotypic correlation
r_g	Genotypic correlation
r_e	Environmental correlation
PCV	Phenotypic Coefficient of Variation
GCV	Genotypic Coefficient of Variation
°C	Degree Celsius
PCR	Polymerase Chain Reaction

Introduction

1. INTRODUCTION

Melon (*Cucumis melo*) is one of the important summer vegetable crops of India. It is grown traditionally in various river beds of India covering about 80 per cent of the area under melon cultivation in India (Nandpuri, 1989).

The species *Cucumis melo* is a polymorphic taxon encompassing a large number of botanical and horticultural varieties or groups. It includes dessert as well as cooking and salad types (Naudin, 1859). They are good sources of vitamin C, sugars and minerals (Ramayya and Azeemoddin, 1983). Melon plants also contain various bioactive principles including elaterin, stigmasterol, spinosterol and the antitumour principle cucurbitacin B (Duke and Ayensu, 1985).

There are several local varieties of melon grown in different regions of India (Nandpuri, 1989). The non dessert or culinary forms of *Cucumis melo* L. is a distinct group distributed and adapted well essentially under humid tropics of South India (Seshadri and More, 1996). Several non dessert types like 'Vellari' of Kerala (eaten both as salad cucumber and cooked cucumber) 'Vellarikkai' of Tamilnadu (eaten like salad cucumber), 'Nakadosakai' and 'Budamkai' of Andhra Pradesh (eaten as cooked cucumber) 'Phoot' and 'Kachri' of Rajasthan and Bihar (eaten as dessert melon with sugar) are distributed in South (Seshadri and Chatterjee, 1996).

Vellari is a traditional as well as popular vegetable crop of Kerala. Truly analyzing this is a non-dessert melon (*C. melo* L.) distributed throughout the humid tropical region of South India, with a variety of common names viz., Vellari, Melon, Pickling melon, Preserving melon, Oriental pickling melon, Culinary melon etc.

Melons of Kerala have large variability in fruit shape and size, skin characters, flesh colour, cavity, keeping quality and reaction towards pest and disease incidence. However, no authentic reports are available on the characterization of these landraces.

The increasing number of varieties and morphological similarities among *Cucumis melo* have necessitated the use of precise systems for their identification and characterization.

Characterization of varieties is generally being done based on morphological and agronomic characters. Use of protein profile is one such system for this purpose. Seed protein variants migrating at different rates under electrophoresis have been extensively used as molecular genetic markers for characterization of species and cultivars. DNA based molecular markers have been widely used for genetic characterization. These techniques are not affected by environmental conditions. Several molecular markers namely, Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) etc. are being used for this purpose. Among these, RAPD marker technique is quick, reliable and widely applicable. It is used for characterization of genetic variability, determination of somaclonal variants and hybrids, taxonomic studies, sex determination and linkage map etc.

In this context, the present study was undertaken with the following objectives:

To characterize the landraces of melon through morphological traits by Mahalanobis D^2 analysis.

To characterize the landraces of melon through protein profile by SDS-PAGE method.

To characterize the landraces of melon through molecular markers by RAPD technique.

To compare these three methods of characterization in forty landraces of melon.

Review of Literature

2. REVIEW OF LITERATURE

Cucumis melo L. commonly known as ‘Vellari’ is a traditional as well as popular vegetable crop of Kerala. The crop has its origin in tropical and subtropical Africa (Grubben, 1977) where many wild types occur. India has also a long history of cultivation of melons which was introduced by the Mughal rulers from Central Asia (Nandpuri, 1989). Melons of India have large variability in fruit shape, skin characters, flesh colour, flesh thickness, sweetness, seed cavity, seed size etc. (Seshadri and Chatterjee, 1996). Distinct forms in terms of fruit shape, colour and keeping quality can be even within Kerala. However, no authentic reports are available on the characterization of these landraces. The available literature on *Cucumis melo* L. and other crops relevant to the present study is reviewed under the following heads.

2.1 Genetic variability and genetic divergence

2.2 Biochemical characterization

2.3 Molecular characterization

2.1 GENETIC VARIABILITY AND GENETIC DIVERGENCE

2.1.1 Variability Studies

The effectiveness of selection for any character does not depend on the amount of phenotypic variability alone. It is of great interest to the breeder to determine the amount of phenotypic variability present in a character that is heritable.

The genotypic coefficient of variation indicates the relative magnitude of genetic diversity present in the plant material and helps compare the genetic variability for different characters.

The success of any crop improvement programme depends to a great extent upon the magnitude of genetic variability existing in the germplasm.

Naudin (1859) classified *Cucumis melo* L. varieties into different horticultural groups based on the fruit characteristics and their uses. Robinson *et al.* (1976) opined that the word melon referred to the fruits of different botanical varieties of *Cucumis melo* L. According to them, the cultivated forms of *Cucumis melo* L. are varied in many characters and are difficult to classify clearly.

According to Deol *et al.* (1981) the vine length ranged from 76.9 to 209.3 cm, with a mean of 130.2cm. Swamy *et al.* (1985) reported that the main vine length ranged from 50.00 to 279.00 cm with a mean of 168.00 cm.

Chhonkar *et al.* (1979) reported that in muskmelon primary branches ranged from 10.75 to 15.00 with a mean of 12.11 at Varanasi. Swamy *et al.* (1985) reported that the number of primary branches per plant were between 2.3 and 8.3 with a mean of 5.7.

Nandpuri *et al.* (1976) studied the performance of three musk melon varieties under green house and field conditions in Ludhiana and observed significant varietal differences for number of days taken from sowing to both first male and female flower production and anthesis. Deol *et al.* (1981) also observed highly significant differences between varieties for days to first female flower production. The range of variation for this trait was 32.7 to 53.1 days.

Swamy *et al.* (1985) observed considerable variation among 45 genotypes of musk melon for days to first harvest. They observed a range of 75 to 96.6 days with a mean of 84.6 days. Nandpuri *et al.* (1975) reported that the yield per plant ranged from 672 to 4811g with a general mean of 2821g.

Regarding the number of fruits per plant, Nandpuri *et al.* (1975) reported a range of 11.6 to 7.3 with a mean of 3.6 whereas Deol *et al.* (1981) reported a low value ranging from 1.3 to 4.2 with a mean of 2.

Fruit weight varied widely in muskmelon. Ranges of 338g to 2064g (Nandpuri *et al.*, 1975), 200g to 1010g (Gurudeep *et al.*, 1977) and 314g to 1517g with a mean of 907g (Swamy *et al.*, 1985) have been recorded.

From Varanasi, Chhonkar *et al.* (1979) reported that thickness of the pulp ranged from 1.25 to 3.15 cm with a mean of 2.85 cm. More *et al.* (1987) reported a range of 0.34 to 1.57 for flesh cavity ratio.

In an attempt to study the varietal response to date of planting, Nandpuri and Lal (1978) observed considerable variation among the varieties for the number of days taken from transplanting to fruit maturity irrespective of the planting date.

Solanki and Seth (1980) observed a wide range of variation among 24 genotypes of cucumber for plant height, leaves per plant, internode length, male flowers per plant, days to fruit maturity, female flowers per plant, fruits per plant and yield per plant.

Gopalakrishnan *et al.* (1983) conducted an evaluation trial of varietal collections from Western Ghats and coastal Kerala and identified the cultivar 'Mudikkode Local' as the highest yielder.

Elkner and Krysiak (1984) compared the physical and chemical characteristics of fruits of 14 melon varieties and found significant differences. The varieties 'Cristel' and 'Muskotaly' had the best biochemical and organoleptic qualities. Nakamura and Ischiuchi (1985) reported that pickling melons (*Cucumis melo* L.) were very similar to pickling cucumber in taste, flavour and processing suitability. In an evaluation of local varieties of *C. melo* var. *conomon* in Japan, the local variety, 'Ohama' was the best for yield and quality (Nakamura and Ischiuchi, 1985). 'Aurora' a melon cultivar with very large fruits surpassed other cultivar in yield, transporting quality and culinary quality (Norton *et al.*, 1985). Birdsnest type cultivars from Iran, which have a compact plant habit, reduced apical dominance, good fruit set and concentration of maturity are potentially valuable for once over harvesting (Mc Collum *et al.*, 1987).

Nerson *et al.* (1988) reported the development of 'melofon', a genotype of *Cucumis melo* suitable for pickle production. Mariappan and Pappiah (1990)

studied 45 diverse cucumber genotypes and reported a wide range of variation for all the traits except for leaves per plant.

Knavel (1991) found considerable genotypic differences among the muskmelon cultivars tested with respect of canopy architecture. According to him, the genotype ‘Main Dwarf’ provided a greater percentage of plant leaf area exposed to sunlight whereas genotype Ky-P reported less secondary stem branching with few potential fruiting sites on the stem.

A wide range of variability among 22 cucumber accessions was observed by Satyanarayana (1991) for all the characters except branches per vine and flesh thickness.

Chacko (1992) observed significant difference among the genotypes of muskmelon for percentage of germination, number of days to first male/female flower production, days to first harvest, yield per vine, volume of fruits, length of vine, number of branches and reaction towards pests and diseases.

In an experiment with four cultivars of muskmelon in Spain, Artes *et al.* (1993) reported that the cultivar ‘Piel de Sapo’ was highest in weight, caliber, edible portion and were the most oval shaped.

A wild melon *C. melo* var. *callosus* was characterized by earliness and multiple fruiting habits (Rana, 1993).

Considerable variation for yield and earliness was reported among six slicing cucumber cultivars from an observational trial in Kerala. Among the cultivars tested EC 179394 and ‘Sheetal’ were found promising for yield and local preference (KAU, 1996).

Wehner and Cramer (1996) reported genetic variance for total, early and marketable fruits per plot, fruit shape and fruit weight in three slicing cucumber populations.

High variability was observed for vine length, number of male and female flowers per vine and number of fruits per vine, while the genetic variation was lowest for the number of fruits per vine (Choudhary *et al.*, 1985).

Nagaprasuna and Rama Rao (1988) observed high Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) for percentage of fruit set, number of fruits per vine and yield per vine. Variability studies by Rastogi and Aryadeep (1990) with 25 varieties of cucumber revealed a good percentage of PCV for most of the characters like vine length, single fruit weight and number of male and female flowers per vine and they also observed high GCV for yield per vine.

Abusaleha and Dutta (1990) observed a moderate level of PCV and GCV for node position of first female flower, days to first female flowering, vine length and yield per vine. They also reported maximum GCV for number of fruits per vine. A high variability for days to first female flowering was observed by Krishna Prasad and Singh (1991), while Saikia *et al.* (1995) reported high variability in yield per vine, node number of first female flower, fruit length, fruit girth, single fruit weight and number of fruits per vine.

Sriramamurthy (2000) observed high variability for the characters like node numbers of first female flower, node number of first male flower, fruit length, fruit girth and yield per vine in 16 genotypes of cucumber were observed.

Rakhi (2001), observed significant differences among the 42 genotypes of landraces of culinary melon (*Cucumis melo* L.) for yield per plant, fruit weight, fruits per plant, keeping quality of fruits and 1000 seed weight.

In muskmelon, Kalloo and Dixit (1983) observed high significant genetic variation for vine length, days to first female flowering and node number of first female flower, which is in agreement with the findings of Singh *et al.* (1989).

2.1.2 Heritability and Genetic Advance

Heritability influences the selection programme to a greater extent. According to Johnson *et al.* (1955a), the genetic advance is more useful in predicting the actual value of selection than heritability; the value indicates the relative effectiveness of selection based on phenotypic expression of the character.

Srivastava and Srivastava (1976) observed high estimates of heritability for single fruit weight, number of male and female flowers per vine and percentage of fruit set. Solanki and Seth (1980) reported high heritability with moderate to low genetic advance for vine length, number of male and female flowers per vine, single fruit weight and yield per vine. High heritability for fruit length, fruit girth and low genetic advance for number of fruits per vine and yield per vine were reported by Joshi *et al.* (1981).

Deol *et al.* (1981) reported high heritability for main vine length in muskmelon (70.64%) but genetic gain was low (36.24%). Number of primary branches per plant showed moderate heritability (50.59%) and low genetic gain (19.79%). Days to first harvest also showed moderate heritability (42.7%) and very low genetic gain (7.4%). High heritability (85.23%) and high genetic gain (77.39%) was recorded for number of fruits per plant. Average fruit weight recorded high heritability and moderate genetic gain (66.29%).

Kaloo *et al.* (1982) reported high heritability and high genetic advance for yield per plant under North Indian conditions. However, Lippert and Hall (1982) reported a low heritability value of less than 13 per cent for this character under glass house condition in Europe.

In cucumber, Mariappan and Pappiah (1990) observed high heritability associated with genetic advance for fruit girth, days for first staminate flowering, number and weight of seeds per fruit indicating the action of additive genes for the expression of these characters.

Lal and Singh (1997) reported highest heritability in characters like node at which first female flower appeared and days from transplanting to first fruit harvest in melons. Node at which first female flower appeared, showed the highest expected genetic advance.

In a genetic study of 13 varieties of cucumber, it was reported that yield had the lowest heritability and lateral branches had the highest among the characters investigated (Sheng and Staub, 1999).

Owens *et al.* (1983) revealed high heritability for fruit length and single fruit weight which indicated that there are more number of additive factors for these characters and further improvement in the yield could be brought out by selection based on phenotypic observations.

Investigations carried out by Choudhary *et al.* (1985) revealed high heritability estimates for vine length, days to first female flowering and low genetic advance for number of male and female flowers per vine. Nagaprasanna and Rama Rao (1988) reported that the magnitude of heritability estimates in broad sense was high for majority of the characters *viz.*, number of female flowers per vine, percentage of fruit set, number of fruits per vine and single fruit weight.

Rastogi and Aryadeep (1990) observed high heritability as well as high genetic advance for characters like fruit yield per vine, single fruit weight and number of fruits per vine, which might be due to the additive gene action.

High genetic advance with higher heritability was reported for vine length and node position of first female flower, while number of fruits per vine, fruit length and marketable yield showed moderate genetic advance (Abusaleha and Dutta, 1990).

Saikia *et al.* (1995) reported that high heritability coupled with high genetic advance as percentage of mean exhibited was by the characters like vine length, node of first female flower, number of fruits per vine, single fruit weight, flesh thickness and yield per vine.

A high heritability with genetic advance among 16 cucumber accessions was observed by Sriramamurthy (2000) for all the characters excepting number of male flowers per vine.

Rakhi (2001) stated that high heritability coupled with high genetic advance was noted for fruit length, 1000 seed weight, average fruit weight and keeping quality of fruits.

In muskmelon, Deol *et al.* (1974) reported low heritability for node to first female flower, fruit length and girth, whereas Chhonkar *et al.* (1979) and Kalloo

and Dixit (1983) reported high heritability for fruit number, flesh thickness and single fruit weight. Singh *et al.* (1989) observed moderate heritability for single fruit weight, number of fruits per vine and yield per vine.

2.1.3 Correlation Studies

Yield is the most important complex character, which depends upon a number of direct and indirect components. An understanding of correlation among important characters provides a useful tool in evaluating and planning future breeding programme.

Gurudeep *et al.* (1977) reported significant positive correlation of flesh thickness with fruit weight in muskmelon. Similarly, fruit length, fruit diameter and seed cavity diameter were found to be correlated with fruit weight in cucumber (Imam *et al.*, 1977).

Singh and Nandpuri (1978) reported that days to first fruit maturity was positively correlated phenotypically as well as genotypically with days to opening of first female flower, TSS, fruit weight and total yield per vine. Flesh thickness was positively correlated both phenotypically and genotypically with total yield.

Chhonkar *et al.* (1979) reported that in muskmelon, the length of the main creeper had a positive association both phenotypically and genotypically with fruit weight, fruit yield and the number of primary branches was very strongly and positively associated with the number of nodes on the main creeper.

Solanki and Seth (1980) observed positive correlation between number of male flowers per vine, number of fruits per vine and single fruit weight.

Deol *et al.* (1981) found a positive and highly significant correlation for vine length with the number of branches per plant. They also observed a positive and significant correlation of the number of days to first female or bisexual flower with the number of days to fruit picking. Flesh thickness did not exhibit significant correlation with any of the traits *viz.*, TSS, vine length and number of branches. Yield per plant showed a highly significant positive correlation with fruit weight and negative correlation with number of days to first female flower.

Correlation studies on some character associated with yield by Salk (1982) in melons found that the total fruit yield per plant was positively correlated with number of fruits per plant. Fruit number was negatively correlated with fruit weight. Positive correlations were found between flesh thickness, fruit weight and fruit diameter.

Cerne (1984) reported that in cucumber, yield components were in positive correlation with number of main roots, vine length and leaf area. Choudhary *et al.* (1985) found the female flowers/vine, fruit length, fruit diameter and weight were positively associated with yield. They also observed negative association of days to first female flower opening with fruits and yield per vine. Haribabu (1985) observed fruit yield to be positively correlated with fruit weight, fruits per vine and vine length. Vine length was correlated with branches per vine and branches/vine with fruits per vine. Significant correlations between yield and its four components were noticed in five monoecious lines and their hybrids (Prudek and Wolf, 1985).

Swamy *et al.* (1985) observed in muskmelon that yield per plant was positively correlated with number of fruits, average fruit weight, number of nodes on the main stem, stem length, internodal length, number of primary branches and fruit shape index.

Studies conducted by Choudhary and Mandal (1987) revealed significant genotypic and phenotypic correlation of yield with fruits per plant, female flowers per plant, fruit length, fruit weight and fruit diameter in cucumber. More *et al.* (1987) reported negative correlation of shape index with flesh to cavity ratio in oblong fruits in muskmelon. They also found flesh area production to be directly influenced by shape index. Vijay (1987) reported that fruits per vine and fruit weight were positively correlated with yield per vine in muskmelon.

Abusaleha and Dutta (1988) reported positive and significant associations between yield and fruit length, fruits per vine, fruit girth and flesh thickness in cucumber. Days to male and female flowering exhibited negative association with yield.

According to Kuo *et al.* (1988), there exists some correlation between flower type and fruit shape but varietal differences were present in muskmelon.

Among nine lines of water melon evaluated for 14 characters, fruit yield was correlated with vine length and vine girth (Laltaprasad *et al.*, 1988).

Singh and Singh (1988) evaluated eleven diverse genotypes of water melon at Sabour and found that yield was positively correlated with number of fruits per vine and negatively with fruit weight and number of days for the appearance of first female flower.

Cerne (1989) found that total fruit weight was positively correlated with parameters of vegetative development in cucumber. Prasunna and Rao (1989) observed positive correlation of fruit yield with node to first female flower, days to first female flower opening, female flowers/ vine, sex ratio, fruits per vine, average fruit weight and primary branches per vine.

Nagaprasuna and Rama Rao (1989) observed that number of fruits per vine, sex ratio and single fruit weight had high positive direct effects on yield at genotypic and phenotypic level. Hence, these traits are considered to be important parameters in any selection programme for yield improvement.

Solanki and Shah (1989) suggested that in any selection programme, maximum weightage should be given to number of female flowers per vine, number of fruits per vine and single fruit weight, since their direct and indirect effects on fruit yield is highly significant.

A study conducted by Rastogi and Aryadeep (1990) with 25 cucumber cultivars also revealed positive correlation of total yield per plant with fruit per plant, fruit weight and fruit length.

Krishna Prasad and Singh (1991) observed positive direct effect of number of fruits per vine on the yield per vine.

Satyanarayana (1991) reported a positive correlation of yield with vine length, nodes/vine, fruits per vine and marketable yield per vine in cucumber. Significant positive correlation was observed in muskmelon between percentage

of germination and yield per vine and also with number of fruits per vine (Chacko, 1992). He also reported high heritability in conjunction with high genetic advance for percentage of germination, yield per vine and vine length.

Prasad and Singh (1992) conducted correlation studies in cucumber and observed a significant and positive correlation of yield per plant with vine length, fruit length, fruit weight, fruit breadth and flesh thickness.

Rajendran and Thamburaj (1993) reported the interassociation of various yield components in watermelon. The average fruit weight had significant positive association with number of fruits per vine, flesh seed ratio and number of seeds/fruit. The number of fruits per vine had significant negative relationship with days to first female flower production.

Studies on correlation in eight genotypes of cucumber by Saikia *et al.* (1995a) showed that yield per plant had strong positive association with main vine length, number of secondary branches, leaf area, fruits per plant, fruit weight and fruit length.

In watermelon, yield per plant exhibited significant positive correlation with number of branches/plant, number of fruits per plant and weight of individual fruits. Earliness was positively correlated with node and days to first female flower production, length of vine, node at which first fruit produced and 100 seed weight (Shibukumar, 1995).

Total fruit yield per vine possessed highly significant positive correlations with flesh thickness, marketable fruit yield per vine, seed cavity size and weight per fruit in melon (Lal and Singh, 1997).

Sriramamurthy (2000) noted that vine length, fruit length and number of fruits per vine had positive correlation with yield.

Rakhi (2001) reported that vine length, leaf area index, fruits per plant, average fruit weight, fruit length and fruit girth had high positive correlation with yield.

In muskmelon, Kalloo *et al.* (1979) stated that fruit length and girth showed positive direct effect on yield, whereas Somkuwar *et al.* (1997) reported positive indirect effect of fruit length and girth on yield.

2.1.4 Path Analysis

Linear correlations between yield and various structural or growth components present a confusing picture because of their interrelationships. The standard partial correlation and regression (path coefficient) is known to offer a much more realistic interpretation of the factors involved. The technique of path coefficient is useful as a means of separating direct and indirect effects. The use of this technique requires a cause and effect of situation among the variables.

Gopalakrishnan *et al.* (1980) conducted path coefficient analysis in pumpkin and reported that length of vine had maximum direct effect of fruit yield per vine. Vijay (1987) reported that number of fruits per vine and weight of individual fruit in muskmelon had strong direct positive effects on yield and recommended them as selection criteria.

In watermelon, Singh and Singh (1988) reported that number of fruits per vine and TSS had the highest direct as well as indirect effect on yield.

Fruit number, female flowers per plant, fruit length, fruit weight and fruit diameter are the important character determining yield in cucumber (Choudhary and Mandal, 1987). Abusaleha and Dutta (1988) also reported highest direct effect for fruit per vine and fruit length. They also found direct negative effect of days to female flowering and percentage of unmarketable yield on total fruit yield. Indirect positive and significant effect of vine length, branches per vine, fruit girth and flesh thickness on yield was also reported. Prasunna and Rao (1989) observed fruits per vine and average fruit weight as the most important yield contributing factors. The number of fruits and early yield per plant had the highest direct positive effect on yield per plant (Pandita *et al.*, 1990). A significant positive effect was found between fruits per vine and yield and branches per vine and yield (Rajput *et al.*, 1991).

Path analysis of yield and its components in 23 genotypes of cucumber by Prasad and Singh (1992) revealed the positive direct effect of vine length, days to female flower appearance, fruit weight and fruit length on yield. Internodal length, number of female flowers and days to maturity has positive and highly significant direct effect on fruit yield (Solanki and Shah, 1992).

Among the various yield components, the average fruit weight had maximum direct influence on the yield of fruits per vine (Rajendran and Thamburaj, 1993).

Chen *et al.* (1994) compared seven monoecious cucumber cultivars for yield components. There were significant positive direct effects of fruits per vine, female flowers/vine and average fruit weight on yield per plant. Saikia *et al.* (1995a) also revealed fruits per plant to have maximum direct effect on yield followed by fruit weight. In Kerala, fruit girth exhibited maximum positive effect on fruit yield followed by average fruit weight (Gayathri, 1997). Meng *et al.* (1999) reported that the longest direct positive action on early yield were average fruit weight, number of harvested fruits per plant and average fruit length.

Sriramamurthy (2000) reported that fruit length, fruit set percentage, days to first male flowering, flesh thickness and vine length had exerted positive direct effect on yield.

Rakhi (2001) revealed that fruits per plant, length of fruit and girth of fruit as primary contributions to yield.

2.1.5 Cluster Analysis Through Mahalanobis D^2 Statistics

Genetic diversity is the base on which programmes of improvement for desired attributes are planned. It plays an important role in the process of decision making. The genetic potential is usually indirectly measured through phenotypic values as genotypes express themselves only in an environment. Diversity measures, which utilize corrections for environmental components would be more efficient than those based on phenotypic variation alone. Large number of measures has been tried, of which Mahalanobis D^2 distance statistics merits

application. This distance statistics is a multivariate analogue of the Euclidean distance in two dimensions.

Mahalanobis D^2 statistics technique which is based on multivariate analysis (Rao, 1952) of quantitative traits is a powerful tool for measuring genetic divergence. The degree of genetic diversity can be worked out between any two populations over all the characters taken under study. Genetically divergent populations fall into different groups on clustering, thus enabling selection of parents. Patil and Bhapkar (1987) reported that the geographic distributions and genetic diversity as estimated by D^2 statistic were not related. They were also of the opinion that very often these two exhibited no parallelism.

Genetic diversity studies on 5 inbred and their 20 F_1 's were carried out by Miller (1985) in cucumber. The study revealed the importance of the character days to fifty percent flowering, which contributed to the maximum for genetic diversity. In a later study, Miller and Quisenberry (1986) showed that days to first female flowering contributed much to the genetic divergence in cucumber.

Studies on genetic diversity of 31 genotypes of cucumber by Rao *et al.* (2003), revealed that sixteen clusters formed based on 15 characters. Genotypes differed significantly. The pattern of distribution of genotypes from different regions into various clusters was at random, demonstrating that the geographical isolation may not be the only factor for causing genetic diversity. Sriramamurthy (2000) grouped 26 genotypes into 9 distinct clusters by D^2 clustering techniques. From the clustering pattern, it was revealed that genetic origin was different from geographic origin and the genetic diversity.

In watermelon, Krishna Prasad *et al.* (2002) found that 48 watermelon inbreds fell in ten different clusters and the geographical distribution was not necessarily related. Yield per plant, days to first female flower and TSS contributed to total divergence.

2.2 BIOCHEMICAL CHARACTERIZATION

In recent years, protein or isozymatic analysis by polyacrylamide gel electrophoresis (PAGE) has been considered as a unique and powerful technique for ascertaining gene homology at the molecular level because of its superior capability for component resolution. Further, PAGE provides a tool for species identification and delimitation and has been particularly useful in deducing somatic relationships between groups where morphological and cytological data were not corollary. In spite of this, innumerable chemotaxonomists have successfully established the phylogenetic relationships employing protein electrophoresis studies in major crops like rice, wheat, barley, soyabean, broad bean chickpea, cotton etc. (Ladizinsky and Hymovitz, 1979).

Data from protein electrophoresis seem to give more accurate information on phylogenetic relationships than isozymes. Proteins separated by electrophoretic methods are thought to undergo the process of evolution with relative slowness due to their “non essential” nature (Margoliash and Fitch, 1968) while enzymes are thought to be extremely sensitive to selection pressures in evolution and thus to the survival of the organism (Mc Daniel, 1970).

Sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS-PAGE) study by Yadav *et al.* (1998) has revealed that 53 genotypes of muskmelon resolved into a total of 13 bands distributed in four zones. Based on the differences 53 germplasm lines could be grouped into five dissimilar protein profile groups and thus it was possible to distinguish certain germplasm lines on the basis of protein profiles.

Choudhary and Hari Har Ram (2000) reported that 65 accessions of muskmelon were characterized using SDS-PAGE of seed proteins extract. A total of 15 seed protein bands could be resolved which were distributed into four distinct zones. The 65 genotypes were classified into 7 distinct groups based on protein profiles. Three morphological indistinguishable genotypes (oblong fruit) were identical on the basis of their seed protein profiles. It was also observed that

genotypes having contrasting morphological traits (Round Vs Oblong fruit) possessed similar protein profile.

PAGE of water soluble seed proteins conducted in 14 inbred lines and one F₁ of bottlegourd demonstrated the presence of eight electrophoretic bands separated into two zones. They were divided into seven groups, each having a different protein profile. Three morphologically indistinguishable varieties were distinguished on the basis of protein band patterns by Upadhyay *et al.* (1998).

Polyacrylamide gel electrophoretic study by Lubis *et al.* (1977) revealed that, on the basis of protein banding, samples of *Vigna unguiculata* belonging to taxonomic grouping *Sesquipedalis*, *Sinensis* and *Cylindrica* were indistinguishable. So they concluded that these three belonged to a single species.

Yaaska (1984) reported that electrophoretic analysis of the enzyme extracted from seedlings of *Phaseolus vulgaris*, *Phaseolus coccineus*, *Vigna mungo* and *Vigna unguiculata* revealed three main isozymes in each of the species except *P. coccineus* which had an additional isozyme. The three isozymes common to all four species differed in resistance to acidity and heat, intracellular location, and electrophoretic variability, indicating that they are genetically independent isozymes.

The advantages of using electrophoretic pattern of seed globulin is that this technique is non expensive and easy to perform in developing countries where cowpea is extensively cultivated and germplasm is collected and stored (Singh and Ntare, 1985).

Rao *et al.* (1992) analysed the seed storage proteins of ten *Vigna* species by means of SDS-PAGE and reported great variation both in number and molecular weight (MW) of the polypeptides. They also reported that proteins extracted from different accessions of the same species revealed the presence of an electrophoretic pattern typical for each species and these species specific bands allow the identification of 10 *Vigna* spp. analysed.

Oghiakhe *et al.* (1993) has reported that no inter varietal differences existed for total protein content but water soluble seed proteins proved useful in distinguishing cultivars. A key for the classification of the fifteen cultivars into five groups was developed based on the presence or absence of three proteins following PAGE of the water soluble proteins.

Valliamcourt *et al.* (1993) compared cultivated and wild cowpea for their isozyme diversity and reported that cultivated cowpea accessions were characterized by very low genetic diversity ($H_t = 0.029$) with only six polymorphic loci. The cultivated groups could not be differentiated from the domesticated cowpea. Wild cowpea was highly diverse with 19 out of 26 loci polymorphic. And six wild accessions displayed identity with the cultivated cowpea.

Zope *et al.* (1992) on evaluation of the globulin fraction of two morphological mutants reported no correlation the banding patterns of the mutants with morphological characters.

Gregova *et al.* (1999) stated that analysis of alleles encoding seed storage proteins was used for identification and molecular characterization of 51 durum wheats (*Triticum durum* Desf.). Composition of alleles at the Glu-A1 and Glu-B1 loci was revealed by SDS-PAGE. Nine of the genotypes were heterogeneous in glutenin patterns. It was allowed to assess and select durum wheat genotypes possessing alleles with positive or negative effect on technological quality. Furthermore, 32 pairs of durum wheat maintained in active and work collections were compared for their glutenin composition with the aim to verify their identity and to compare genotypes with identical or very similar names.

Yan *et al.* (2003) expressed Allelic variation of the high molecular weight glutenin in *Aegilops tanschi* of 1986 accessions detected by sodium dodecyl sulfate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis. The results showed that characterization of wheat HMW-GW

was facilitated by using CE which provides high resolution and increases the speed of analysis in conjunction with the traditional gel electrophoretic methods.

The characterization and identification of crop cultivars by electrophoresis was reported by Cooke (1984). Electrophoretic protein analysis was used in the ornamental crops viz. rose (Kuhns and Fretz, 1978), gladiolus (Horoki *et al.*, 1986), carnation (Messequer and Arnas, 1985; Singh *et al.*, 2002) and petunia (Natarella and Sink, 1975; Merrenijk *et al.*, 1986) and zinnia (Venkatachalam and Jayabalan, 1992).

Soluble protein profiles of the *Lathyrus sativus* somaclones along with the parent cultivar P-24 analysed by SDS-PAGE at different developmental stages did not reveal any major differences either between the parent and the somaclones or among the somaclones except in two cases. In seeds of Bio R-24 at 30DAF a prominent band of 27 RD was absent which was present in all other and a band of 22RD was additional in Bio L-57 and P-24 at 45 DAF). The result reported that banding patterns of the somaclones showed correlation with morphological characters in such cases (Chakraparti *et al.*, 1999). Identification of tomato cultivars by polyacrylamide isoelectric focusing was reported by Hen *et al.* (1992).

2.3 MOLECULAR MARKERS

Molecular markers are genotypic markers (Bretting and Widrlechner, 1995). They are used to study the differences among strains at molecular level. Molecular markers constitute biochemical constituents (secondary metabolites in plants) and macromolecules (protein, DNA). Biochemical markers have been used since long for the characterization of variation in a plant, now considered to be inappropriate as universal markers (Cooke, 1994).

Molecular markers have been shown to be useful for diversity assessment in a number of plant species (Waugh and Powell, 1992). Molecular markers are direct manifestation of genetic content

(Weising *et al.*, 1995). They serve as reliable indices of genetic variation. In the past decade, molecular markers have very rapidly complemented the classical strategies.

The genetic markers are used for clonal identification, linkage mapping, population diversity, taxonomy, evolutionary studies, determining the genetic fidelity during micropropagation, germplasm conservation etc. (Bretting and Widrlechner, 1995).

2.3.1 Isozymes

Numerous attempts have been made to use isozyme polymorphism as genetic markers in *Cucumis* (Kennard *et al.*, 1994, Knerr and Staub., 1992, Meglic *et al.*, 1994, Sujatha *et al.*, 1991, Staub *et al.*, 1997; Staub *et al.*, 1998).

6-Phosphogluconate dehydrogenase isozyme was used to construct a genetic map of melon (Baudracco – Arnas and Pitrat, 1996).

Allelic variation was detected at a total of nine loci of five isozymes among 114 melon accessions. Geographical variation was detected in two enzymes, APS and 6-PGDH. Pgd-11 and Ap-31 were frequent in India and Myanmar, while most of the melons in Laos, China, Korea and Japan carried Pgd-13 and Ap-33, except var. inodorus in China. It was also suggested that the small seed type with wet tolerance originated in Central India and was selected under wet conditions in the east revealed by Akashi *et al.* (2002).

Angelica *et al.* (2003) studied the genetic diversity among 28 accessions of Cassava Active Germplasm Bank through the isoenzymatic systems $\alpha_1\beta$ - Esterase (EST), Peroxidase (POX), Glutamate Oxalacetic Transaminase (GOT) and Acid Phosphatase (ACP). The dendrogram suggested a similarity between leaf tissue and a morphological characterization indicating that highly inherited characters are good cassava descriptors.

Genetic analysis of glutamate dehydrogenase isozymes in cultivated and wild species of section *Cepa* in *Allium* was studied Shigyo *et al.* (1995).

The use of peroxidase polymorphism in the identification of Malling Merton apple rootstocks was expressed by Vinterhalter and Jances (1986).

The enzyme coding loci do not constitute a random sample of genes and they are not randomly dispersed throughout the genome. Electrophoresis will detect only portion of the actual variability present in amino acid sequences (Hills and Moritz, 1990). Some enzymes are unstable markers during plant development and standardization of sampling procedures is sometimes difficult. Therefore, the isozymes have been replaced by DNA based molecular markers (Anolles and Trigiano, 1997).

2.3.2 DNA Markers

The term DNA finger printing was introduced for the first time by Jeffrey *et al* (1985). Presently the term DNA finger printing / profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These included characterization of genetic variability, genome finger printing, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics.

With the advent of molecular biology techniques, DNA based marker have replaced enzyme markers in germplasm identification and characterization as well as in gene tagging. Because of its plasticity, ubiquity and stability, DNA is the ideal molecule for such analysis (Caetano – Anolles *et al.*, 1991). Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization based markers and polymerase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

2.3.2.1 Hybridization Based DNA Markers

The hybridization based DNA marker techniques utilize labelled nucleic acid molecules as hybridization probes (Anolles *et al.*, 1991). Probe molecules range from synthetic oligonucleotides to cloned DNA. Some of the important hybridization based DNA techniques are Restriction Fragment Length Polymorphism (RFLP), Hypervariable Sequences and Variable Number of Tandem Repeats (VNTRs).

2.3.2.1.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism analysis involves digesting the genome with restriction enzymes, separating the fragments electrophoretically and then preferentially visualizing. The fragments containing particular homologous sequences by hybridizing them to a specific DNA probe (Deverna and Alpert, 1990).

Genetic diversity in *Cucumis* species was documented by using RFLP (Baudracco – Arnas and Pitrat, 1996). Thirty four RFLPs were analysed for linkage in 218 F₂ plants derived from two divergent cultivars. RFLP markers detected similar polymorphism levels. RFLPs were largely due to base substitutions rather than insertions, deletions. Twelve per cent of markers showed distorted segregation.

Three different types of molecular markers, RFLP, RAPD and AFLP were used to measure genetic diversity among six genotypes of *Cucumis melo* L. Clustering analysis separated the genotypes into two main groups: (i) the sweet type cultivated melons and (2) the exotic type, not cultivated melons (Garcia – Mas *et al.*, 2000).

Zheng *et al.* (1999) carried out molecular variation in melon as revealed by RFLP marker as was reported by Silberstein *et al.* (1999). RFLP markers were used to construct linkage map to the Fom-2 fusarium wilt resistance gene in melon.

In tomato phylogenetic relationships and genetic variation analysed using RFLP marker was expressed by Miller and Tanksley (1990).

RFLP analysis used for the construction of linkage map in disease resistance like root-knot nematode in *Solanum bulbocastanum* (Brown *et al.*, 1996), bacterial canker resistance in *Lycopersicon peruvianum* (Sandbrink *et al.*, 1995) and an aphid resistance gene in Cowpea (Myers *et al.*, 1996).

2.3.2.1.2 Hypervariable Sequences and Variable Number of Tandom Repeats (VNTRs)

Kaemmer *et al.* (1993) used oligonucleotide probes to differentiate *Musa* cultivars in various genomic groups. Bhat *et al.* (1995) found that DNA fingerprinting using oligonucleotide probes was useful for cultivar identification and for overall genome analysis to establish relatedness among the various accessions of *Musa* germplasm. The presence of hypervariable sequence was confirmed in plants and animals by Gupta *et al.* (1996). Studies by Crouch *et al.* (1999) to compare different PCR based marker system (Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandom Repeats (VNTRs) and Amplified Fragment Length Polymorphism (AFLP) for the analysis of breeding population of *Musa* showed that VNTR analysis detected the highest levels of polymorphism.

2.3.2.2 Polymerase Chain Reaction (PCR) Based DNA Marker Techniques

These are fingerprinting techniques that use an *in vitro* enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleic acid molecules (Anolles and Trigiano, 1997).

Among the PCR based marker techniques, the important ones are Amplified Fragment Length Polymorphism, Microsatellite, Sequence Characterized Amplified Region and Random Amplified Polymorphic DNA.

2.3.2.2.1 Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism is based on PCR amplification of restriction fragment generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases (Vos *et al.*, 1995).

Perin *et al.* (2002) reported by a reference map of *Cucumis melo* based on two recombinant inbred line populations by using AFLP method.

Garcia – Mas *et al.* (2000) studied measure the genetic diversity of six genotypes of *Cucumis melo* by using AFLP, RAPD and RFLP.

Wang *et al.* (1997) revealed that RAPD, microsatellite and AFLP markers were evaluated for linkage analysis in melon (*Cucumis melo* L.) varieties MR-1 (resistant to fusarium, powdery and downy mildew) and Ananos Yokneum (Ay; susceptible to these diseases), to construct a detailed genetic map. AFLP markers were more efficient in mapping the melon genome than RAPD or microsatellite makers. The map contains 197 AFLPs, six RAPDs and one microsatellite marker assigned to 14 major and six minor linkage groups. The map had immediate utility for identifying markers linked to disease resistance genes that are suitable for marker assisted breeding.

Genetic diversity of eight selected Argentinean garlic clones with AFLP produced the dendrogram showed 6 arbitrary groups. The garlic clones were clustered according to the physiological group and bulb colour. The potential use of AFLP could allow not only the differentiation among species, but also between botanical varieties and well defined ecotype groups as reported by Garcia Lampasona *et al.* (2003).

The gms gene of Chinese Cabbage (*Brassica campestris* ssp. *Chinesis*) conferring a recessive genetic male sterility was mapped with AFLP markers. Four markers were tightly linked to that gene. The AFLPs were cloned and sequenced. The sequence tagged site (STS) markers can be used for marker

assisted selection of male sterile plants among segregating populations (Ying *et al.*, 2003).

AFLP markers were used to fingerprint and to examine genetic diversity among a set of cultivars having Sharka resistance (Hurtado *et al.*, 2002).

Genetic diversity of strawberry cultivars using AFLP (Tyrka *et al.*, 2002) in six cultivars of *Fragaria* X *Ananassa* (Duch) was evaluated. 116 markers were polymorphic and could be used to distinguish all analyzed materials. Cluster analyses revealed 2 main groups of clones and divided strawberry cultivars (CUL) and tested F₁ hybrids of 'Sweet Heart' (HYB). Results suggest that AFLP method is sufficient for effective identification and useful for assessing the level of genetic diversity in strawberry cultivars and breeding lines.

2.3.2.2 Microsatellite

The term microsatellite was coined by Litt and Luty (1989). DNA sequences with short repeated motifs (2–6 bp) are called Simple Sequence Repeats (SSRs), microsatellite (Epplen *et al.*, 1991) because microsatellites are highly polymorphic, randomly distributed in the genome and easily analysed as a general and novel source of genetic markers (Thottappilly *et al.*, 2000).

Microsatellite consists of randomly arranged di-tri-tetra nucleotide repeats, which are hypervariable and ubiquitously distributed throughout eukaryotic genomes. Microsatellite DNA markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellite (Weber and May, 1989).

Katzir *et al.* (1996) carried out the seven SSRs which were used to test a diverse sample of cucurbitaceae, including 8 melon, 11 cucumber, 5 squash, 1 pumpkin, and 3 watermelon genotypes. Five of the seven SSRs detected length polymorphism among the 8 melon genotypes with gene diversity values ranging from 0.53 to 0.75 Microsatellite. Four of the seven SSRs detected polymorphism among the 11 cucumber genotypes with gene diversity values ranging between

0.18 and 0.64. Primers specific to SSRs of *C. melo* and *C. sativus* also amplified DNA extracted from genotypes belonging to other genera of the Cucurbitaceae family.

Staub *et al.* (2000) have successfully employed seventeen SSR markers that were used to characterize genetic relationship among 46 accessions in two *Cucumis melo* L. subsp. *melo* (*Cantalupensis*, *Inodorus*) and subsp. *agrestis* (*Conomon* and *Flexuosus*) groups. Empirical estimations of variances associated with each marker type in the accessions examined indicated that, per band, lower coefficients of variation can be attained in the estimation of genetic diversity when using RAPDs compared to SSRs.

Damin-Poleg *et al.* (2001) expressed that sixty one *Cucumis* SSR markers were developed, most of them (46) from melon (*Cucumis melo* L.) genomic libraries. Forty of the markers (30 melons and 10 cucumber SSRs) were evaluated for length polymorphism in a sample of 13 melon genotypes and 11 cucumber (*Cucumis sativus* L.) genotypes. SSR data were applied to phylogenetic analysis among the melon and cucumber genotypes. A clear distinction between the ‘exotic’ groups and the sweet cultivated groups was demonstrated in melon. In cucumber, separation between the two subsp. *C. sativus* var. *sativus* and *C. sativus* var. *hardwickii* was obtained.

Paris *et al.* (2003) compared forty-five accessions of *Cucurbita pepo* for presence or absence of 448 AFLP, 147 ISSR and 20 SSR bands. Clustering was in accordance with the division of *C. pepo* into three subspecies, *Fraternal*, *Texana* and *Pepo*. The subsp. *texana* cluster consisted of six sub-clusters, one each for the representatives of its five cultivar-groups (Acorn, Crookneck, Scallop, Straightneck and Ovifera Gourd) and wild gourds. The smallest-fruited accession, ‘Miniature Ball’, appeared to occupy a genetically central position within *C. pepo*.

A partial linkage map of melon was constructed by using SSR, from a cross between P1414723 and Dulce. Twenty-two SSR were analyzed in a F₂/F₃ population to produce a map spanning 14 linkage groups. One SSR marker was

tightly linked to fruit flesh P_H. SSR marker of this map, two of the three postulated ZYMV resistance genes were located using a BC1 population (PI414723 recurrent parent). One SSR marker was tightly linked to a ZYMV resistance gene, designated ZYM-1 (Damin-Poleg *et al.*, 2001).

Lopez – Sese *et al.* (2002) analysed 15 genotypes of spanish melon (*C. melo* L.) in allele variation, at 12 microsatellite (SSR) loci. Many SSR loci suggested that some populations were in genotypic disequilibrium. Moreover, a high level of genetic variation was observed between cassaba market classes than within accessions. Resulted bulk sampling technique coupled with molecular analysis technique that employ a unique array of discriminating markers can provide information leading to effective.

The use of SSR to assess genetic diversity and to determine the relationship among cultivars of *capsicum* (Sanwen *et al.*, 2001), *common bean* (Galvan *et al.*, 2003), *soyabean* (Giancola *et al.*, 2002), *citrus* (Corazza – Nunes *et al.* 2002), *almond* (Martinez-Gomez *et al.*, 2003), *strawberry* (Arnan *et al.*, 2003) and *prunus* rootstocks (Serrano *et al.*, 2002).

2.3.2.2.3 Sequence Characterized Amplified Region (SCAR)

Sequence characterized amplified region DNA analysis was developed to produce reliable PCR based results. Efficiency of RAPD to SCAR marker conversion and comparative PCR sensitivity in cucumber was reported by Horejsi *et al.* (1999) and sex determination in Papaya by using SCAR primers (Deputy *et al.*, 2002).

SCAR markers were used to detect linkage of Fom-2 fusarium wilt resistance in melon (Zheng *et al.*, 1999)

Bautista *et al.* (2003) used this technique to identified olive trees. Parent and Page (1998) used this technique to identify raspberry cultivars. Damasco *et al.* (1998) used marker based on SCAR to detect dwarf off type of *in vitro* grown Cavendish bananas.

2.3.2.2.4 Random Amplified Polymorphic DNA (RAPD)

Polymerase chain reaction in conjunction with random primers, was used for finger printing genomes (Welsh and Mc Clelland, 1990), population biology studies (Astley, 1992), identification of genome specific markers and other uses (Williams *et al.*, 1990; Erlich *et al.*, 1991). Several authors have applied the RAPD technique to investigate genetic variability and found the technique very efficient and reliable (Brown *et al.*, 1993; Munthali *et al.*, 1996). Analysis of RAPDs offers several advantages compared to RFLP. The most important advantage is that RAPD is not a labour intensive procedure. It is not necessary to construct or maintain a genomic library. RAPD requires smaller quantities of genomic DNA than RFLP analysis. Also it is less costly compared to RFLP. Generation of RAPD is quicker than RFLP and can be used to detect even single gene mutation (Williams *et al.*, 1990).

2.3.2.2.4.1 RAPD and Linkage Maps

RAPD assay has been used by several groups as an efficient tool for identification of markers linked to agronomically important traits which are introgressed during the development of mere isogenic lines. Traits of interests studied include jointless pedicel in tomato (Wing *et al.*, 1994), wound-response genes (Cortes *et al.*, 2002), downy mildew resistance in cucumber (*Cucumis sativus*) (Horejsi *et al.*, 2000), Fusarium crown and root rot resistance (Frl) in tomato (Fazio *et al.*, 1999), tomato mosaic virus in tomato (Ohmori *et al.*, 1995 and Ohmori *et al.*, 1996), spotted wilt virus resistance in tomato (Stevens *et al.*, 1995), late blight resistance in tomato (Chunwongse *et al.*, 2002), fusarium wilt, melon necrotic spot virus and aphid infection resistant in *Cucumis melo* (Baudracco – Arnas and Pitrat, 1996), ZYMV resistance in melon (Poleg *et al.*, 2002), fusarium wilt resistance gene in melon (*Cucumis melo* L.) (Zheng *et al.*, 1999 and Wechter *et al.*, 1995), downy mildew resistance genes in lettuce (Paran and Michelmore, 1993), common bacterial blight resistance in tepary bean (Park *et al.*, 1998), golden mosaic virus resistance in common bean

(Urrea and Miklas, 1996), anthracnose resistance in common bean (Roberto *et al.*, 1996), root knot nematode resistance in sweet potato (Ekoskit *et al.*, 1997) and clubroot resistance locus in *Brassica rapa* L. (Kuginuki *et al.*, 1997).

Multilateral branching in cucumber (*Cucumis sativus* L.) was identified with 2 RAPD markers W7-2 and BC-551 (Fazio *et al.*, 2003). Statistical analysis showed significant association of multilateral branching with these markers.

The genetic linkage map have been created in *Cucumis melo* L. (Baudracco – Arnas and Pitrat, 1996), cucumber (*Cucumis sativus* L.) (Fazio *et al.*, 2003a), banana (Faure *et al.*, 1993), sweet cherry (Stockinger *et al.*, 1996), citrus (Christophani *et al.*, 1999), rose (Debener and Mattiesch, 1999), oilplam (Moretzsohn *et al.*, 2000) and in onion (Van He Usden *et al.*, 2000) using RAPD.

Flesh adhesion to the stone in peach was identified with two RAPD markers viz., OPB05 and UBC439 (Jun *et al.*, 2002). Significant association of flesh adhesion with these markers was revealed.

2.3.2.2.4.2 RAPD and Taxonomic Studies

RAPD markers have been widely used for taxonomic and related studies. Demeke *et al.* (1992) investigated the potential use of RAPDs for taxonomic studies using brassica, sinapsis and *Raphanus* taxa. Analysis of the RAPD bands revealed the relationship between diploid and amphidiploid *Brassica* taxa. Results showed that the *Raphanus sativus* and *Sinapsis alba* were distinct from the *Brassica* taxa.

Duneman *et al.* (1994) investigated the use of RAPD markers for taxonomic studies in *Malus*. Eighteen accessions of wild species and twenty-seven apple cultivars were tested with 29 pre-selected primers. The analysis of the bands using unweighted pair group method and arithmetic average showed the relationship among the cultivars which was in agreement with the known linkage. A dendrogram generated for wild species gave relationships that were in accordance with the known phylogenetic information.

The technical simplicity of the RAPD technique has facilitated its use in the analysis of phylogenetic relationship in several plants like roses (Debener *et al.*, 1996), blue berry (Levi and Rowland, 1997) and cymbidium (Obara-Okeyo and Kako, 1998).

The genetic closeness of various species of *Vanda* was determined using RAPD markers. Strip-leaved *Vanda* sp. (*Vanda sanderiana*) and *Ascocentrum miniatum* were more closely related to each other than to the terete leaved *Vanda* species studied. RAPD analysis supported the suggestion that terete leaved *Vanda* and *V. hookeriana* be classified in the separate genus *Papilionanthe* and that *V. sanderiana* should remain in the genus *Vanda* (Lim *et al.*, 1999).

2.3.2.2.4.3 RAPD and Somaclones

RAPD analysis was used to detect genetic variation in micro propagated Cavendish banana (Damasco *et al.*, 1996). A RAPD marker specific to the dwarf off-type from micro propagation of Cavendish group cultivars was identified following an analysis of 57 normal and 59 dwarf plants generated from several different micro propagation events. Of the 66 random decamer primers used in the initial screen, 28.8 per cent revealed polymorphisms between normal and dwarf plants. Use of this marker could facilitate early detection and elimination of dwarfs from batches of micro propagated bananas. Results of studies by Hammerschlag *et al.* (1996) showed the feasibility of using tissue culture methods to generate fruit trees with increased level of disease resistance. RAPD was used to study genetic variation at the DNA level among somaclonal variants in banana plants (*Musa* AAA cv. 'Grand Naine'). Four different types of somaclonal variants were identified and characterized in banana plants generated by meristem culture (Walther *et al.*, 1997). Tissue cultured off types did not display any visual differences during *invitro* culture. But after six weeks of hardening in a commercial nursery, the field established plants showed significant phenotypic differences. RAPD

analysis of somaclonal variants revealed the presence of polymorphic bands with at least one set of primers. This enabled early detection of somaclonal variants. This allows the elimination of off types before planting of micro propagated plants in the field. RAPD markers were found to be useful for confirmation of genetic fidelity in micro propagated plants (Gupta *et al.*, 1996).

Martin *et al.* (2002) reported that RAPD markers were used for the assessment of somaclonal variation in *Chrysanthemum* varieties.

Somaclonal variants were reported in beet (Munthali *et al.*, 1996) peach (Hashmi *et al.*, 1997), tomato (Hong *et al.*, 1999) and grapes (Vendisson *et al.*, 1999) using RAPDs

Plants regenerated by somatic embryogenesis from long term callus culture of five garlic cultivars were subjected to RAPD analysis (Al-Zahim *et al.*, 1999). Certain changes were observed in the RAPD profiles of the regenerants of different cultivars, suggesting the existence of somaclonal variants.

RAPD analysis was done by Babu (2000) to access the genetic stability in tissue culture derived black pepper plants. Monomorphic banding pattern was observed for the tissue culture regenerants, compared with their respective source plants. Uniformity was confirmed at both stages of development studies. Thus genetic stability and clonal fidelity were ensured for the tissue culture regenerants and the viability of the protocol was confirmed.

2.3.2.2.4.4 RAPD and Hybrids

RAPD technique has been used for the identification of hybrids and their parent determination as well. Wang *et al.* (1994) proposed RAPD finger printing as a convenient tool for the identification, protection and parentage determination of plant hybrids. In their study, DNA from three families of rice plants selected in Northern China (each comprising the

male sterile, the restorer, the hybrid F₁s and the maintainer lines) was extracted and amplified by RAPD technique. The results obtained were useful for identification of each single plant line.

Truksa and Prochazka (1996) reported different banding pattern based on the DNA polymerase used for testing three lines of cucumber for the production of hybrid seeds. Low level of polymorphism was obtained which indicated that RAPD was not suitable for verifying the hybridity of seeds. RAPD markers have been successfully used to test the paternity of Japanese pear hybrid (Banno *et al.*, 2000).

2.3.2.2.4.5 RAPD for Identification of Somatic Hybrids

One of the limiting factors for the efficient exploitation of protoplast fusion is the difficulty of unequivocally identifying nuclear hybrids. RAPDs have been used to characterize both interspecific and intraspecific somatic hybrids. Baird *et al.* (1992) proposed RAPDs for the identification of hybrids at an early stage following fusion in potato. Inter and intraspecific somatic hybrids of potato were characterized by using RAPD along with sexual hybrids.

Xu *et al.* (1993) used RAPD assay for the identification of somatic hybrids between *Solanum tuberosum* and *S. brevidens*. Somatic hybrids showed a combination of the parental banding pattern with four of the five primers screened whereas regenerants from one of the parents had a similar banding pattern as that of the parent.

2.3.2.2.4.6 RAPD in Sex Determination

Early identification of sex in nutmeg (Shibu *et al.*, 2000) and papaya (Somri, 1998) is useful for growers. RAPD markers were used to differentiate between the sexual forms of 3 commercial papaya cultivars belonging to the solo group (Lemos *et al.*, 2002). The BC 210 primer were able to detect hermaphrodites in all of the cultivars tested.

Deputy *et al.* (2002) have successfully employed three RAPD products that have been cloned and a portion of their DNA was sequenced. Based on these sequence SCAR primers were synthesized. SCART12 and SCARW11 produced products in hermaphrodite. SCART1 produces a product in all papayas regardless of plant sex. The sexing technique, using SCART12 and SCART1 as a positive control, was used to correctly predict hermaphrodite papaya plants in a population of seedlings with an overall accuracy of 99.2%.

RAPD primers were tested on dioecious and monoecious hemp cultivars to identify sex specific molecular markers. Two primers (OPD05 and UBC 354) generated specific bands in male plants. These markers were hemp MADC3 and MADC4 (Male associated DNA from *Cannabis sativa*) as reported by Torjek *et al.* (2002).

RAPD markers were used for the identification of two pigeonpea cytoplasmic genetic male sterile lines. Amplification product of 600 bp amplified by primer OPC 11 was observed in both the cytoplasmic male sterile lines, which was absent in maintainer lines and the putative R line expressed by Souframanien *et al.* (2003).

Genotypic and morphogenetic differences among three female varieties of *Piper longum*, one variety each from Assam and Calicut and one variety released from Kerala, were investigated using RAPD analysis and it was revealed that these varieties were genetically different. In *Piper longum*, RAPD technique was used to investigate the molecular basis of genotypic differentiation between the male and female parents (Banerjee *et al.*, 1999). As a result male sex associated RAPD markers were identified for the first time in *longum*.

2.3.2.2.4.7 RAPD Detection of Genetic Variability

RAPD markers have been used to characterize cocoa clones representing the three main cultivated sub populations viz. Criollo, Forestro and Trinitario (Wilde *et al.*, 1992). The use of single primers of

arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments, which were unique to the individual cocoa clones studied.

Lashermes *et al.* (1996) successfully employed RAPD markers to analyse genetic diversity among cultivated and sub spontaneous accessions of *Coffea arabica*. The narrow genetic bases of commercial cultivars were confirmed by their study. On the other hand, relatively large genetic diversity was observed within the germplasm collection. Results suggested an East-West differentiation in Ethiopia, the primary centre of diversification of *Coffea arabica*.

Duran *et al.* (1997) analysed 48 coconut types belonging to East African Tall types by different DNA marker techniques including RAPDs, micro satellite primed PCR and Inter Specific Tandem Repeats (ISTR) analysis. All three approaches detected large number of DNA polymorphism among the set of genotypes and allowed the identification of single genotypes by individual specific fingerprints. The cluster and principal coordinate analyses were done and the observed clustering and association of individuals corroborated the expectations based on the known geographical origin and parental relationships.

Varghese *et al.* (1997) evaluated the applicability of RAPD markers in the cultivated rubber tree, using forty three primers in a set of twenty four clones selected from different South-East Asian Countries. Out of the total 220 fragments amplified, eleven were polymorphic. The statistical analysis indicated the absence of a distinct geographical grouping because of the breeding history of *Hevea*.

Pattanayak *et al.* (2002) characterized 24 tetraploid Indian potato cultivars by using RAPD to access diversity within and between late blight resistant and susceptible cultivars. Most of the diversity was detected within varieties, with 88% of variation being within and 12% being between the resistant and susceptible cultivars.

Nkongolo (2003) revealed the use of RAPD marker to determine the variation within and among cowpea populations from different agrological zones. The analysis of molecular variance revealed that within region or types variation accounted for 96% of the total molecular variance. This result indicated an uncontrolled gene flow among populations.

Vidal *et al.* (1999) expressed the genetic relationship among 32 white grape vine varieties (*Vitis vinifera* L.) grown in different French and Spanish regions surveyed by using RAPD markers. Three groups of clearly defined varieties were separated in clear geographical groups with Atlantic or Mediterranean influence. Some of them were classified according to a common cultivation area and ampelogeaphic characters, suggesting a common origin.

DNA fingerprints generated by RAPD and Inter Simple Sequence Repeat Polymerase Chain Reaction (ISSR-PCR) analysis were used to compare the four most widely planted *Vitis vinifera* cultivars in Chile. Both the techniques were able to distinguish the cultivars, although the resolving power of ISSR profiles was higher than that of RAPDs. The results indicated that no variation was found within the Chilean Merlot clone using either ISSR or RAPD analysis (Herrera *et al.*, 2002).

The genetic variability of 38 grapefruit (*Citrus paradisi* Maof) and three pummelo (*C. maxima* (Burn.) Merr.) accessions were evaluated using RAPD, and single sequence repeat (SSR) analyses. The experimental result expressed 2 main grapefruit groups (Corazza – Nunes *et al.*, 2002).

RAPD, AFLP and SSR markers were used to characterize and differentiate of 100 soybean varieties. It was taken as a leading case study for plant variety protection (PVP) purposes in soybean. Results suggested that these three techniques described genetic variability in different and specific ways. A combination of SSR and morphological descriptors show the best compromise genetic relationships (Giancola *et al.*, 2002).

RAPD markers were used to determine the genetic diversity among 90 cashew accessions. A dendrogram confirmed that the diversity of Indian cashew collections can be considered to be moderate to high (Dhanaraj *et al.*, 2002). Cashew varieties obtained from diverse geographical locations around the world. These lines were involved in RAPD polymorphisms. Accessions from India Mozambique and Tanzanian showed the closest relationship with lines from Brazil being the most distinct from the other provenances. A specific RAPD-PCR product was linked with cashew lines from the Cook Islands (Mneney *et al.*, 2001). Fifty cassava clones were studied using RAPD technique. Genetic diversity of these genotypes was studied using four primers. Out of these 46 different bands, 90% to 100% were polymorphic bands. Statistical analysis indicated that interpopulational genetic divergence ranged from 0.069 to 0.203. Rate of nucleotide substitution among the landraces was 9.8 per cent site per year, while that for the improved varieties was 15 per cent (Asante and Offei, 2003). Twenty four selections and eleven hybrids of cashew were employed in combination of RAPD and ISSR primers. The statistical analysis showed no correlation between the relationships based on molecular data and the pedigree of the varieties. Difference in the average similarity coefficient between selections and hybrids was low indicating the need and scope for identification of more parental lines in enhancing the effectiveness of hybridization programme reported by Archak *et al.* (2003).

RAPD technique was used to investigate the genetic diversity of the *Peruvianum* complex (PC) and a *Lycopersicon* species. Tested accessions were clearly divided into 4 main clusters consisting of the PC, the self compatible EC, *L. pennellii* and *L. hirsutum* (Egashira *et al.*, 2000). Archak *et al.* (2002) reported the genetic diversity of 27 tomato cultivars analysed with RAPD markers generated by 42 random primers. Interestingly old introductions and locally developed cultivars of the 1970s exhibited significantly greater genetic variation than the ones released during the 1990s. RAPD markers were used for finger printing genotypes of *Solanum anguiri* L. and *S. aethiopicum* L. (Stedje and Bukenya-Ziraba, 2003).

Prakash *et al.* (2002) studied 41 genotypes of guava involving in RAPD experiment. The experiment revealed maximum genetic distance of 54% between *P. guajava* and *P. quadrangularis* while the minimum distance was only 11% between SWY-1 and GR-1 Navalur selections.

Shigyo *et al.* (2002) identified two cultivated and related species of sections *Cepa* and *Phyllodolon* in *Allium* by using 60 RAPD markers. Some of the RAPDs were effective for identifying inter specific hybrids. A total of 393 RAPDs were detected between the cultivated species, *A. fistulosum* and shallot. These RAPDs will be useful as genetic markers in the two sections.

Nair *et al.* (2002) carried out genetic diversity in 28 Indian sugarcane varieties using 25 RAPD markers. The parentage of the varieties did not contribute significantly to the clustering pattern. Varieties belonging to same parentage were grouped under different clusters while varieties from different parentage were grouped into same cluster. The study revealed the limited genetic base of the current Indian commercial varieties and the need to diversify the genetic base by using new resource from the germplasm.

The use of RAPD markers were frequently used for the finger printing of the chrysanthemum varieties was reported by Martin *et al.* (2002).

Landry *et al.* (1994) used RAPD markers to fingerprint 8 commercial available apple rootstocks. Phylogeny analysis used to draw the genetic relationship between these lines using only RAPD markers data. The resulting cladogram was compared to the true genetic relationship between these lines in order to assess the efficiency of RAPDs in determining accurately the phylogenetic relationship. DNA finger printing system based on 13 informative RAPD loci amplified by 5 RAPD primers that allowed the rapid identification of apple rootstocks.

The genetic diversity of twenty seven superior tea accessions (*Camellia sinensis* var. *sinensis*) from Korea, Japan and Taiwan was evaluated by Kaundun *et al.* (2000) using RAPD-PCR markers. Out of the

fifty primers screened seventeen primers generated fifty eight polymorphic and reproducible bands. A minimum of three primers was sufficient to distinguish all the twenty seven accessions studied

An experiment was carried out on five new strawberry cultivars and one cultivated variety to study genetic variability based on RAPD markers and protein (PAGE) pattern using eleven different RAPD primers. Two hundred and four polymorphic DNA fragments with a high potential to differentiate strawberry genotypes could be produced. A dendrogram displaying the relative genetic similarities among the genotypes showed the existence of genetic diversity among the six varieties. From this study, the fingerprints at protein level or DNA-RAPD were found to be very important to distinguish strawberry cultivars (El-Tarras *et al.*, 2001).

An analysis of a Brazilian oil palm (*Elaeis oleifera*) germplasm collection was carried out using RAPD markers. A sample of hundred and seventy five accessions obtained along the Amazon River Basin was analysed and compared to seventeen accessions of oil palm from Africa. Ninety six RAPD markers were used in this analysis, of which fourteen were shown to be specific to oil palm, while twelve were specific to Brazilian oil palm. Results showed that the Brazilian oil palm accessions have moderate level of genetic diversity compared to African oil palm accessions (Moretzsohn *et al.*, 2002).

Random amplified polymorphic DNA markers were extensively used for the molecular characterization of various crop species. RAPD markers have been used to characterize germplasm in several important crop species including *Carica papaya* (Stiles *et al.*, 1993) and apple (Gaulao *et al.*, 2001).

Shimada *et al.* (1999) investigated the genetic diversity of forty two plum varieties by RAPD analysis. Twenty primers discriminated all plum varieties. Genetic diversity among the examined strains was duly characterized. The results found that North American plums were

genetically distant to the other strains, and the Taiwanese plums differed from Japanese cultivated plums.

Phylogenetic relationships among nine *Mangifera* species were studied using RAPDs (Schnell *et al.*, 1993). Analysis was conducted using average taxonomic distance, un-weighted pair group method using arithmetic averages (UPGMA) and principal component analysis. Ten selected primers produced 109 usable bands.

Eighteen commercial mango cultivars were selected to assess genetic relatedness using RAPD markers. Thirty arbitrary 10-mer primers were used, of these twenty seven primers amplified mango genomic DNA. None of these primers produced unique band pattern for each cultivars. RAPD data were used to calculate a squared Euclidean distance matrix, and based on this cluster analysis was done using a minimum variance algorithm (Ravishankar *et al.*, 2000). Fifty mango cultivars were screened using RAPD markers with decamer primers of arbitrary sequences (Hemanth Kumar *et al.*, 2001). Out of the eighty primers screened, ten were selected which gave 139 clear and bright fragments. A dendrogram based on Jaccards coefficient of similarity implied a moderate degree of genetic diversity among the cultivars.

According to Nayar (2001) a total of 134 RAPDs were generated when PCR amplification was carried out using forty decamer primers (Operon Inc., CA, USA) of Kit A and Kit B. 130 bands were polymorphic which accounted to an average of 3.2 polymorphic bands per primer. OPA-06, OPB-10, OPB-14 produced no amplification. OPA-20, OPB-08, OPA-13 and OPB-06 were identified as promising primer for RAPD analysis. OPA-20 produced five intense bands and three faint bands when subjected to amplification reaction with the genomic DNA of the mother plant and this was the primer selected. No marked difference was found in RAPD banding pattern (using OPA-20) as primer between the three subcultures and the mother plant of Red banana. Simi (2001) characterized eleven ecotypes of Nendran banana using RAPD

technology. A total of 106 RAPDs were generated. Of these 100 bands were polymorphic which accounted to an average of 2.5 polymorphic bands per primer. Eight primers (OPA-01, OPA-03, OPA-13, OPB-01, OPB-06, OPB-10, OPB-12 and OPB-18) produced reproducible banding pattern. These primers yielded 42 scorable bands with an average of 5.25 bands per primer. The similarity coefficient values ranged from 0.3333 to 0.9355 and the genetic distance varied from 0.042 to 0.349. From the dendrogram, it was grouped into five clustered groups. Kahangi *et al.* (2002) examined seventeen *Musa* cultivars for RAPD markers using PCR with ten 10-mer primers. The study included five reference cultivars of genomic groups AA, AB, AAA, AAB and ABB. The ten primers generated 69 genetic markers that were used for estimation of genomic groups and for cultivar identification. The pair-wise RAPD distance analysis of the data and subsequent generation of the dendrogram using the “Neighbour” “Joining Tree” program grouped into two clusters depending on their genomic similarities.

Staub *et al.* (1997) analysed variation at isozyme and RAPDs loci in 8 cucumber and 7 melon cultivars to determine genetic variation among population of each species. Empirical estimates of variances associated with each marker type in the cucumber and melon accessions examined indicated that per band, lower coefficients of variation can be attained in the estimation of genetic difference when using RAPDs compared to isozymes.

RAPD markers and agronomic traits were used to determine the genetic relationships among 32 breeding lines of melon belonging to seven varietals types Garcia *et al.* (1998). A total of 115 traits were scored for genetic distance calculation and cluster analysis. RAPD data were highly correlated with the pedigree information already known for the lines and revealed the existence of two clusters for each varietals type that comprised the lines sharing similar agronomic features. These groupings were consistent with the development of breeding programmes trying to generate two separate sets of parental lines for

hybrid production. Nevertheless, the performance of certain hybrids indicated that RAPDs were more suitable markers than agronomic traits in predicting genetic distance among the breeding lines.

Garcia-Mas *et al.* (2000) used three different types of molecular markers RAPD, RFLP and AFLP to measure genetic diversity among 6 genotypes of *Cucumis melo*. Clustering analysis performed with the 3 types of markers separated the genotypes into two main groups, 1. sweet type, cultivated melon and 2. the exotic type, non cultivated melon.

RAPD and SSR markers were used to characterize genetic relationship among 46 accessions in two *Cucumis melo* L. subsp. *melo* (Cantalupensis, Inodorus) and subsp. *agrestis* (Common and Flexuosus) groups (Staub *et al.*, 2000). Empirical estimation of variances associated with each marker type in the accessions examined indicated that per band, lower coefficients of variation can be attained in the estimation of genetic diversity when using RAPDs compared to SSRs. Result of RAPD marker analysis suggests that 80 marker bands were adequate for assessing the genetic variations present in the accessions examined. Lopez-Sese *et al.* (2001) revealed that the population structure of 15 spanish melon (*C. melo* L.) accessions, mostly of group Inodorus, was assessed by the analysis of 16 individuals of each accession using 100 random amplified polymorphic DNA (RAPD) bands produced by 36 primers. A relatively high level of polymorphism was detected using RAPD markers. Moreover, a higher level of genetic variation was observed between cassaba market classes than within accessions. Result of bulk sampling technique coupled with molecular analysis technique that employ a unique array of discriminating markers can provide information leading to effective.

RAPD markers were used to analyse genetic diversity of several melon groups (Staub, 2001, Lopez-Sese and Staub, 2001, Stepansky *et al.*, 1999, Horejsi *et al.*, 1999; Silberstein *et al.*, 1999).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled “Morphological, biochemical and molecular characterization in landraces of melon (*Cucumis melo* L.)” was carried out at the Department of Olericulture and Centre for Plant Molecular Biology, College of Agriculture, Vellayani during 1999-2002. Each experiment was described as follows.

3.1 COLLECTION OF LANDRACES

A survey was carried out in different melon growing areas of Kerala and Tamilnadu for collecting landraces of melon. Special emphasis was given to locally adapted types from the traditionally melon growing areas of Kerala. It also included few collections from Tamilnadu.

Effective collection was made through the extension personnel in the Department of Agriculture, Kerala Horticultural Development Programme and Krishi Vigyan Kendras. Seed samples of various landraces were collected and the crops were raised in summer season. The details of the accessions of melon with their sources are presented in the table 1.

3.1.1 Characterization of Landraces

The basic materials for the study included 40 accessions of various landraces of melon. They were grown in the experiment field of the Instructional Farm, College of Agriculture, Vellayani (Plate 1). It is situated at 8.5° N latitude, 76.9° E longitude at an altitude of 29 m above MSL. The experimental site was loamy soil.

The experiment was laid out in Randomised Block Design with two replications. Pits of 60 cm diameter and 30 cm depth were taken at a spacing of 2 x 1.5 m. In each pit, four seeds were sown. Sowing was done in such a way that in each replication there was a single row of four plants per accession (micro plots). The cultural and management practices were adopted according to



(A)



(B)

Plate 1. A general view of experimental field: (A) Replication I
(B) Replication II

Table 1. List of landraces of melon used for the study

S. No.	Genotypes	Source
1.	CM 1	Nagarcoil local, Tamilnadu
2.	CM 2	Mettupalayam, Tamilnadu
3.	CM 3	Kottayam, Kerala
4.	CM 4	Kolakkattupudur, Tamilnadu
5.	CM 5	Coimbatore, Tamilnadu
6.	CM 6	Kanyakumari, Tamilnadu
7.	CM 7	Marthandam, Tamilnadu
8.	CM 8	Dhamarikulam, Kerala
9.	CM 9	Kakkamoola, Thiruvananthapuram, Kerala
10.	CM 10	Nemom, Thiruvananthapuram, Kerala
11.	CM 11	Balaramapuram, Thiruvananthapuram, Kerala
12.	CM 12	Kattakada, Thiruvananthapuram, Kerala
13.	CM 13	Aryanad, Thiruvananthapuram, Kerala
14.	CM 14	Nedumangad, Thiruvananthapuram, Kerala
15.	CM 15	Nedumangad, Thiruvananthapuram, Kerala
16.	CM 16	Vembayam, Thiruvananthapuram, Kerala
17.	CM 17	Palapoor, Thiruvananthapuram, Kerala
18.	CM 18	Ochira, Kollam, Kerala
19.	CM 19	Kottarakkara, Kollam, Kerala
20.	CM 20	Chengannur, Kerala
21.	CM 21	Kalavoor, Kottayam, Kerala
22.	CM 22	Manimala, Pathanamthitta, Kerala
23.	CM 23	Thiruvalla, Pathanamthitta, Kerala
24.	CM 24	Madapalli, Kottayam, Kerala
25.	CM 25	Ettumanoor, Kottayam, Kerala
26.	CM 26	Vakathanam, Kottayam, Kerala
27.	CM 27	Velloor, Kottayam, Kerala
28.	CM 28	Ikkattoor, Kottayam, Kerala
29.	CM 29	Thrikkodithanam, Kottayam, Kerala
30.	CM 30	Kattappana, Idukki, Kerala
31.	CM 31	Kattappana, Idukki, Kerala
32.	CM 32	Manjapra, Ernakulam, Kerala
33.	CM 33	Chalakudi, Thrissur, Kerala
34.	CM 34	Anakkayam, Malappuram, Kerala
35.	CM 35	Perithalmanna, Malappuram, Kerala
36.	CM 36	Vadakara, Kozhikode, Kerala
37.	CM 37	Periya, Wayanad, Kerala
38.	CM 38	Edakkad, Kannur, Kerala
39.	CM 39	Kanhangad, Kasaragod, Kerala
40.	CM 40	Sowbagya, KAU, Kerala

the package of practices recommended by the Kerala Agricultural University (KAU, 1996).

3.1.1.1 Observations Recorded

Four plants in each accession were tagged for recording the biometrical observations. The details of the experimental observations are given below.

3.1.1.1.1. Days to First Male Flower

The number of days were counted from the sowing of seeds to the opening of the first male flower and recorded.

3.1.1.1.2 Node Number of First Male Flower

Node of the first male flower was noted by counting from the first true leaf.

3.1.1.1.3 Days to First Female Flower

The number of days taken from the date of sowing to the bloom of the first female flower was recorded.

3.1.1.1.4 Node of the First Female Flower

Nodes were counted from the first true leaf to the one at which first female flower was produced.

3.1.1.1.5 Sex Ratio

Number of male and female flowers were counted starting from the commencement of flowering till its completion and expressed as male to female sex ratio.

$$\text{Sex ratio} = \frac{\text{Number of male flowers}}{\text{Number of female flowers}}$$

3.1.1.1.6 Vine Length

Vine length from the collar region to the tip of the main vine was measured at the time of harvest and expressed in centimetre.

3.1.1.1.7 Number of Primary Branches

The number of primary branches per plant was counted at the stage of full maturity of the plant.

3.1.1.1.8 Number of Secondary Branches

The number of secondary branches per plant was counted at the stage of full maturity of the plant.

3.1.1.1.9 Internodal Length

Distance between two adjacent nodes was taken from the bottom portion, middle and top of the vine and average length was calculated and expressed in centimetre.

3.1.1.1.10 Leaf Petiole Length

Length of petiole of three leaves was measured at random in each plant and their mean was expressed in centimetre.

3.1.1.1.11 Leaf Thickness

Leaf thickness in the middle portion was measured using stage and ocular micrometer. Leaf sections from the randomly selected leaves of the plants were used for recording leaf thickness. Mean was computed and expressed in μ (microns).

3.1.1.1.12 Days to First Harvest

The number of days taken from sowing to the first harvest was computed for each plant and the mean value was taken.

3.1.1.1.13 Fruit Length

The length of the fruits was recorded, average length worked out and expressed in centimetre.

3.1.1.1.14 Fruit Girth

The girth at the middle portion of the fruits was measured and the mean girth was expressed in centimetre.

3.1.1.1.15 Fruit Diameter

The diameter in the middle portion of the fruits was measured and the mean was expressed in centimetre.

3.1.1.1.16 Average Fruit Weight

Weight of four randomly selected fruits from each observational plant was taken and the average value was worked out and expressed in gram.

3.1.1.1.17 Dry Matter Content (%)

Matured entire plant was removed and the fresh weight was measured. Sample plant material was dried in the hot air oven at 40 °C for one day and the dry weight was measured. Dry Matter Content (DMC) was expressed in per cent. Dry matter content was calculated by the formula

$$\text{DMC} = \frac{\text{Fresh plant weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.1.1.1.18 Fruits per Plant

The total of all the fruits obtained from each plant was counted and the mean value was taken.

3.1.1.1.19 Seeds per Fruit

One well ripened fruit from each plant was selected at random and seeds with the mucilage were extracted carefully, keeping them under fermentation for 36 hours. It was washed, cleaned and dried under shade for three days and number of seeds were counted and recorded.

3.1.1.1.20 1000 Seed Weight

A random sample of 1000 fully developed seeds per fruit from each collection was weighed using an electronic weighing balance (sartorius) and weighting was recorded in gram.

3.1.1.1.21 Yield per Plant

Weight of fruits from observational plants at each harvest was taken using a top loading balance and added to get the total and the average value was recorded in kilogram.

3.1.1.1.22 Shape and Colour of Fruits

Shape and colour of fruits in each accession were noted.

3.1.1.2 Statistical Analysis

3.1.1.2.1 Biometrical Techniques Applied

Mean, variance, standard error and coefficient of variation were the basic parameters estimated. The significance of the genotypic differences was tested through analysis of variance technique. The character associations were estimated through correlation coefficient using analysis of covariance technique. Heritability coefficient and genetic advance were estimated. The methodology on the estimation of the parameters is given below. With two characters X and Y measured on 'g' genotypes raised in randomized block design with 'r' replications, the variance covariance analysis (ANACOVA) is as follows.

3.1.1.2.2 Analysis of Variance /Covariance

Source	df	Mean square		
		X	Y	XY
Between genotypes	(g-1)	G_{XX}	G_{YY}	G_{XY}
Error	(r-1)(g-1)	E_{XX}	E_{YY}	E_{XY}

3.1.1.2.3 Estimates of Components of Variance and Covariance

	Genotypic	Environmental	Phenotypic
X	$\sigma_{gx}^2 = \frac{G_{xx} - E_{xx}}{r}$	$\sigma_{ex}^2 = E_{xx}$	$\sigma_{px}^2 = \sigma_{gx}^2 + \sigma_{ex}^2$
Y	$\sigma_{gy}^2 = \frac{G_{yy} - E_{yy}}{r}$	$\sigma_{ey}^2 = E_{yy}$	$\sigma_{py}^2 = \sigma_{gy}^2 + \sigma_{ey}^2$
XY	$\sigma_{gxy} = \frac{G_{xy} - E_{xy}}{r}$	$\sigma_{exy} = E_{xy}$	$\sigma_{pxy} = \sigma_{gxy} + \sigma_{exy}$

3.1.1.2.4 Coefficient of Variation

Phenotypic and genotypic coefficient of variation (PCV and GCV) for a trait X was estimated as:

$$\text{GCV} = \frac{\sigma_{gx}}{\bar{X}} \times 100$$

$$\text{PCV} = \frac{\sigma_{px}}{\bar{X}} \times 100$$

Where,

σ_{gx} = genotypic standard deviation

σ_{px} = phenotypic standard deviation

\bar{X} = Mean of the character under study

3.1.1.2.5 Heritability and Genetic Advance

Heritability (H^2) in broad sense was estimated as the proportion of heritable component of variation.

$$\text{Heritability } (H^2) = \frac{\sigma_{gx}^2}{\sigma_{px}^2} \times 100 \text{ (Jain, 1982)}$$

$$\text{Genetic advance} = k \times H^2 \times \sigma_{px}$$

$$\text{Genetic advance as percentage of mean (GA)} = \frac{kH^2\sigma_{px}}{\bar{X}} \times 100$$

3.1.1.2.6 Correlation Analysis

The correlation coefficient (phenotypic, genotypic and environmental) were worked out as

$$\text{Genotypic correlation } (r_{gxy}) = \frac{\sigma_{gxy}}{\sigma_{gx} \sigma_{gy}}$$

$$\text{Phenotypic correlation } (r_{pxy}) = \frac{\sigma_{pxy}}{\sigma_{px} \sigma_{py}}$$

$$\text{Environmental correlation } (r_{exy}) = \frac{\sigma_{exy}}{\sigma_{ex} \sigma_{ey}}$$

3.1.1.2.7 Path Coefficient Analysis

The direct and indirect effects of component characters on yield per plant were estimated through path analysis technique (Wright, 1954; Dewey and Lu, 1959).

The following scales suggested by Lenka and Mishra (1973) were used for the categorization of direct and indirect effects.

Scale	Category
0.00 to 0.09	Negligible
0.10 to 0.19	Low
0.20 to 0.29	Medium
0.30 to 0.99	High
≥ 1.00	Very high

3.1.1.2.8 Mahalanobis (D^2) Analysis

Mahalanobis D^2 (1936) as applied for classificatory studies by Murthy and Arunachalam (1966) in crop plants, was applied to cluster of 40 genotypes of the *Cucumis melo* L. in experiment.

For i^{th} and j^{th} genotypes, the D^2 value is computed as

$$D^2 = \sum_{i=1}^k (X_{il} - X_{jl})^2$$

where, k is the number of characters.

The genotypes were grouped into several clusters based on these D^2 values as per Tocher's method of clustering (Rao, 1952).

3.2 BIOCHEMICAL CHARACTERIZATION

3.2.1 Estimation of Protein

Total soluble protein content of *Cucumis melo* seed was estimated by using spectrophotometer as per the standard procedure given by Bradford (1976). Ten

seeds of *Cucumis melo* were ground with 2 ml of extraction buffer. Extraction buffer contains 0.0625 N Tris base (0.0756 g), SDS (2g), Glycerol (10ml), 1mM EDTA (0.037g), Mercaptoethanol (2ml) and Double distilled water (100ml). It was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant liquid was transferred to a fresh micro centrifuge tube and stored at -85°C in ultra cool refrigerator. It was used as stock solution.

10 µl of the stock solution was diluted with 200 µl of distilled water. From this 10 µl was taken for protein estimation. It was mixed with 2990 µl of dye solution (Coomassie brilliant blue (G) (30ml), methanol (50ml), orthophosphoric acid (30ml) and make up to 300ml of distilled water) in cuvette. The absorbance at 595nm was taken using spectrophotometer (Systronics UV-VIS). Calculation of the protein in the extract was calculated by comparing with standard curve developed using BSA.

3.2.2 Electrophoretic Analysis of Proteins

Electrophoretic separation of soluble proteins of leaves was carried out as suggested by Laemmli (1970) with slight modifications. Already prepared stock protein samples were used for electrophoretic analysis.

Equal volume of (10 µl) protein sample and sample buffer (10 µl) (Tris buffer (1.5g), Glycerol (20ml), SDS (2g), Mercaptoethanol (2ml), Bromophenol blue (2 mg) and Double distilled water (100 ml)) were mixed. It was heated at 70°C for five minutes using water path. Thus, sample became ready for loading. The protein concentration was adjusted in each sample to strength of 100 µg of protein from Bradford method. Based upon the values, prepared samples were loaded in each wells of the gel (15% consistency). In this analysis, each gel had one well of protein marker. Protein marker was prepared by same steps involved in sample solution preparation.

Reagents

a) Acrylamide stock solution (30%)

Acrylamide	–	29.2 g
Bis-acrylamide	–	0.8 g
Double distilled water	–	100 ml

b) Separating (resolving) gel buffer stock (1.5 M Tris-HCl pH 8.8)

Tris base (18.15g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6N HCl and made up the volume to 100ml with double distilled water and then stored at 4°C.

c) Stacking gel buffer stock (0.5 M Tris-HCl pH 6.8)

Tris base (6.0g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH to 6.8 with 6N HCl and the volume was made upto 100 ml with double distilled water and then stored at 4°C.

d) Polymerising agents

Ammonium persulphate (APS) 10 per cent

0.05 g in 500 µl of double distilled water prepared freshly before use.

TEMED – Fresh from the refrigerator

e) Sodium dodecyl sulphate (SDS) 10 per cent stock solution

1 g of SDS dissolved in 10 ml of double distilled water.

f) Electrode buffer (pH 8.3)

Tris base	3g
Glycine	14.4g
SDS	1g
Double distilled water	1 lit

3.2.2.1 Procedure

Vertical electrophoresis apparatus was used for protein separation. Firstly, glass plates were cleaned and kept in casting unit. Separating gel was first casted followed by stacking gel by mixing the various solutions as indicated below.

a) Preparation of separating gel (15%)

Double distilled water	4.7 ml
Acrylamide stock	10 ml
Tris HCl, pH 8.8	5 ml
SDS 10 %	0.20 ml

The above solution was mixed well and degassed for three minutes and then the following were added immediately.

APS (%) freshly prepared	—	0.01 ml
TEMED	—	0.01 ml

The separating gel was mixed well and poured immediately between glass plates without air bubbles and a layer of water was added above the polymerizing solution to quicken the polymerization process.

a) Preparation of stacking gel (4 %)

Double distilled water	7.5 ml
Acrylamide stock	1.35 ml
Tris HCl, pH 6.8	1 ml
SDS 10 %	0.10 ml

The solution was mixed well, degassed and the following chemicals were added immediately.

APS (10%)	0.05 ml
TEMED	0.01 ml

The water layered over the separating gel was removed and washed with a little electrode buffer and then stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

After polymerization, the comb was removed and the bottom tank was filled with electrode buffer. Then glass plates with polymerized material were fitted in the vertical electrophoresis without air bubbles. The upper tank and wells were also filled with electrode buffer. Previously, prepared samples were loaded into the different wells. The electrophoresis was performed at 100 mV till the dye reached the separating gel. Then the voltage was increased to 200 mV and continued till the dye reached the bottom of the gel. The gel was removed immediately after electrophoresis and incubated in the staining solution (Coomassie brilliant blue (R) (0.1g), Methanol (40ml), Glacial acetic acid (10ml) and double distilled water (50ml)) for over night with uniform shaking. Then the gel was transferred to the destaining solution (staining composition without coomassie brilliant blue). The protein appeared as bands and the gel was photographed after plating it on transilluminator (Appligene Model White / UV TMW-20).

3.3 MOLECULAR CHARACTERIZATION

3.3.1 Protocol for DNA Isolation of Melon (Baudracco – Arnas, 1995, Brown *et al.*, 1998; Staub *et al.*, 1996).

1. Emerging young leaves of grown *Cucumis melo* L. cultivars were collected.
2. Collected leaves were washed with distilled water after that wipe up the water particles from the leaf surface by using tissue paper. Leaves were chopped coarsely.
3. Weighed 1 g of chopped leaf sample was transferred to cool dry mortar and pestle container and after that liquid nitrogen was poured into that container.
4. The leaves were ground well to get fine powder.
5. Entire powdered material was transferred to pre heated, 10 ml of extraction buffer in 100 ml beaker at 60°C in water bath.

6. A volume of 750 μ l of SDS, a pinch of PVP were added into that beaker and mixed gently.
7. The beaker was again transferred to water bath at 70°C for 20 minutes with occasional shaking.
8. Entire solution was transferred to clean, dry centrifuge tube and added equal volume of chloroform: Isoamyl alcohol (IAA) in the ratio of 24:1.
9. Make 10-15 times of gentle inversion that causes thorough mixing of solution, which turns to milky yellow colour.
10. Centrifugation was done at 10,000 rpm for 10 minutes at 4°C.
11. After completion of centrifugation, aqueous phase was transferred to clean and dry centrifuge tube without disturbing interphase layer. Again added equal volume of chloroform: IAA (24:1) in that centrifuge tube and make gentle inversion.
12. Centrifugation was done at 10,000 rpm for 10 minutes in 4°C. After completion of the centrifugation, aqueous layer was transferred to another clean centrifuge tube.
13. Double the volume of 95 % ethanol and 1/10th volume of sodium acetate were added within the tube. Make 10-15 times of gentle inversion that produces dispersion of pellets.
14. Centrifugation was done at 15,000 rpm for 5 minutes in 4°C. After completion of the centrifugation, DNA pellet appeared in the side wall of the centrifuge tube.
15. Drain the supernatant with care. Tube was allowed to air dry until no smell of ethanol remains.
16. DNA pellet was dissolved in 500 μ l of TE buffer. This solution was transferred to eppendorf tube. This sample would contain a mixture of DNA and RNA.

17. Added 3 μl of RNase to each sample. Mixed well and incubated for 15 minutes at 40°C with occasional shaking.
18. 50 μl of 3 *M* sodium acetate and 1 ml of 95 % ethanol were added in each tube and mixed thoroughly till the DNA pellet is clearly visible.
19. Centrifuged at 20000 rpm for 2 minutes in 4°C to pellet the DNA. Poured off the supernatant and the tubes air dried.
20. Resuspend the DNA in 500 μl of TE buffer and stored at 4°C (short term) or at -85°C (long term)

3.3.2 Quantification of DNA

Reliable quantification of DNA is very important for many applications in molecular biology including amplification of target DNA by polymerase chain reaction (PCR). DNA quantification was carried out with the help of UV-VIS spectrophotometer (Spectronic Genesys 5).

The buffer in which the DNA was already dissolved was taken in a cuvette and used for the calibration of Optical Density (OD) Values in the spectrophotometer at 260 nm as well as at 280 nm wavelength.

Since, an OD of 1.0 at 260 nm represents $50\ \mu\text{g}\ \text{ml}^{-1}$ of DNA, the concentration of DNA in the sample was estimated by applying the following formula.

$$\text{Amount of DNA } (\mu\text{g}\ \text{ml}^{-1}) = A_{260} \times 50 \times \text{dilution factor}$$

where A_{260} – absorbance at 260 nm

$$\text{Dilution factor} = \frac{\text{Total volume of cuvette } (\mu\text{l})}{\text{DNA sample taken for spectrophotometer } (\mu\text{l}) \text{ reading}}$$

The quality of DNA could be judged from the ratio of the OD values recorded at 260 nm and 280 nm. A A_{260}/A_{280} ratio between 1.8 and 2.0 indicates best quality of DNA.

where A_{280} – absorbance at 280 nm

3.3.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit supplied by the Bangalore Genei. The required amount of agarose was weighed out (0.9 per cent for visualising the genomic DNA and 1.4 per cent for visualising the PCR products) and added to 1xTAE buffer. Agarose was dissolved by boiling. After cooling to about 50 °C, ethidium bromide was added to a final concentration of 0.5 µg ml⁻¹. The mixture was poured immediately to preset template with appropriate comb. After solidification, the comb and the sealing tapes were removed and the gel was mounted in electrophoresis tank filled with 1xTAE running buffer. The DNA sample was mixed with required volume of gel loading dye (6.0 x loading dye viz., 40 per cent sucrose, 0.25 per cent bromophenol blue). Each well was loaded with 12 µl (10µl sample DNA + 2µl loading dye) of sample DNA mixture. In PCR products, each well was loaded with 18 µl (15µl PCR sample + 3µl loading dye) of amplified product mixture. One of the well was loaded with 5µl of molecular weight marker (2 µl of ladder + 1 µl of loading dye + 3 µl of double distilled water). Electrophoresis unit was connected and operated continuously at 100 volts upto the loading buffer reached 3/4th length of the gel. The running was completed, the gel was visualised using an ultraviolet transilluminator. The amplified products were documented by using Alpha Imager 1200 (Alpha Innotech Inc., USA).

3.3.4 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA analysis was performed following the protocol standardized by Staub *et al.* (2000) for *Cucumis melo*. Forty arbitrarily designed decamer primers supplied by Operon Inc., CA, USA were used.

All polymerase chain reaction (PCR) solutions were purchased from Bangalore Genei. Each PCR had a volume of 15 µl and contained 3 mM MgCl₂, 0.2 mM dNTP (0.05 mM of each dATP, dGTP, dCTP and dTTP), 10 ng of DNA, 5 pM of primer, 1.5 µl of 10 X Taq buffer and one unit of Taq DNA polymerase.

Amplification was carried out in a Programmable Thermal Controller (MJ Research, Inc.) using the following cycling profile.

Thermocycle profile

Denaturation 94 °C / 4 min

3 cycles of

Denaturation 94 °C / 15 sec

Annealing 35 °C / 15 sec

Extension 72 °C/ 75 sec

40 cycles of

Denaturation 94 °C/ 15 sec

Annealing 40 °C / 15 sec

Extension 72 °C / 75 sec

72 °C/ 7 min; indefinite soak at 4 °C

A control PCR tube containing water instead of template DNA was included in each reaction set.

After completion of the PCR, amplified products along with DNA molecular weight marker supplied by US biochemicals were electrophoresed in 1.4% agarose gels, stained with ethidium bromide for 2.5 hrs at 100 Volts. After completion of the running, the gel was immediately illuminated and documented by using Alpha Imager 1200 L Alpha Innotech Inc., USA.

The number of monomorphic bands and number of polymorphic bands was recorded. Those primers used in amplification which produced maximum number of bands were used to amplify the DNA of all the forty samples. All the samples were amplified in PCR reaction using four primers. Total 160 PCR products were electrophoresis by using 1.4% agarose gel. The gels were illuminated by using UV light and documented. The PCR was repeated atleast thrice in order to check the reproducibility.

3.3.5 Data Analysis

Protein and RAPD bands were represented by '+' (for presence) and '-' (for absence). Presence and absence of individual bands was denoted as 1 and 0 respectively. The scores of individual bands were used to create a data matrix as described by Rolf (1997). The similarity index (SI) values were computed as a ratio of number of similar bands to the total number of bands in pair wise comparison of the genotypes. A dendrogram was constructed based on Jaccard's similarity coefficient with unweighted pair group method (UPGMA) using the NTSYS-pc version 2.02 (Exeter Software, New York, USA).

Results

4. RESULTS

The experimental data collected on morphological characters, yield and other yield components were statistically analysed and the results are presented under the following heads:

- 4.1 Genetic variability and genetic divergence.
- 4.2 Biochemical characterization (SDS-PAGE).
- 4.3 Molecular characterization (RAPD).

4.1 GENETIC VARIABILITY AND GENETIC DIVERGENCE

4.1.1 Analysis of Variance

General analysis of variance showed significant differences among the 40 landraces of melon for all the 21 characters studied (Table 2).

4.1.2 Mean Performance of Genotypes

The mean performances of all the genotypes for 21 characters were presented in table 3 to 8.

4.1.2.1 Days to First Male Flower

The genotypes differed significantly for this trait, and it ranged from 26.250 to 34.375 days (Table 3). Earliest flowering (26.250 days) was observed in the genotype CM 28 followed by CM 38 (27.250 days) and CM 18 (27.375 days). While the genotype CM 35 was found to be late male flowering (34.375 days). Seventeen genotypes viz., CM 1, CM 2, CM 3, CM 5, CM 9, CM 10, CM 11, CM 13, CM 14, CM 15, CM 18, CM 23, CM 29, CM 36, CM 37, CM 39 and CM 40 registered values less than the general mean (28.969 days).

4.1.2.2 Node Number of First Male Flower

The genotypes exhibited wide variation for node number of first male flower which ranged from 2.500 to 5.500 (Table 3). The genotype CM 28 produced the first male flower at 2.500th node followed by CM 9 (2.750) and CM 16 (3.375) whereas, the genotype CM 35 produced male flower at 5.500th node. Fifteen genotypes CM 1, CM 3, CM 5, CM 6, CM 7, CM 8, CM 9, CM 10, CM 14,

Table 2. General analysis of variance for various characters in landraces of melon (40 Genotypes)

Source of Variance	df	Mean sum of squares																				
		Days to first male flower	Node number of first male flower	Days to first female flower	Node number of first female flower	Sex ratio	Vine length, cm	Number of primary branches	Number of secondary branches	Internodal length, cm	Leaf petiole length, cm	Leaf thickness, μ	Days to first harvest	Fruit length, cm	Fruit girth, cm	Fruit diameter, cm	Average fruit weight, kg	Dry matter content, %	Fruits per plant	Seeds per fruit	1000 seed weight, g	Yield per plant, kg
Replication	1	0.153	0.378	0.017	0.038	13.316	280.200	0.003	0.001	1.669	0.007	1.600	0.509	0.233	1.835	0.242	0.005	0.053	2.381	10.400	0.048	3.739
Treatment	39	** 5.150	** 0.687	** 7.066	** 2.800	** 113.618	** 4220.468	** 0.180	** 0.678	** 4.299	** 4.787	** 3013.718	** 21.397	** 110.132	** 74.543	** 8.976	** 0.350	** 8.013	** 21.299	** 26265.076	** 44.223	** 24.296
Error	39	0.081	0.102	0.255	0.133	8.816	157.661	0.050	0.100	0.151	0.097	14.472	0.613	2.131	1.258	0.102	0.009	0.692	2.285	171.477	0.486	2.379

** - Significant at 1% level

CM 16, CM 17, CM 19, CM 28, CM 39 and CM 40 registered values less than the general mean (4.075) for this trait.

4.1.2.3 Days to First Female Flowering

Among the genotypes, the number of days taken for first female flowering ranged from 33.000 to 40.000 days (Table 3). The genotype CM 36 was the earliest flowering (33.000 days) followed by CM 11 (33.125 days) and CM 21 (33.250 days), while the genotypes CM 5 and CM 35 were late flowering (40.000 days). Twenty five genotypes *viz.*, CM 4, CM 6, CM 7, CM 9, CM 10, CM 11, CM 12, CM 13, CM 14, CM 16, CM 17, CM 21, CM 22, CM 23, CM 26, CM 27, CM 28, CM 29, CM 30, CM 31, CM 32, CM 36, CM 37, CM 38 and CM 39 recorded values less than the general mean (35.041 days).

4.1.2.4 Node Number of First Female Flower

The node number of first female flower ranged from 6.375 to 12.625 (Table 3). The genotypes CM 9 produced the first female flower at 6.375th node followed by CM 2, CM 7 (7.125) and CM 14, CM 16 (7.250), whereas CM 5 produced the first female flower at 12.625th node. Twenty four genotypes *viz.*, CM 1, CM 4, CM 6, CM 7, CM 8, CM 9, CM 10, CM 11, CM 12, CM 13, CM 14, CM 16, CM 17, CM 18, CM 20, CM 21, CM 22, CM 24, CM 25, CM 27, CM 28, CM 32, CM 33 and CM 40 exhibited lower values for this trait when compared to the grand mean (8.709).

4.1.2.5 Sex Ratio

Significant differences were observed for this trait among the different genotypes and the sex ratio ranged from 10.500 to 38.700 (Table 3). Among the genotypes, the broadest sex ratio was observed in the genotype CM 4 (38.700) followed by CM 32 (38.550) and CM 15 (37.900), while the genotype CM 18 exhibited the narrowest sex ratio (10.500). Totally, twenty three genotypes *viz.*, CM 1, CM 4, CM 8, CM 9, CM 11, CM 12, CM 15, CM 17, CM 21, CM 23, CM 25, CM 26, CM 27, CM 28, CM 29, CM 30, CM 32, CM 33, CM 34, CM 35, CM 36, CM 38 and CM 40 registered greater values for sex ratio than general mean (29.502).

Table 3. Mean performance of genotypes for days to first male flower, node number of first male flower, days to first female flower, node number of first female flower and sex ratio

Genotypes	Days to first male flower	Node number of first male flower	Days to first female flower	Node number of first female flower	Sex ratio
CM 1	28.250	3.500	36.125	8.250	36.400
CM 2	28.500	4.125	39.250	11.000	19.300
CM 3	28.000	3.500	38.250	9.500	23.100
CM 4	29.250	4.250	33.375	7.875	38.700
CM 5	28.375	3.500	40.000	12.625	23.500
CM 6	32.750	3.625	34.500	8.500	26.700
CM 7	29.375	3.750	34.125	7.125	26.000
CM 8	29.500	3.750	35.625	7.500	34.400
CM 9	23.625	2.750	33.375	6.375	30.700
CM 10	28.375	3.500	34.375	8.250	19.500
CM 11	28.500	4.000	33.125	8.250	34.800
CM 12	29.500	4.250	34.875	8.500	36.600
CM 13	28.750	4.500	34.250	8.500	18.800
CM 14	28.625	3.500	33.875	7.250	37.200
CM 15	28.250	4.500	37.875	9.500	37.900
CM 16	29.375	3.375	34.750	7.250	28.200
CM 17	29.125	4.000	34.000	8.125	34.400
CM 18	27.375	4.250	35.625	8.375	10.500
CM 19	30.500	3.500	35.500	9.250	18.100
CM 20	29.500	4.250	35.875	7.125	15.350
CM 21	29.250	4.500	33.250	8.250	33.200
CM 22	30.000	4.250	34.625	8.250	27.400
CM 23	28.500	4.750	33.500	9.875	33.300
CM 24	29.500	4.500	37.750	8.500	28.500
CM 25	30.000	4.250	35.750	8.250	31.600
CM 26	29.125	4.500	33.500	9.375	33.900
CM 27	29.375	4.500	35.000	8.375	37.400
CM 28	26.250	2.500	34.125	7.625	34.400
CM 29	28.500	5.000	33.375	10.000	34.500
CM 30	29.750	4.500	33.500	9.500	35.200
CM 31	29.375	4.250	33.625	9.125	22.300
CM 32	29.250	4.500	34.125	8.375	38.550
CM 33	30.500	4.750	36.000	8.250	36.000
CM 34	29.250	4.250	35.250	9.000	35.800
CM 35	34.375	5.500	40.000	11.250	29.600
CM 36	28.500	4.500	33.000	9.375	33.600
CM 37	28.125	4.250	34.250	9.125	25.000
CM 38	27.250	4.250	33.500	9.500	35.100
CM 39	28.125	3.625	33.375	8.750	14.300
CM 40	28.250	3.500	35.375	8.500	30.300
Mean	28.969	4.075	35.041	8.709	29.502
CD (0.05)	0.576	0.648	1.022	0.737	6.006

4.1.2.6 Vine Length

The vine length ranged from 103.125 to 299.375cm among the various genotypes (Table 4). The genotype CM 4 exhibited the vine longest (299.375 cm) followed by CM 39 (260.000 cm) and CM 19 (56.875 cm). The shortest vine was recorded in CM 2 (103.125 cm). Among the forty genotypes, twenty four genotypes *viz.*, CM 1, CM 4, CM 6, CM 8, CM 9, CM 10, CM 12, CM 13, CM 15, CM 17, CM 18, CM 19, CM 21, CM 22, CM 23, CM 26, CM 27, CM 31, CM 32, CM 34, CM 35, CM 38, CM 39 and CM 40 exceeded the general mean (201.284 cm) for this trait.

4.1.2.7 Number of Primary Branches

Among the genotypes, the number of primary branches ranged from 2.250 to 3.625 (Table 4). The genotype CM 14 (3.625) expressed the maximum number of primary branches followed by CM 7, CM 23, CM 31 (3.500) and CM 4, CM 11, CM 18, CM 33 (3.375). The least performance for this trait was recorded in CM 36 (2.250). Among the forty genotypes, twenty one genotypes *viz.*, CM 1, CM 4, CM 7, CM 10, CM 11, CM 12, CM 14, CM 15, CM 18, CM 19, CM 21, CM 22, CM 23, CM 24, CM 29, CM 30, CM 31, CM 32, CM 33, CM 35 and CM 40 registered greater values for number of primary branches than general mean (3.081).

4.1.2.8 Number of Secondary Branches

Among the genotypes, the number of secondary branches ranged from 2.375 to 5.375 (Table 4). The genotype CM 32 (5.375) had maximum number of secondary branches followed by CM 38 (4.500) and CM 1(4.375). The least performance for this trait was recorded in CM 18 (2.375). Twenty genotypes *viz.*, CM 1, CM 3, CM 4, CM 7, CM 8, CM 12, CM 14, CM 15, CM 17, CM 19, CM 20, CM 21, CM 26, CM 28, CM 29, CM 32, CM 33, CM 36, CM 38 and CM 40 registered greater values for number of secondary branches than general mean (3.447).

Table 4. Mean performance of genotypes for vine length, number of primary branches, number of secondary branches and internodal length

Genotypes	Vine length, cm	Number of primary branches	Number of secondary branches	Internodal length, cm
CM 1	219.875	3.125	4.375	8.070
CM 2	103.125	2.625	3.375	4.900
CM 3	188.125	3.000	3.750	10.075
CM 4	299.375	3.375	4.125	11.700
CM 5	105.625	2.875	2.875	4.140
CM 6	220.625	3.000	3.000	9.190
CM 7	188.125	3.500	3.500	9.330
CM 8	239.875	3.000	4.250	8.425
CM 9	213.750	3.000	3.000	10.390
CM 10	233.750	3.250	3.125	9.225
CM 11	186.875	3.375	3.250	9.075
CM 12	207.500	3.250	3.500	10.445
CM 13	211.250	2.750	2.750	7.600
CM 14	182.625	3.625	4.000	10.150
CM 15	215.750	3.125	3.750	10.430
CM 16	186.875	3.000	3.125	8.310
CM 17	224.375	2.500	3.625	11.350
CM 18	204.375	3.375	2.375	9.660
CM 19	256.875	3.250	3.750	10.210
CM 20	179.375	3.000	3.750	8.285
CM 21	203.750	3.250	3.500	9.130
CM 22	218.125	3.125	2.875	10.185
CM 23	238.125	3.500	2.875	9.200
CM 24	172.500	3.125	3.000	9.035
CM 25	126.875	3.000	2.875	8.355
CM 26	223.750	2.500	3.500	9.885
CM 27	233.125	2.625	3.375	8.875
CM 28	191.250	3.000	3.625	8.895
CM 29	161.250	3.250	3.500	10.090
CM 30	108.750	3.250	3.250	9.335
CM 31	224.375	3.500	3.250	8.375
CM 32	228.750	3.250	5.375	8.320
CM 33	126.875	3.375	3.625	9.710
CM 34	256.250	3.000	3.000	10.710
CM 35	219.375	3.250	3.125	10.900
CM 36	199.375	2.250	4.250	9.550
CM 37	110.625	2.875	2.875	8.225
CM 38	228.750	2.875	4.500	10.135
CM 39	260.000	3.000	2.750	8.150
CM 40	251.375	3.250	3.500	10.710
Mean	201.284	3.081	3.447	9.218
CD (0.05)	25.398	0.450	0.640	0.786

4.1.2.9 Internodal Length (cm)

The internodal length ranged from 4.140 to 11.700 cm among the various genotypes (Table 4). The genotype CM 4 exhibited the longest internode (11.700 cm) followed by CM 17 (11.350 cm) and CM 35 (10.900 cm). The least performance for this trait was recorded in CM 5 (4.140 cm). Among the forty genotypes, twenty one genotypes *viz.*, CM 3, CM 4, CM 7, CM 9, CM 10, CM 12, CM 14, CM 15, CM 17, CM 18, CM 19, CM 22, CM 26, CM 29, CM 30, CM 33, CM 34, CM 35, CM 36, CM 38 and CM 40 exceeded the general mean (9.218 cm) for this trait.

4.1.2.10 Leaf Petiole Length (cm)

Among the various genotypes, the leaf petiole length ranged from 6.890 to 14.110 cm (Table 5). The genotype CM 4 exhibited the longest petioles (14.110 cm) followed by CM 39 (13.150 cm) and CM 26 (13.030 cm). The least performance for this trait was recorded in CM 2 (6.890 cm). Totally, 18 genotypes *viz.*, CM 1, CM 3, CM 4, CM 9, CM 10, CM 19, CM 20, CM 22, CM 25, CM 26, CM 28, CM 30, CM 33, CM 34, CM 35, CM 36, CM 37 and CM 39 exceeded the general mean (10.755 cm) for this trait.

4.1.2.11 Leaf Thickness (μ)

This trait differed significantly among the genotypes and it ranged from 253.000 to 418.125 μ (Table 5). The genotype CM 2 (418.125 μ) had the highest leaf thickness followed by CM 5 (400.379 μ) and CM 27 (384.500 μ). CM 19 had the thinnest leaves (253.000 μ). Among the forty genotypes, eighteen genotypes *viz.*, CM 4, CM 5, CM 11, CM 12, CM 14, CM 17, CM 20, CM 22, CM 26, CM 27, CM 29, CM 32, CM 33, CM 36, CM 37, CM 38 and CM 40 registered greater values for leaf thickness than the general mean (329.897 μ).

4.1.2.12 Days to First Harvest

Among the various genotypes, days to first harvest ranged from 50.400 to 66.100 days (Table 5). The genotype CM 39 was earliest to first harvest (50.400 days) followed by CM 29 (50.500 days) and CM 22 (51.000 days), while the genotype took maximum days to first harvest CM 2 (66.100 days). Twenty

Table 5. Mean performance of genotypes for leaf petiole length, leaf thickness and days to first harvest

Genotypes	Leaf petiole length, cm	Leaf thickness, μ	Days to first harvest
CM 1	10.810	312.625	52.800
CM 2	6.890	418.125	66.100
CM 3	12.310	324.625	52.100
CM 4	14.110	371.250	58.700
CM 5	7.565	400.375	64.200
CM 6	10.270	320.250	56.500
CM 7	9.460	286.125	54.800
CM 8	10.175	311.250	52.800
CM 9	11.075	319.000	54.000
CM 10	12.385	302.625	51.300
CM 11	10.110	341.125	54.300
CM 12	8.710	375.625	51.800
CM 13	10.625	308.625	53.600
CM 14	9.215	353.250	53.000
CM 15	10.000	321.625	52.300
CM 16	8.150	360.000	54.500
CM 17	10.135	362.500	53.100
CM 18	9.475	308.500	52.100
CM 19	12.550	253.000	53.000
CM 20	11.850	335.125	55.500
CM 21	10.340	296.125	53.200
CM 22	12.425	322.250	51.000
CM 23	10.030	299.875	53.700
CM 24	10.535	272.125	52.700
CM 25	12.360	281.250	56.300
CM 26	13.030	372.750	56.500
CM 27	9.495	384.500	52.800
CM 28	12.265	294.250	55.400
CM 29	10.000	355.000	50.500
CM 30	11.210	286.250	52.600
CM 31	10.500	309.000	54.800
CM 32	9.990	353.125	53.600
CM 33	10.910	375.000	53.400
CM 34	11.360	308.500	55.200
CM 35	12.470	286.500	54.000
CM 36	11.960	350.000	60.800
CM 37	11.765	372.500	51.200
CM 38	9.970	375.000	54.500
CM 39	13.150	275.500	50.400
CM 40	10.585	340.750	53.300
Mean	10.755	329.897	54.310
CD (0.05)	0.630	7.695	1.584

six genotypes viz., CM 1, CM 3, CM 8, CM 9, CM 10, CM 11, CM 12, CM 13, CM 14, CM 15, CM 17, CM 18, CM 19, CM 21, CM 22, CM 23, CM 24, CM 27, CM 29, CM 30, CM 32, CM 33, CM 35, CM 37, CM 39 and CM 40 recorded values less than the general mean (54.310 days).

4.1.2.13 Fruit Length (cm)

The genotypes differed significantly for this trait. The range for fruit length varied from 17.900 to 46.730 cm among the forty genotypes (Table 6). The longest fruit was observed in CM 10 (46.730 cm), which was followed by CM 4 (44.150 cm) and CM 13 (41.540 cm). The shortest fruit 17.9 cm was observed in CM 27. Twenty genotypes viz., CM 1, CM 4, CM 6, CM 8, CM 9, CM 10, CM 11, CM 12, CM 13, CM 14, CM 15, CM 20, CM 21, CM 23, CM 24, CM 25, CM 26, CM 29, CM 32 and CM 38 exhibited higher values than the general mean (29.689 cm) for this trait.

4.1.2.14 Fruit Girth (cm)

The data presented in table 6 revealed that the genotypes differed significantly for this trait. The range for fruit girth varied from 20.120 to 51.50 cm. The genotype CM 39 recorded the highest fruit girth (51.510cm) followed by CM 10 (38.310 cm) and CM 20 (36.240 cm), whereas the lowest was observed in CM 3 (20.120cm). Totally fifteen genotypes viz., CM 1, CM 7, CM 10, CM 17, CM 18, CM 19, CM 20, CM 24, CM 28, CM 30, CM 31, CM 32, CM 35, CM 39 and CM 40 registered greater values for fruit girth than the general mean (27.748 cm).

4.1.2.15 Fruit Diameter (cm)

Fruit diameter ranged from 6.200 cm to 15.710cm among the various genotypes (Table 6). The genotype CM 39 exhibited the highest fruit diameter (15.710cm) followed by CM 2 (13.220cm) and CM 5 (12.860 cm), whereas the lowest was observed in CM 3 (6.200 cm). Sixteen genotypes viz., CM 1, CM 2, CM 5, CM 7, CM 10, CM 16, CM 17, CM 18, CM 19, CM 20, CM 24, CM 30, CM 32, CM 35, CM 39 and CM 40 registered greater values for fruit diameter than the general mean (9.146 cm).

Table 6. Mean performance of genotypes for fruit length, fruit girth, fruit diameter and average fruit weight

Genotypes	Fruit length, cm	Fruit girth, cm	Fruit diameter, cm	Average fruit weight, kg
CM 1	34.000	28.400	10.160	1.560
CM 2	19.250	21.660	13.220	0.481
CM 3	29.580	20.120	6.200	0.850
CM 4	44.150	25.840	9.000	1.093
CM 5	18.640	22.330	12.860	0.620
CM 6	34.020	21.690	7.150	0.722
CM 7	18.220	32.530	10.480	0.705
CM 8	29.960	22.030	7.170	0.773
CM 9	34.930	26.640	8.580	1.935
CM 10	46.730	38.310	12.680	1.828
CM 11	33.640	23.330	7.300	0.874
CM 12	41.020	24.160	7.710	0.879
CM 13	41.540	23.120	7.390	0.911
CM 14	34.440	21.640	6.670	0.641
CM 15	30.680	25.710	8.510	0.916
CM 16	27.840	27.500	9.360	0.966
CM 17	23.890	31.510	9.860	0.949
CM 18	26.690	32.450	10.600	1.112
CM 19	26.920	36.180	11.410	0.941
CM 20	32.890	36.240	11.700	1.740
CM 21	32.670	27.310	8.900	0.977
CM 22	18.930	27.570	8.510	0.972
CM 23	34.290	20.170	6.410	0.793
CM 24	33.070	36.110	11.310	1.626
CM 25	31.490	22.910	7.150	1.500
CM 26	33.660	24.090	7.220	0.872
CM 27	17.900	24.920	7.600	0.710
CM 28	29.320	27.970	8.670	0.915
CM 29	31.790	24.840	7.440	0.753
CM 30	27.140	30.530	9.410	0.853
CM 31	26.600	28.540	9.060	0.878
CM 32	33.730	31.210	10.040	2.125
CM 33	26.140	27.510	8.660	0.939
CM 34	25.060	25.560	8.200	0.599
CM 35	28.880	29.490	9.500	1.026
CM 36	19.080	25.860	7.930	0.768
CM 37	25.320	25.050	7.470	0.622
CM 38	42.130	23.260	7.370	1.956
CM 39	18.140	51.510	15.710	0.623
CM 40	23.210	34.100	11.270	1.030
Mean	29.689	27.748	9.146	1.026
CD (0.05)	2.953	2.269	0.648	0.197

4.1.2.16 Average Fruit Weight (kg)

The genotypes exhibited wide variation for average fruit weight which ranged from 0.481g to 2.125g (Table 6). Among the genotypes, CM 32 (2.125g) recorded the highest average fruit weight followed by CM 38 (1.950g) and CM 9 (1.935g), whereas less weight appeared in CM 2 (0.481g). Totally twelve genotypes *viz.*, CM 1, CM 4, CM 9, CM 10, CM 18, CM 20, CM 24, CM 25, CM 32, CM 35, CM 38 and CM 40 exceeded the general mean (1.026g) for this trait.

4.1.2.17 Dry Matter Content (%)

Dry matter content ranged from 51.200 to 60.800% among the various genotypes (Table 7). The genotype CM 36 (60.800%) exhibited highest dry matter content followed by CM 2 (60.100%) and CM 5 (58.30%), whereas the lowest dry matter content was observed in CM 37 (51.200%). Totally, twenty genotypes *viz.*, CM 2, CM 5, CM 6, CM 7, CM 9, CM 11, CM 16, CM 20, CM 22, CM 23, CM 25, CM 26, CM 28, CM 29, CM 31, CM 34, CM 36, CM 37, CM 38 and CM 39 exceeded the general mean (54.470%) for this trait.

4.1.2.18 Fruits Per Plant

Significant differences were observed for this trait among the different genotypes (Table 7). The mean yield per plant in number ranged from 1.300 to 14.600. The genotype CM 36 recorded the maximum fruits per plant (14.600) followed by CM 15 (13.100) and CM 12 (12.300), while the genotype CM 5 recorded the minimum fruits per plant (1.300). Totally twenty genotypes *viz.*, CM 1, CM 3, CM 4, CM 8, CM 11, CM 12, CM 14, CM 15, CM 16, CM 17, CM 21, CM 23, CM 27, CM 28, CM 29, CM 32, CM 33, CM 34, CM 36 and CM 40 registered values more than the general mean of 7.857.

4.1.2.19 Seeds per Fruit

Seeds per fruit ranged from 401.500 to 980.600 among the various genotypes (Table 7). The genotype CM 4 (980.600) exhibited the highest seeds per fruit followed by CM 10 (884.200) and CM 38 (852.900), whereas the lowest seeds per fruit were observed in CM 2 (401.500). Totally thirteen genotypes

Table 7. Mean performance of genotypes for dry matter content, fruits per plant, seeds per fruit, 1000 seed weight and yield per plant

Genotypes	Dry matter content, %	Fruits per plant	Seeds per fruit	1000 seed weight, g	Yield per plant, Kg
CM 1	52.300	9.200	733.100	21.460	13.540
CM 2	60.100	1.400	401.500	36.680	1.016
CM 3	53.600	8.300	539.200	17.580	6.553
CM 4	54.300	10.300	980.600	20.150	10.550
CM 5	58.300	1.300	437.300	23.180	0.939
CM 6	56.500	3.400	477.300	15.550	2.514
CM 7	54.800	5.500	402.900	20.400	4.929
CM 8	52.700	10.300	584.700	24.300	7.525
CM 9	54.700	7.200	734.900	22.160	12.193
CM 10	54.100	6.500	884.200	10.430	11.371
CM 11	54.600	11.300	586.600	18.560	9.557
CM 12	52.900	12.300	558.600	25.420	9.551
CM 13	52.900	2.800	519.800	18.220	2.364
CM 14	53.000	11.800	567.200	12.500	7.462
CM 15	52.200	13.100	565.200	19.470	10.228
CM 16	54.500	8.600	525.600	18.430	7.489
CM 17	53.100	12.200	534.200	23.360	10.561
CM 18	53.600	2.800	542.100	16.190	3.140
CM 19	53.500	7.500	549.700	19.430	5.772
CM 20	55.500	7.100	604.200	18.530	12.241
CM 21	53.200	9.700	589.300	18.780	9.560
CM 22	55.500	3.400	573.900	21.370	3.040
CM 23	54.500	8.800	505.400	13.460	6.133
CM 24	52.700	4.800	567.900	17.000	7.571
CM 25	56.700	5.400	572.500	10.550	7.917
CM 26	56.400	7.600	605.400	24.080	7.170
CM 27	52.700	10.600	484.600	13.890	6.873
CM 28	55.400	10.000	572.600	16.860	7.760
CM 29	55.500	11.500	621.100	19.510	8.028
CM 30	52.600	7.300	529.900	15.790	5.403
CM 31	54.700	4.300	572.300	12.800	4.326
CM 32	53.600	9.700	570.700	20.670	15.874
CM 33	53.400	9.300	579.500	16.570	8.147
CM 34	55.200	9.400	534.900	16.940	5.452
CM 35	54.000	7.500	557.000	24.110	6.849
CM 36	60.800	14.600	503.100	21.810	10.332
CM 37	51.200	7.800	567.400	23.510	3.963
CM 38	54.500	7.200	852.900	18.610	12.139
CM 39	55.800	4.500	589.300	20.510	3.160
CM 40	53.200	8.000	597.200	19.180	7.018
Mean	54.470	7.857	581.895	19.200	7.405
CD (0.05)	1.682	3.058	26.487	1.410	3.120

viz., CM 1, CM 4, CM 8, CM 9, CM 10, CM 11, CM 20, CM 21, CM 26, CM 29, CM 38, CM 39 and CM 40 exceeded the general mean of (581.895).

4.1.2.20 1000 Seed Weight (g)

The 1000 seed weight ranged from 10.430 to 36.680g among the various genotypes (Table 7). The genotype CM 2 (36.680g) exhibited the highest 1000 seed weight followed by CM 12 (25.420g) and CM 8 (24.300g), whereas the lowest 1000 seed weight was observed in CM 10 (10.430g). Totally nineteen genotypes viz., CM 1, CM 2, CM 4, CM 5, CM 7, CM 8, CM 9, CM 12, CM 15, CM 17, CM 19, CM 22, CM 26, CM 29, CM 32, CM 35, CM 36, CM 37 and CM 30 exceeded the general mean of 19.200g.

4.1.2.21 Yield per Plant (kg)

The genotypes showed significant differences for this trait (Table 7) and it ranged from 0.939 kg to 15.874 kg. Among the genotypes, CM 32 produced the highest yield (15.874 kg) per plant (Plate 2). This was followed by the genotypes CM 1 (13.540 kg) and CM 20 (12.241 kg), whereas the lowest yield per plant was recorded in the genotype CM 5 (0.939 kg). Totally twenty one genotypes viz., CM 1, CM 4, CM 8, CM 9, CM 10, CM 11, CM 12, CM 14, CM 15, CM 16, CM 17, CM 20, CM 21, CM 24, CM 25, CM 28, CM 29, CM 32, CM 33, CM 36 and CM 38, exceeded the general mean of 7.405kg.

4.1.2.22 Fruit Shape and Colour

Shape and colour of fruit in each accession were presented in table 8 and Plate 3.

4.1.3 Variability Studies

The phenotypic variance, genotypic variance and coefficient of variation (PCV and GCV) for the twenty one characters are presented in table 9 and Fig. 1.

Seeds per fruit expressed the highest genotypic variance (13046.8) followed by vine length (2031.403) and leaf thickness (1499.623). Lowest value (0.065) was recorded for number of primary branches followed by average fruit weight (0.170) and number of secondary branches (0.289).



Plate 2. CM 32 – High yielding superior landrace

Table 8. Characterization of the landraces of melon in terms of fruit shape and fruit colour

S. No.	Genotypes	Fruit shape	Fruit colour
1.	CM 1	Oblong	White in green
2.	CM 2	Round netted	Yellow
3.	CM 3	Cylindrical	White in green
4.	CM 4	Slender	Yellowish green
5.	CM 5	Round netted	Yellow
6.	CM 6	Cylindrical	White in green
7.	CM 7	Round	Yellow
8.	CM 8	Cylindrical	White in green
9.	CM 9	Cylindrical	Green in white
10.	CM 10	Cylindrical	Green in white
11.	CM 11	Cylindrical	White in green
12.	CM 12	Cylindrical	White in green
13.	CM 13	Cylindrical	White in green
14.	CM 14	Cylindrical	Green in white
15.	CM 15	Cylindrical	Green in white
16.	CM 16	Oblong	White in green
17.	CM 17	Cylindrical	White in green
18.	CM 18	Oblong	White in green
19.	CM 19	Cylindrical	Yellow in green
20.	CM 20	Oblong	White in green
21.	CM 21	Cylindrical	White in green
22.	CM 22	Cylindrical	White in green
23.	CM 23	Cylindrical	White in green
24.	CM 24	Oblong	Yellow in green
25.	CM 25	Oblong	White in green
26.	CM 26	Oblong	White in green
27.	CM 27	Cylindrical	White in green
28.	CM 28	Cylindrical	Yellow in green
29.	CM 29	Cylindrical	White in green
30.	CM 30	Cylindrical	Yellow in green
31.	CM 31	Cylindrical	Green in white
32.	CM 32	Oblong	Yellow in green
33.	CM 33	Cylindrical	White in green
34.	CM 34	Cylindrical	Yellow in green
35.	CM 35	Cylindrical	White in green
36.	CM 36	Cylindrical	White in green
37.	CM 37	Cylindrical	White in green
38.	CM 38	Cylindrical	Green in white
39.	CM 39	Round	White in green
40.	CM 40	Cylindrical	Yellow in green

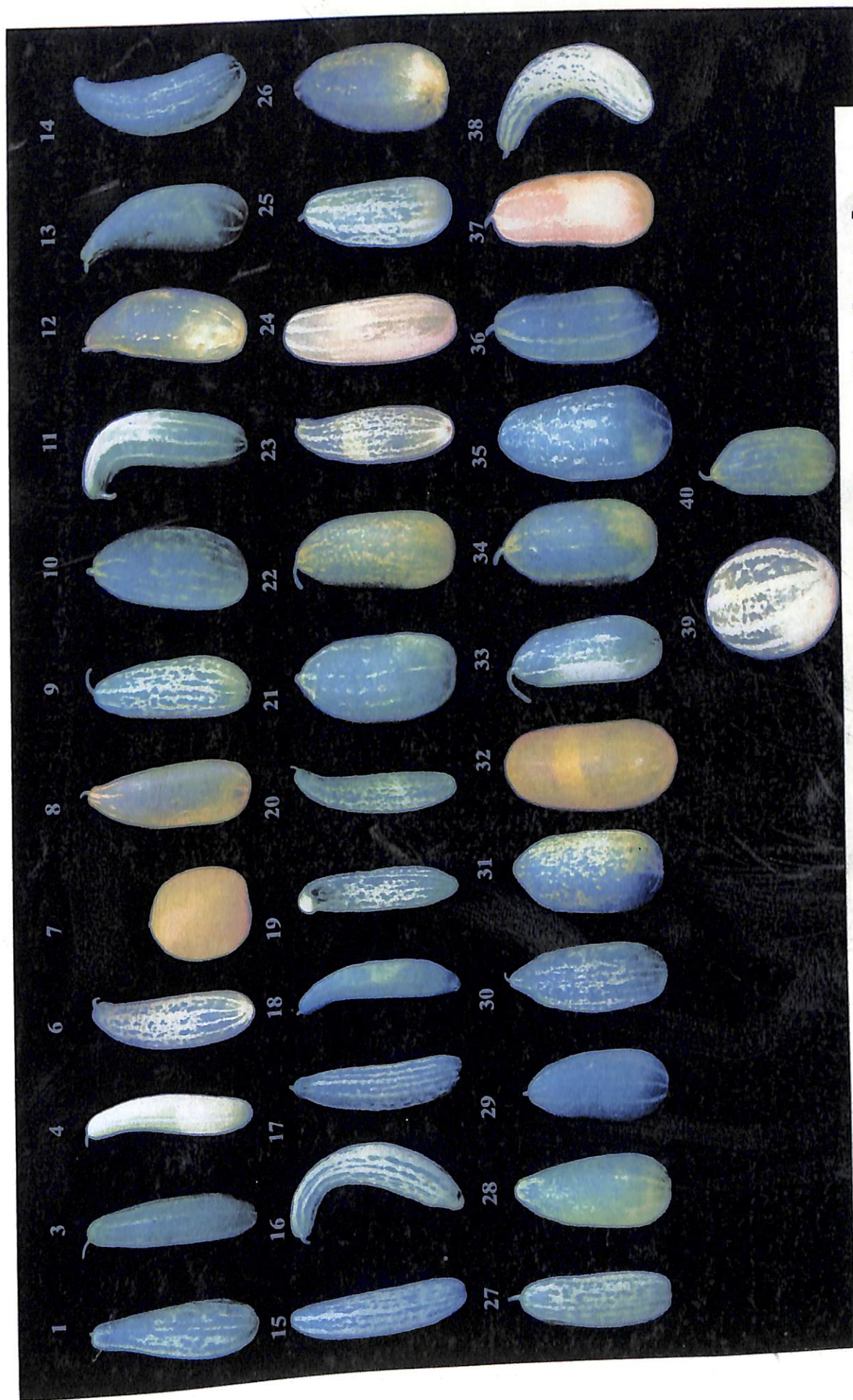


Plate 3.A comprehensive representation of landraces of melons used in the study

Table 9. Phenotypic and genotypic coefficient of variation for various characters in landraces of melon

S. No.	Characters	Phenotypic variance, σ_p	Genotypic variance, σ_g	Environmental variance, σ_e	Coefficient of variation	
					Phenotypic, %	Genotypic, %
1.	Days to first male flower	2.535	2.616	0.081	5.58	5.49
2.	Node number of first male flower	0.292	0.395	0.102	15.42	13.27
3.	Days to first female flower	3.406	3.661	0.255	5.46	5.27
4.	Node number of first female flower	1.334	1.467	0.133	13.90	13.26
5.	Sex ratio	52.401	61.217	8.816	26.52	24.54
6.	Vine length, cm	2031.403	2189.065	157.662	23.24	22.39
7.	Number of primary branches	0.065	0.115	0.050	11.00	8.30
8.	Number of secondary branches	0.289	0.389	0.100	18.09	15.59
9.	Internodal length, cm	2.074	2.225	0.151	16.18	15.62
10.	Leaf petiole length, cm	2.345	2.442	0.097	14.53	14.24
11.	Leaf thickness, μ	1499.623	1514.095	14.472	11.79	11.74
12.	Days to first harvest	54.000	56.131	2.131	6.11	5.94
13.	Fruit length, cm	36.642	37.901	1.258	25.23	24.75
14.	Fruit girth, cm	4.437	4.539	0.103	22.19	21.82
15.	Fruit diameter, cm	0.170	0.180	0.009	23.29	23.03
16.	Average fruit weight, kg	3.661	4.352	0.692	41.32	40.22
17.	Dry matter content, %	9.507	11.792	2.285	3.83	3.51
18.	Fruits per plant	10.959	13.338	2.379	43.70	39.24
19.	Seeds per fruit	13046.800	13218.276	171.477	19.76	19.63
20.	1000 seed weight, g	21.869	22.355	0.486	24.62	24.36
21..	Yield per plant, kg	10.392	11.005	0.613	49.32	44.70

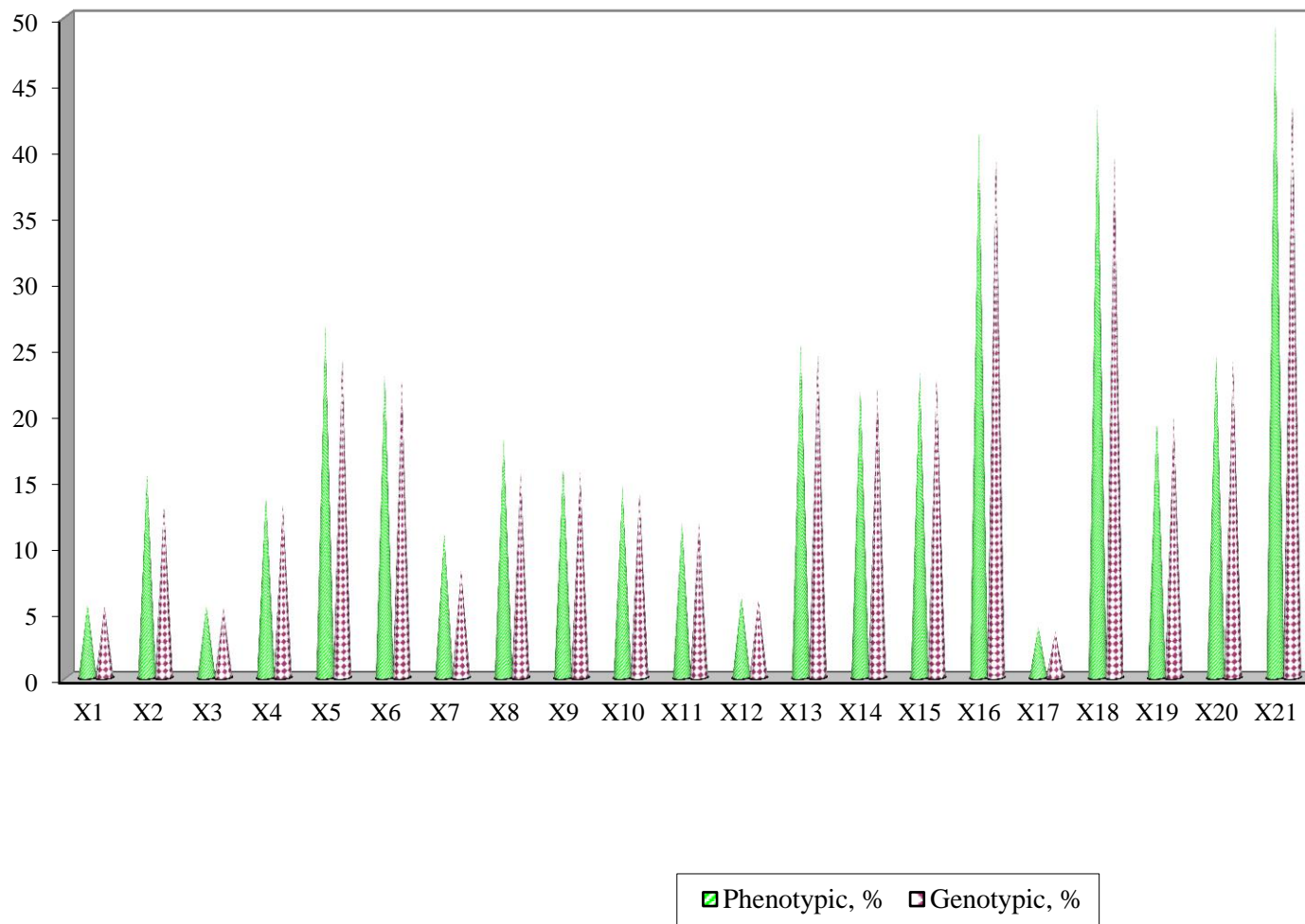


Fig. 1. Phenotypic and genotypic coefficient of variation for various characters in landraces of melon

The phenotypic variance was also highest for seeds per fruit (13218.276) followed by vine length (2189.065). Least phenotypic variance was observed in number of primary branches (0.115) followed by average fruit weight (0.180).

Very high values of PCV were observed for yield per plant (49.32) followed by fruits per plant (43.70) and average fruit weight (41.32). Moderate PCV was shown in sex ratio (26.52) followed by fruit length (25.23), 1000 seed weight (24.62), fruit diameter (23.29), vine length (23.24) and fruit girth (22.19). Lowest values of PCV was noticed for dry matter content (3.83) followed by days to first female flower (5.46), days to first male flower (5.58) and days to first harvest (6.11).

Maximum GCV was observed for yield per plant (44.70) followed by average fruit weight (40.22) and fruits per plant (39.24). Moderate GCV was noted for fruit length (24.75), followed by sex ratio (24.54) and 1000 seed weight (24.36). Lowest GCV was obtained for dry matter content (3.51), followed by days to first female flower (5.27), days to first male flower (5.49) and days to first harvest (5.94).

4.1.4 Heritability and Genetic Advance

Heritability and genetic advance was estimated for twenty one characters of forty landraces of melon and the results are presented in table 10 and Fig. 2.

4.1.4.1 Heritability

High estimates of heritability in broad sense was recorded for almost all the traits such as leaf thickness (99.04%), seeds per fruit (98.70%), 1000 seed weight (97.83%), fruit diameter (97.74%), days to first male flower (96.90%), fruit girth (96.68%), fruit length (96.20%), leaf petiole length (96.02%), average fruit weight (94.74%), days to first harvest (94.43%), internodal length (93.22%), days to first female flower (93.03%), vine length (92.80%), node number of first female flower (90.94%), sex ratio (85.60%), dry matter content (84.11%), yield per plant (82.16%), fruits per plant (80.62%), number of secondary branches (74.25%) and node number of first male flower (74.04%). The trait number of primary branches recorded moderate heritability (56.86%).

Table 10. Estimates of heritability, genetic advance and genetic advance as percentage of mean for various characters in landraces of melon

S. No.	Characters	Heritability, %	Genetic advance	Genetic advance as percentage of mean
1.	Days to first male flower	96.90	8.23	11.14
2.	Node number of first male flower	74.04	0.96	23.52
3.	Days to first female flower	93.03	3.66	10.46
4.	Node number of first female flower	90.94	2.27	26.05
5.	Sex ratio	85.60	13.76	46.76
6.	Vine length, cm	92.80	89.41	44.43
7.	Number of primary branches	56.86	0.40	12.89
8.	Number of secondary branches	74.25	0.95	27.67
9.	Internodal length, cm	93.22	2.86	31.07
10.	Leaf petiole length, cm	96.02	3.09	28.74
11.	Leaf thickness, μ	99.04	79.42	24.06
12.	Days to first harvest	94.43	6.45	11.88
13.	Fruit length, cm	96.20	14.85	50.01
14.	Fruit girth, cm	96.68	12.26	44.19
15.	Fruit diameter, cm	97.74	4.29	46.90
16.	Average fruit weight, kg	94.74	0.83	80.65
17.	Dry matter content, %	84.11	3.61	6.63
18.	Fruits per plant	80.62	5.70	72.58
19.	Seeds per fruit	98.70	233.56	40.17
20.	1000 seed weight, g	97.83	9.53	49.62
21.	Yield per plant, kg	82.16	6.18	83.47

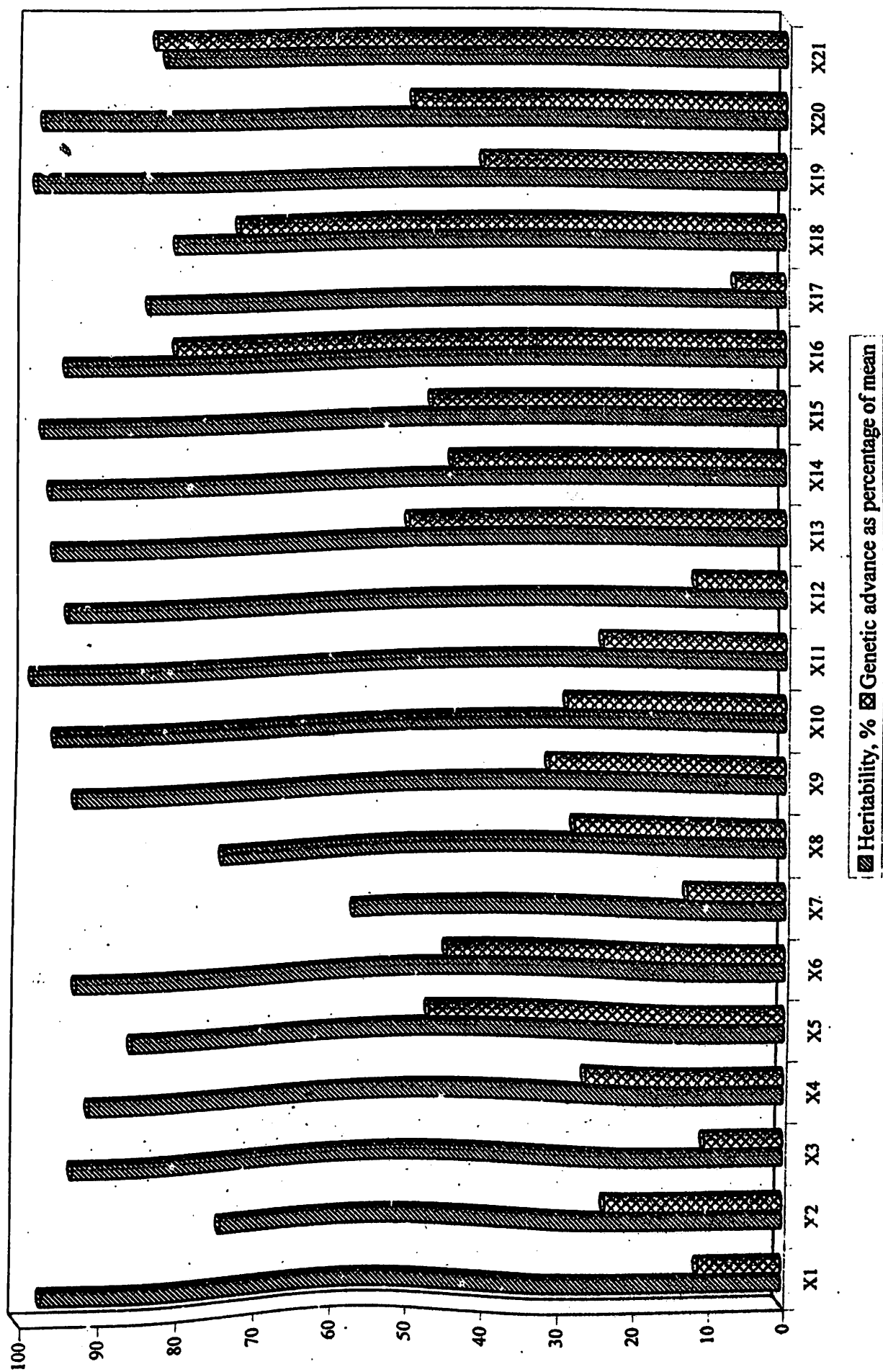


Fig. 2. Heritability and genetic advance as percentage of mean for various characters in landraces of melon.

4.1.4.2 Genetic Advance

Estimates of genetic advance was the highest for the traits seeds per fruit (233.56), vine length (89.41) and leaf thickness (79.42). Moderate estimates of genetic advance was observed for the traits like fruit length (14.85), sex ratio (13.76) and fruit girth (12.26). Rest of the traits recorded least estimates of genetic advance.

4.1.4.3 Genetic Advance as Percentage of Mean (Genetic gain)

Expected genetic advance as percentage of mean was maximum for yield per plant (83.47) followed by average fruit weight (80.65), fruits per plant (72.58), fruit length (50.01), 1000 seed weight (49.62), fruit diameter (46.90), sex ratio (46.76), vine length (44.43), fruit girth (44.19), leaf petiole length (28.74), number of secondary branches (27.67), node number of first female flower (26.05), leaf thickness (24.05) and node number of first male flower (23.52).

Moderate genetic advance as percentage of mean was obtained for the character number of primary branches (12.89) followed by days to first harvest (11.88), days to first male flower (11.14) and days to first female flower (10.46). Dry matter content (6.63) recorded comparatively lower value of genetic advance as percentage of mean.

4.1.5 Correlation Studies

The phenotypic, genotypic and environmental correlation coefficient between yield and its components and their inter correlation are presented in table 11, 12 and 13. The genotypic correlation coefficients were high for all the characters studied.

4.1.5.1 Correlation between Yield and Its Components

The characters which significantly contributed to yield were node number of first female flower, sex ratio, vine length, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruit. Number of secondary branches exhibited the highest positive and significant association with yield ($r_p = 0.642$, $r_g = 0.748$, $r_e = 0.268$) followed by average fruit weight ($r_p = 0.689$, $r_g = 0.739$, $r_e = 0.383$), fruits per plant ($r_p = 0.649$,

X1 – Days to first male flower

X2 – Node number of first male flower

X3 – Days to first female flower

X4 – Node number of first female flower

X5 – Sex ratio

X6 – Vine length

X7 – Number of primary branches

X8 – Number of secondary branches

X9 – Internodal length

X10 – Leaf petiole length

X11 – Leaf thickness

X12 – Days to first harvest

X13 – Fruit length

X14 – Fruit girth

X15 – Fruit diameter

X16 – Average fruit weight

X17 – Dry matter content

X18 – Fruits per plant

X19 – Seeds per fruit

X20 – 1000 seed weight

X21 – Yield per plant

Table 11. Phenotypic correlation matrix of various characters in landraces of melon

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21
X1	1.000																				
X2	0.497**	1.000																			
X3	0.277	0.084	1.000																		
X4	0.254	0.366*	0.511**	1.000																	
X5	0.028	0.152	-0.228	-0.102	1.000																
X6	0.016	-0.083	-0.315*	-0.295	0.098	1.000															
X7	0.096	0.006	-0.061	-0.193	0.035	0.090	1.000														
X8	-0.019	-0.016	-0.093	-0.122	0.489**	0.203	-0.002	1.000													
X9	0.087	0.135	-0.341*	-0.344*	0.356*	0.521**	0.213	0.147	1.000												
X10	0.094	0.022	-0.245	-0.173	-0.044	0.365*	-0.006	0.006	0.460**	1.000											
X11	-0.140	0.089	0.077	0.215	0.248	-0.257	-0.328*	0.236	-0.217	-0.441**	1.000										
X12	0.015	-0.060	0.322*	0.394**	-0.048	-0.290	-0.337*	0.066	-0.511**	-0.281	0.442**	1.000									
X13	-0.083	0.008	-0.233	-0.241	0.189	0.260	0.243	0.199	0.278	0.181	-0.059	-0.216	1.000								
X14	0.008	-0.119	-0.087	-0.200	-0.429**	0.250	0.134	-0.097	0.085	0.343*	-0.450**	-0.338*	-0.161	1.000							
X15	-0.031	-0.196	0.296	0.147	-0.537**	-0.022	0.017	-0.126	-0.372*	-0.040	-0.117	0.189	-0.299	0.772**	1.000						
X16	-0.217	-0.077	-0.062	-0.318*	0.035	0.155	0.082	0.327*	0.128	0.178	-0.148	-0.179	0.531**	0.248	0.122	1.000					
X17	-0.016	-0.059	0.095	0.318*	-0.191	-0.196	-0.340*	-0.055	-0.369*	-0.073	0.232	0.742**	-0.302	-0.116	0.199	-0.152	1.000				
X18	-0.079	0.071	-0.308*	-0.235	0.693**	0.228	0.006	0.501**	0.512**	0.100	0.149	-0.252	0.169	-0.155	-0.415**	-0.027	-0.243	1.000			
X19	-0.258	-0.101	-0.302	-0.265	0.189	0.422**	0.157	0.278	0.387*	0.470**	0.009	-0.209	0.668**	0.144	-0.008	0.576**	-0.220	0.182	1.000		
X20	-0.032	0.048	0.321*	0.307*	-0.035	-0.146	-0.377*	0.164	-0.242	-0.243	0.436**	0.413**	-0.266	-0.096	0.249	-0.206	0.270	-0.052	-0.189	1.000	
X21	-0.195	-0.020	-0.245	-0.383*	0.512**	0.262	0.052	0.642**	0.370*	0.174	0.051	-0.216	0.494**	0.087	-0.126	0.689**	-0.231	0.649**	0.548**	-0.096	1.000

* 5% significant; ** 1 % significant

X1 – Days to first male flower

X2 – Node number of first male flower

X3 – Days to first female flower

X4 – Node number of first female flower

X5 – Sex ratio

X6 – Vine length

X7 – Number of primary branches

X8 – Number of secondary branches

X9 – Internodal length

X10 – Leaf petiole length

X11 – Leaf thickness

X12 – Days to first harvest

X13 – Fruit length

X14 – Fruit girth

X15 – Fruit diameter

X16 – Average fruit weight

X17 – Dry matter content

X18 – Fruits per plant

X19 – Seeds per fruit

X20 – 1000 seed weight

X21 – Yield per plant

Table 12. Genotypic correlation matrix of various characters in landraces of melon

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21
X1	1.000																				
X2	0.560**	1.000																			
X3	0.300	0.095	1.000																		
X4	0.260	0.457**	0.555**	1.000																	
X5	0.016	0.213	-0.241	-0.123	1.000																
X6	0.028	-0.093	-0.347*	-0.295	0.104	1.000															
X7	0.161	0.022	-0.109	-0.205	0.021	0.121	1.000														
X8	-0.041	-0.000	-0.153	-0.168	0.544**	0.258	-0.079	1.000													
X9	0.094	0.161	-0.374*	-0.371*	0.426**	0.578**	0.269	0.172	1.000												
X10	0.105	0.036	-0.253	-0.176	-0.033	0.400**	-0.051	0.027	0.483**	1.000											
X11	-0.147	0.102	0.083	0.228	0.265	-0.266	-0.424**	0.268	-0.226	-0.449**	1.000										
X12	0.008	-0.076	0.334*	0.431**	-0.065	-0.306*	-0.473**	0.060	-0.549**	-0.284	0.446**	1.000									
X13	0.082	0.038	-0.255	-0.251	0.207	0.280	0.299	0.228	0.278	0.182	-0.059	-0.217	1.000								
X14	0.009	-0.132	-0.100	-0.207	-0.471**	0.269	0.130	-0.114	0.080	0.341*	-0.457**	-0.345*	-0.193	1.000							
X15	-0.038	-0.225	0.299	0.161	-0.584**	-0.015	-0.018	-0.150	-0.397**	-0.046	-0.123	0.193	-0.322*	0.775**	1.000						
X16	-0.238	-0.073	-0.087	-0.350*	0.045	0.174	0.101	0.353*	0.128	0.199	-0.151	-0.188	0.537**	0.251	0.119	1.000					
X17	-0.021	-0.045	0.087	0.387*	-0.218	-0.223	-0.564**	-0.107	-0.427**	-0.059	0.254	0.805**	-0.345*	-0.125	0.217	-0.206	1.000				
X18	-0.080	0.152	-0.347*	-0.264	0.750**	0.265	-0.041	0.612**	0.585**	0.108	0.157	-0.282	0.171	-0.187	-0.482**	-0.027	-0.289	1.000			
X19	-0.268	-0.109	-0.316*	-0.284	0.194	0.439**	0.211	0.309*	0.411**	0.488**	0.009	-0.222	0.691**	0.154	-0.006	0.599**	-0.244	0.207	1.000		
X20	-0.028	0.044	0.330*	0.316*	-0.044	-0.156	-0.480**	0.167	-0.257	-0.246	0.440**	0.423**	-0.272	-0.093	0.258	-0.029	0.307*	-0.069	-0.193	1.000	
X21	-0.234	0.013	-0.287	-0.439**	0.527**	0.310*	0.040	0.748**	0.417**	0.189	0.058	-0.232	0.511**	0.066	-0.159	0.739**	-0.262	0.647**	0.613**	-0.120	1.000

* 5% significant; ** 1 % significant

X1 – Days to first male flower

X2 – Node number of first male flower

X3 – Days to first female flower

X4 – Node number of first female flower

X5 – Sex ratio

X6 – Vine length

X7 – Number of primary branches

X8 – Number of secondary branches

X9 – Internodal length

X10 – Leaf petiole length

X11 – Leaf thickness

X12 – Days to first harvest

X13 – Fruit length

X14 – Fruit girth

X15 – Fruit diameter

X16 – Average fruit weight

X17 – Dry matter content

X18 – Fruits per plant

X19 – Seeds per fruit

X20 – 1000 seed weight

X21 – Yield per plant

Table 13. Environmental correlation matrix of various characters in landraces of melon

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21
X1	1.000																				
X2	0.249	1.000																			
X3	-0.163	0.038	1.000																		
X4	0.190	-0.054	0.004	1.000																	
X5	0.209	-0.090	-0.129	0.060	1.000																
X6	-0.217	-0.042	0.104	-0.296	0.054	1.000															
X7	-0.198	-0.024	0.109	-0.231	0.080	0.011	1.000														
X8	0.181	-0.059	0.255	0.105	0.288	-0.082	0.148	1.000													
X9	-0.054	0.008	0.112	-0.037	-0.244	-0.228	0.102	0.033	1.000												
X10	-0.224	-0.081	-0.109	-0.137	-0.176	-0.235	0.241	-0.162	0.069	1.000											
X11	0.199	0.027	-0.106	-0.060	0.125	-0.076	-0.161	0.126	0.001	-0.150	1.000										
X12	0.174	0.027	0.138	-0.073	0.121	-0.049	0.061	0.127	0.065	-0.242	0.444**	1.000									
X13	-0.104	-0.247	0.161	-0.096	0.015	-0.079	0.171	0.070	0.284	0.163	-0.059	-0.190	1.000								
X14	-0.009	-0.083	0.159	-0.111	-0.013	-0.094	0.319*	-0.012	0.203	0.396**	-0.148	-0.188	0.710**	1.000							
X15	0.237	-0.062	0.260	-0.092	-0.060	-0.188	0.306*	0.020	0.159	0.146	0.266	0.094	0.454**	0.653**	1.000						
X16	0.274	-0.136	0.312*	0.097	-0.061	-0.139	0.052	0.266	0.137	-0.269	-0.084	-0.013	0.416**	0.182	0.222	1.000					
X17	0.048	-0.117	0.169	-0.173	-0.040	0.009	0.192	0.149	0.085	-0.249	0.014	0.257	0.111	-0.047	0.041	0.350*	1.000				
X18	-0.104	-0.205	-0.066	-0.070	0.422**	-0.007	0.115	0.124	0.048	0.057	0.196	-0.057	0.214	0.125	0.204	-0.035	-0.028	1.000			
X19	0.168	-0.131	0.021	0.122	0.254	0.085	-0.012	0.233	-0.230	-0.197	-0.004	0.182	-0.246	-0.321*	-0.162	-0.134	0.060	-0.061	1.000		
X20	-0.191	0.144	0.151	0.194	0.093	0.082	-0.200	0.288	0.088	-0.183	0.185	0.169	-0.088	-0.217	-0.160	-0.131	-0.142	0.147	0.006	1.000	
X21	0.191	-0.138	0.051	-0.029	0.437**	-0.076	0.089	0.268	0.043	0.070	-0.032	-0.121	0.486**	0.373*	0.256	0.383*	-0.048	0.660**	-0.089	0.180	1.000

* 5% significant; ** 1 % significant

$r_g = 0.647$, $r_e = 0.660$), seeds per fruit ($r_p = 0.548$, $r_g = 0.613$, $r_e = -0.089$), sex ratio ($r_p = 0.512$, $r_g = 0.527$, $r_e = 0.437$), fruit length ($r_p = 0.494$, $r_g = 0.511$, $r_e = 0.486$), internodal length ($r_p = 0.370$, $r_g = 0.417$, $r_e = 0.043$) and vine length ($r_p = 0.262$, $r_g = 0.310$, $r_e = -0.076$). The character node number of first female flower exhibited negative association with yield ($r_p = -0.383$, $r_g = -0.439$, $r_e = -0.029$)

4.1.5.2 Inter Correlation among the Components

Days to first male flower exhibited significant association with yield through node number of first male flower ($r_g = 0.560$). Node number of first male flower exhibited significant association with yield through node number of first female flower ($r_g = 0.457$).

Days to first female flower exhibited significant positive association with yield through node number of first female flower, days to first harvest, and 1000 seed weight ($r_g = 0.555$, 0.334 and 0.330 respectively). Significant negative association was observed among vine length, internodal length and fruits per plant and seeds per fruit ($r_g = -0.347$, -0.374 , 0.347 and -0.316 respectively).

Node number of first female flower exhibited significant positive association with yield through days to first harvest, dry matter content and 1000 seed weight ($r_g = 0.431$, 0.387 , and 0.316 respectively). Negative correlation with internodal length and average fruit weight ($r_g = -0.371$ and -0.350) was significant.

Sex ratio was positively correlated with number of secondary branches, internodal length and fruits per plant ($r_g = 0.544$, 0.426 and 0.750 respectively). Significant negative association was noticed between fruit girth and fruit diameter ($r_g = -0.471$ and -0.584).

Vine length was positively correlated with internodal length, leaf petiole length and seeds per fruit ($r_g = 0.578$, 0.400 and 0.439 respectively). Significant negative association was noticed in days to first harvest ($r_g = -0.306$).

Number of primary branches exhibited significant negative association with yield through leaf thickness, days to first harvest, dry matter content and 1000 seed weight ($r_g = -0.424$, -0.473 , -0.564 and -0.480 respectively). Number

of secondary branches exhibited significant positive association with yield through average fruit weight, fruits per plant and seeds per fruit ($r_g = 0.353, 0.612$ and 0.309 respectively).

Internodal length exhibited significant positive association with yield through leaf petiole length, fruits per plant and seeds per fruit ($r_g = 0.483, 0.585$ and 0.411 respectively). Significant negative association was observed among fruit diameter and dry matter content ($r_g = -0.397$ and -0.427).

Leaf petiole length exhibited significant positive association with yield through seeds per fruit ($r_g = 0.488$). Significant negative association was observed in leaf thickness ($r_g = -0.449$). Leaf thickness exhibited significant positive association with yield through days to first harvest and 1000 seed weight ($r_g = 0.446$ and 0.440). Significant negative association was observed in fruit girth ($r_g = -0.457$).

Days to first harvest exhibited significant positive association with yield through dry matter content and 1000 seed weight ($r_g = 0.805$ and 0.423). Significant negative association was observed in fruit girth ($r_g = -0.345$).

Fruit length exhibited significant positive association with yield through average fruit weight and seeds per fruit ($r_g = 0.537$ and 0.691). Significant negative association was observed in fruit diameter and dry matter content ($r_g = -0.322$ and -0.345). Fruit girth was positively correlated with fruit diameter ($r_g = 0.775$). Fruit diameter was negatively correlated with fruits per plant ($r_g = -0.482$).

Average fruit weight exhibited significant positive correlation with yield through seeds per fruit ($r_g = 0.599$). Dry matter content was positively correlated with 1000 seed weight ($r_g = 0.307$).

4.1.6 Path Coefficient Analysis

The genotypic correlations among yield and its component characters were partitioned into different components to find out the direct and indirect Contribution of each character on yield per plant (Table 14 and Fig.3). The

Table 14. Direct and indirect effects of the component characters on yield in landraces of melon

Characters	Node number of first female flower	Sex ratio	Vine length, cm	Number of secondary branches	Internodal length, cm	Fruit length, cm	Average fruit weight, kg	Fruits per plant	Seeds per fruit	Genotypic correlation with yield
Node number of first female flower	-0.0290	-0.0040	-0.0020	-0.0120	0.0270	-0.0004	-0.2040	-0.1430	-0.0130	-0.4387**
Sex ratio	0.0030	0.0380	0.0010	0.0490	-0.0280	0.0003	0.0230	0.4180	0.0090	0.5274**
Vine length, cm	0.0080	0.0040	0.0070	0.0200	-0.0400	0.0004	0.0990	0.1390	0.0210	0.3104**
Number of secondary branches	0.0030	0.0190	0.0010	0.1000	-0.0110	0.0003	0.2100	0.3040	0.0140	0.7483**
Internodal length, cm	0.0100	0.0140	0.0040	0.0150	-0.0780	0.0004	0.0830	0.3060	0.0190	0.4178**
Fruit length, cm	0.0070	0.0070	0.0020	0.0200	-0.0210	0.0015	0.3410	0.1030	0.0330	0.5111**
Average fruit weight, kg	0.0090	0.0010	0.0010	0.0330	-0.0100	0.0008	0.6420	-0.0170	0.0290	0.7392**
Fruits per plant	0.0070	0.0260	0.0020	0.0500	-0.0390	0.0003	-0.0180	0.6070	0.0090	0.6472**
Seeds per fruit	0.0080	0.0070	0.0030	0.0280	-0.0300	0.0010	0.3700	0.1100	0.0500	0.6129**

Bold values indicate direct effects

Residual effect 0.0708

X1 – Node number of first female flower

X2 – Sex ratio

X3 – Vine length

X4 – Number of secondary branches

X5 – Internodal length

X6 – Fruit length

X7 – Average fruit weight

X8 – Fruits per plant

X9 – Seeds per fruit

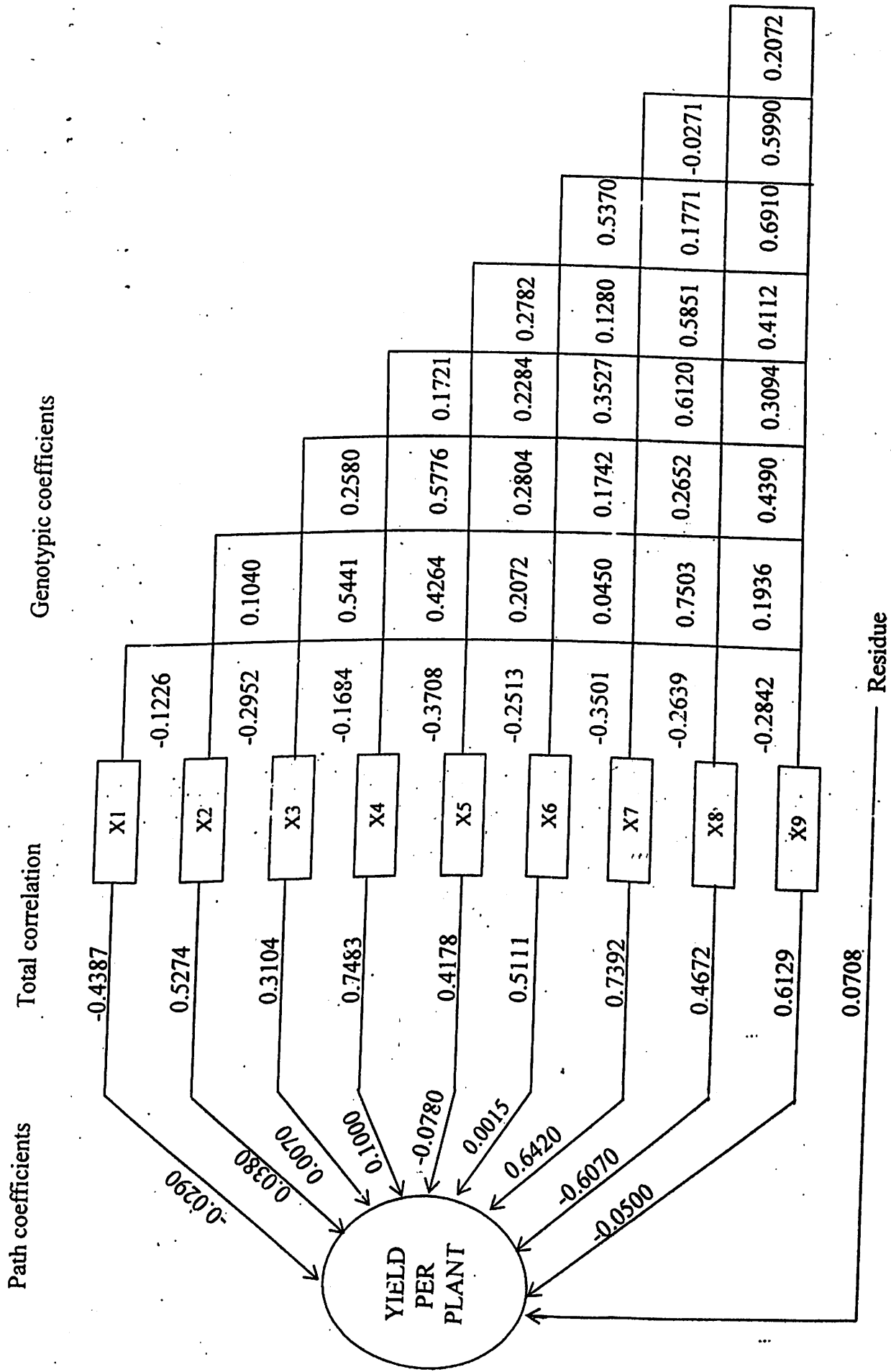


Fig. 3. Path diagram showing direct effects and interrelationships in landraces of melon

characters like node number of first female flower, sex ratio, vine length, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruit were selected in the present study for path coefficient analysis.

The path analysis revealed that average fruit weight exerted maximum positive direct effect on yield per plant (0.6420) followed by fruits per plant (0.6070). Number of secondary branches, seeds per fruit, sex ratio, vine length and fruit length also exerted positive direct effect on yield (0.1000, 0.0500, 0.0380, 0.0070 and 0.0015 respectively). Node number of first female flower and internodal length showed a negative significant direct effect on yield.

4.1.6.1 Indirect Effects

The indirect effects of node number of first female flower through average fruit weight, fruits per plant, seeds per fruit, number of secondary branches, sex ratio, vine length and fruit length was negative and high (–0.2040, –0.1430, –0.0130, –0.0120, –0.0040, –0.0020 and –0.0004 respectively). The node number of first female flower exerted a positive indirect effect through internodal length (0.0270).

Though the positive indirect effect of sex ratio through fruits per plant, number of secondary branches, average fruit weight, seeds per fruit, node number of first female flower, vine length and fruit length (0.4180, 0.0490, 0.0230, 0.0090, 0.0030, 0.0010 and 0.0003 respectively) was positive. Sex ratio exerted the indirect negative effect through internodal length (–0.0280).

Vine length exerted a positive indirect effect through fruits per plant, average fruit weight, seeds per fruit, number of secondary branches, node number of first female flower, sex ratio and fruit length (0.1390, 0.0990, 0.0210, 0.0200, 0.0080, 0.0040 and 0.0004 respectively). Vine length exerted a negative indirect effect through internodal length (–0.0400).

Path analysis revealed that number of secondary branches exerted a positive indirect effect through fruits per plant, average fruit weight, sex ratio, seeds per fruit, node number of first female flower, vine length and fruit length (0.3040,

0.2100, 0.0190, 0.0140, 0.0030, 0.0010 and 0.0003 respectively). Number of secondary branches exerted a negative indirect effect through internodal length (-0.0110).

The indirect effect of internodal length through fruits per plant, average fruit weight, seeds per fruit, number of secondary branches, sex ratio, node number of first female flower, vine length and fruit length was positive (0.3060, 0.0830, 0.0190, 0.0150, 0.0140, 0.0100, 0.0040 and 0.0004 respectively).

From the path analysis, it was revealed that fruit length exerted a positive indirect effect through average fruit weight, fruits per plant, seeds per fruit, number of secondary branches, node number of first female flower, sex ratio and vine length (0.3410, 0.1030, 0.0330, 0.0200, 0.0070, 0.0070 and 0.0020 respectively). Negative indirect effect of fruit length was obtained in internodal length (-0.0210).

The indirect effect of average fruit weight through number of secondary branches, seeds per fruit, node number of first female flower, sex ratio, vine length and fruit length was positive (0.0330, 0.0290, 0.0090, 0.0010, 0.0010 and 0.0008 respectively). Average fruit weight exerted a negative indirect effect through fruits per plant (-0.0170) and internodal length (-0.0100).

Path analysis revealed that fruits per plant exerted a maximum positive indirect effect through number of secondary branches (0.0500) followed by sex ratio (0.0260) seeds per fruit (0.0090), node number of first female flower (0.0070), vine length (0.0020) and fruit length (0.0003). It exerted a maximum negative indirect effect through internodal length (-0.0390) followed by average fruit weight (-0.0180).

Seeds per fruit exerted a positive indirect effect through average fruit weight (0.3700), fruits per plant (0.1100), number of secondary branches (0.0280), node number of first female flower (0.0080), sex ratio (0.0070), vine length (0.0030) and fruit length (0.0010). The negative indirect effect of seeds per fruit was through internodal length (-0.0300).

The residual effect due to the unknown causal factors influencing yield per plant was 0.0708, indicating that the characters holding important role in determining the total fruit yield are included in the present study.

4.1.7 Cluster Analysis Through Mahalanobis D^2 Analysis

By the application of clustering technique, the 40 genotypes *Cucumis melo* were grouped into several clusters and the results are presented below.

4.1.7.1 Group Constellations

The forty *Cucumis melo* accessions were grouped into twenty clusters (Rao, 1952). The clustering pattern is given in table 15.

The cluster I the largest having three genotypes and the cluster ranging from II to XIX having two genotypes each. Cluster XX was smallest with only a single genotype.

The cluster I genotypes contained which high (CM 1) to moderate yielding, earliest female flowering (CM 21) and with medium length (CM 30). Cluster II were moderate yielder (CM 11, CM 29). Cluster III had genotypes with high sex ratio and number of fruits per plant (CM 15) and high internodal length (CM 35). Cluster IV contained genotypes with moderate yielders (CM 17, CM 40) and long internodes (CM 17). The cluster V included genotypes with medium length of fruit (CM 33, CM 37). Cluster VI contained highest yielder with highest fruit weight (CM 32, CM 20). The cluster VII included poor yielder with smallest plants (CM 18, CM 31). Cluster VIII contained poor yielder (CM 13, CM 23) and long fruits (CM 13). Cluster IX contained moderate yielder (CM 3, CM 14). Cluster X contained poor yielder (CM 19) and XI contained moderate yielder with earlier female flowering (CM 16, CM 27). The cluster XII contained moderate yielder with more seed weight (CM 8) and poor yielder with early yielder (CM 22). The cluster XIII contained high yielders (CM 36, CM 28), earlier female flowering with earliest yielder (CM 36). The cluster XIV contained poor yielder, shortest plants with rounded fruits (CM 2, CM 5) (Plate 4). The cluster XV contained moderate yielder with more number of fruits (CM 12, CM 26). The cluster XVI contained highest sex ratio and longest fruit (CM 4, CM 38). The

Table 15. Composition of D^2 cluster in landraces of melon

Cluster Number	Number of genotypes	Name of the genotypes
I	3	CM1, CM21, CM30
II	2	CM11, CM29
III	2	CM15, CM35
IV	2	CM17, CM40
V	2	CM33, CM37
VI	2	CM20, CM32
VII	2	CM18, CM31
VIII	2	CM13, CM23
IX	2	CM3, CM14
X	2	CM19, CM24
XI	2	CM16, CM27
XII	2	CM8, CM22
XIII	2	CM28, CM36
XIV	2	CM2, CM5
XV	2	CM12, CM26
XVI	2	CM4, CM38
XVII	2	CM9, CM10
XVIII	2	CM6, CM35
XIX	2	CM7, CM25
XX	1	CM39



Plate 4. Immature melon fruit of genotype CM 2

cluster XVII contained high yielder with fruit weight (CM 9, CM 10). The cluster XVIII contained poor yielder with late female flowering (CM 6, CM 35). The cluster XIX contained poor yielder with earlier female flowering (CM 7) and moderate yielder with high fruit weight (CM 25). The cluster XX was unique with single genotype (CM 39) which is poor yield with longest vines.

The intra and inter cluster D^2 values are presented in table 16. The intra cluster distance (D^2) was highest in cluster XIX (1851.161) followed by cluster XVIII (1385.377) and cluster XVII (1236.628). The inter cluster distance was maximum between cluster XVIII and XX followed by clusters XVII and XVIII (6829.191) and IX and XX (6599.451).

The genetic distance between cluster II and IX was minimum (496.405). With the help of average inter cluster D^2 values, cluster diagram showing the inter relationship has been prepared (Fig. 4).

4.2 BIOCHEMICAL CHARACTERIZATION

Biochemical characterization of forty landraces of melon was carried out by using SDS-PAGE method.

4.2.1 Protein Analysis

Total soluble protein content of forty landraces of melon seed was estimated by Bradford (1976) method (Table 17).

The protein yield of 40 landraces of melon ranged from $0.858 \mu\text{g seed}^{-1}$ (CM10) to $3.200 \mu\text{g seed}^{-1}$ (CM 20).

4.2.2 Electrophoretic Analysis of Protein

The electrophoretic assay of protein samples were carried out using 15% polyacrylamide gel consistency. For each lane $100\mu\text{g}$ of protein was loaded.

Totally, twenty protein bands (18 bands) were produced in the landraces of melons. Based on their total bands, maximum number of 18 bands were present in CM 7 and CM 40. 17 bands were obtained in CM 10 and CM 37. 16 bands were seen in CM 12, CM 16, CM 22 and CM 38. CM 24 and CM 31 produced

Table 16. Intra and Inter cluster distance in landraces of melon

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
I	23.215 (538.920)	30.475 (928.712)	24.174 (584.396)	32.652 (1066.185)	40.574 (1646.246)	27.385 (749.935)	24.735 (611.833)	32.328 (1045.094)	37.483 (1404.938)	27.294 (744.963)	41.648 (1734.576)	29.884 (893.036)	29.776 (886.592)	58.558 (3428.986)	41.556 (1726.879)	52.089 (2713.260)	44.446 (1975.465)	50.283 (2528.340)	31.183 (972.362)	56.333 (3173.396)
II		14.968 (224.055)	23.578 (555.899)	30.561 (933.986)	22.834 (521.414)	30.032 (901.931)	28.521 (813.446)	30.902 (954.929)	22.280 (496.405)	46.024 (2118.248)	26.525 (703.561)	30.382 (923.042)	24.566 (1194.784)	52.224 (2727.343)	25.997 (675.837)	45.734 (2091.616)	51.853 (2688.701)	50.014 (2501.423)	40.471 (1637.933)	71.582 (5124.043)
III			16.146 (260.698)	29.399 (864.307)	34.217 (1170.802)	30.854 (951.953)	22.795 (519.607)	26.684 (712.012)	25.882 (669.861)	33.424 (1117.149)	32.359 (1047.080)	27.639 (763.924)	27.460 (754.029)	53.985 (2914.427)	35.294 (1245.683)	53.025 (2811.671)	51.861 (2689.613)	44.897 (2015.710)	30.878 (953.429)	63.377 (4016.586)
IV				16.925 (286.450)	32.117 (1031.508)	23.415 (548.263)	27.008 (729.452)	47.839 (2288.575)	40.744 (1660.076)	40.683 (1655.076)	30.301 (918.151)	33.224 (1103.832)	31.216 (974.417)	52.899 (2798.346)	40.080 (1606.397)	51.697 (2672.568)	47.541 (2260.163)	59.752 (3570.306)	42.084 (1771.063)	50.734 (2573.909)
V					16.981 (288.348)	24.422 (1184.880)	38.836 (1508.209)	44.680 (1996.292)	30.266 (916.033)	53.078 (2817.223)	24.971 (623.532)	32.050 (1027.172)	43.828 (1920.907)	51.554 (2657.821)	27.285 (744.444)	53.328 (2843.879)	61.706 (3807.610)	51.405 (2642.467)	47.541 (2260.104)	75.178 (5651.672)
VI						18.014 (324.521)	26.953 (726.440)	42.614 (1815.913)	40.741 (1659.845)	36.962 (1366.180)	36.084 (1302.075)	32.667 (1067.110)	30.117 (907.023)	56.034 (3139.787)	38.837 (1508.295)	45.254 (2047.920)	40.322 (3559.008)	58.046 (3369.360)	39.727 (1578.205)	53.886 (2903.664)
VII							18.705 (349.876)	33.953 (1152.787)	34.439 (1186.037)	33.670 (1133.676)	33.083 (1094.510)	35.574 (1265.474)	26.274 (690.316)	52.150 (2757.288)	44.400 (1971.397)	54.644 (2985.922)	45.444 (2065.150)	56.510 (3193.389)	32.173 (1035.125)	53.821 (2896.658)
VIII								22.192 (492.498)	28.985 (840.112)	42.046 (1767.857)	43.682 (1908.080)	37.719 (1422.719)	39.169 (1534.180)	59.606 (3552.826)	39.893 (1591.420)	58.701 (3445.782)	59.657 (3559.008)	44.581 (1987.450)	36.421 (1326.507)	77.798 (6052.593)
IX									23.581 (556.072)	50.743 (2574.882)	29.997 (899.847)	36.261 (1314.832)	40.369 (1629.623)	56.356 (3175.975)	33.126 (1097.334)	54.893 (3013.195)	61.359 (3764.897)	48.289 (2331.844)	41.748 (1742.884)	81.237 (6599.451)
X										24.020 (576.941)	53.278 (2838.565)	35.091 (1231.393)	37.399 (1398.716)	66.453 (4415.960)	53.411 (2852.733)	65.290 (4262.808)	53.455 (2857.480)	49.482 (2448.460)	32.672 (1067.444)	48.803 (2381.725)
XI											25.634 (657.097)	38.333 (1469.402)	40.905 (1673.225)	48.387 (2341.319)	37.154 (1380.434)	59.798 (3575.848)	62.720 (3933.746)	56.816 (3228.018)	45.259 (2048.413)	72.527 (5260.176)
XII												27.966 (782.087)	38.366 (1471.963)	57.902 (3352.672)	32.317 (1044.413)	56.612 (3204.946)	58.394 (3409.891)	39.287 (1543.496)	36.638 (1342.356)	64.720 (4188.653)
XIII													28.172 (793.669)	55.828 (3116.763)	46.627 (2174.117)	54.943 (3018.737)	46.383 (2151.372)	60.024 (3602.871)	35.489 (1259.500)	53.935 (2909.026)
XIV														29.005 (841.287)	57.128 (3263.585)	81.085 (6574.709)	78.733 (6198.963)	71.039 (5046.518)	59.684 (3562.136)	78.105 (6100.381)
XV															30.689 (941.823)	49.454 (2445.687)	62.604 (3919.308)	46.030 (2118.750)	50.288 (2528.875)	80.458 (6473.499)
XVI																31.652 (1001.831)	42.081 (1770.774)	75.780 (5742.537)	67.857 (4604.539)	80.873 (6540.442)
XVII																	35.166 (1236.628)	82.639 (6829.191)	59.590 (3550.936)	59.235 (3508.806)
XVIII																		37.221 (1385.377)	49.717 (2471.804)	86.943 (7559.050)
XIX																			43.025 (1851.161)	62.302 (3881.517)
XX																				0.000 (0.000)

Intra and Inter cluster D² value in paranthesis

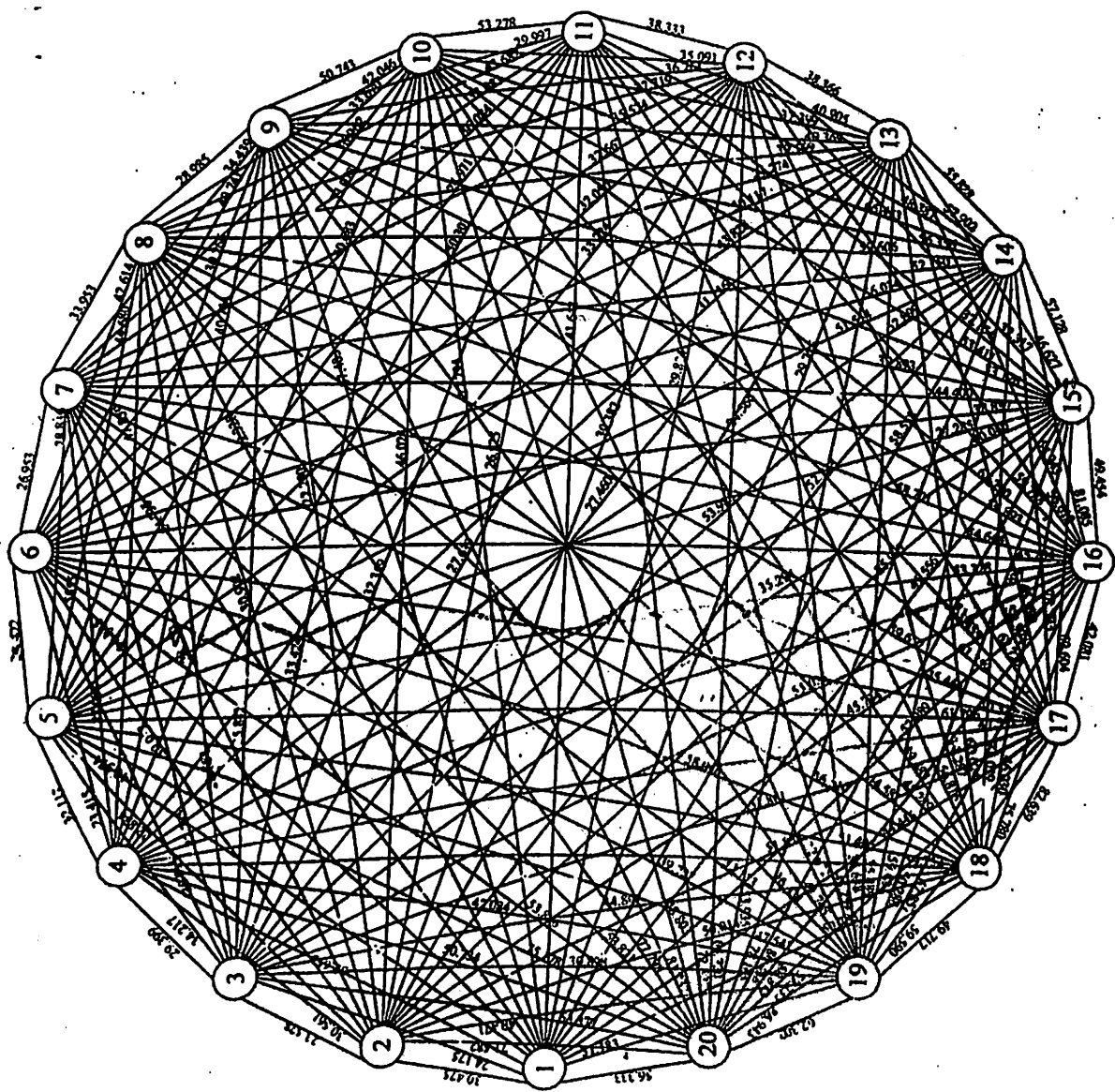


Fig. 4. Diagrammatic representation of clustering of 40 genotypes of melon (*Cucumis melo* L.)

Table 17. List of protein quantity and number of bands present for electrophoresis of various protein samples of melon

S. No.	Genotypes	Protein quantity, $\mu\text{g seed}^{-1}$	Number of bands present
1.	CM 1	2.039	14
2.	CM 2	2.273	5
3.	CM 3	2.212	11
4.	CM 4	1.692	11
5.	CM 5	2.222	11
6.	CM 6	1.937	13
7.	CM 7	1.733	18
8.	CM 8	2.385	12
9.	CM 9	1.988	12
10.	CM 10	0.858	17
11.	CM 11	2.609	13
12.	CM 12	2.253	16
13.	CM 13	2.222	13
14.	CM 14	2.253	9
15.	CM 15	2.619	10
16.	CM 16	1.957	16
17.	CM 17	2.640	12
18.	CM 18	2.548	12
19.	CM 19	2.090	13
20.	CM 20	3.200	11
21.	CM 21	2.202	13
22.	CM 22	1.906	16
23.	CM 23	1.101	14
24.	CM 24	1.611	15
25.	CM 25	1.367	10
26.	CM 26	1.703	13
27.	CM 27	1.652	8
28.	CM 28	1.550	14
29.	CM 29	2.008	11
30.	CM 30	1.937	14
31.	CM 31	1.978	15
32.	CM 32	2.446	14
33.	CM 33	1.570	13
34.	CM 34	1.244	10
35.	CM 35	2.120	12
36.	CM 36	1.631	14
37.	CM 37	1.815	17
38.	CM 38	1.611	16
39.	CM 39	1.051	8
40.	CM 40	1.652	18

15 bands. 14 bands were produced in CM 1, CM 23, CM 28, CM 30 and CM 32. CM 6, CM 11, CM 13, CM 19, CM 21, CM 26, CM 33 and CM 36 gave 13 bands each. 12 bands were obtained in CM 8, CM 9, CM 17, CM 18 and CM 35. CM 3, CM 4, CM 5, CM 20 and CM 29 showed 11 bands each. 10 bands were produced in CM 15, CM 25 and CM 34. Nine bands were seen in CM 14, 8 bands were obtained in CM 27 and CM 39. CM 2 gave minimum number of 5 bands (Plate 5).

4.2.3 Data Analysis

The banding pattern from protein analysis for each genotype was scored by visual observation. Reproducible bands were scored for their presence (1) or absence (0) for all the *Cucumis melo* accessions studied. The protein analysis data were subjected to cluster analysis using NTSYS program for all the accessions, to estimate similarity indices and genetic relatedness among the accessions. The similarity index (SI) values were computed as a ratio of number of similar bands to the total number in pair wise comparison of the accessions. The SI values obtained for each pair wise of protein bands among the 40 landraces of melon are shown in table 18. Based on the protein analysis data, the genetic distances were used to construct a dendrogram for all the 40 accessions following the UPMGA method using NTSYS program (Rolf, 1997). Option SAHN was performed which resulted in the dendrogram shown in Fig. 5.

Forty landraces of melon were grouped into two major clusters namely, A and B. The major cluster A was comprised of 39 accessions *viz.*, CM 1, CM 3, CM 4, CM 5, CM 6, CM 7, CM 8, CM 9, CM 10, CM 11, CM 12, CM 13, CM 14, CM 15, CM 16, CM 17, CM 18, CM 19, CM 20, CM 21, CM 22, CM 23, CM 24, CM 25, CM 26, CM 27, CM 28, CM 29, CM 30, CM 31, CM 32, CM 33, CM 34, CM 35, CM 36, CM 37, CM 38, CM 39 and CM 40. The major cluster B has CM 2.

The cluster A was divided into two minor clusters namely, A1 and A2. The minor cluster A1 consisted of 7 accessions *viz.*, CM 1, CM 29, CM 8, CM 3, CM 17, CM 34 and CM 39.

Lanc M – Protein marker
Lane 1 – Protein profile of CM 1 genotype
Lane 2 – Protein profile of CM 2 genotype
Lane 3 – Protein profile of CM 3 genotype
Lane 4 – Protein profile of CM 4 genotype
Lane 5 – Protein profile of CM 5 genotype
Lane 6 – Protein profile of CM 6 genotype
Lane 7 – Protein profile of CM 7 genotype
Lane 8 – Protein profile of CM 8 genotype
Lane 9 – Protein profile of CM 9 genotype
Lane 10 – Protein profile of CM 10 genotype
Lane 11 – Protein profile of CM 11 genotype
Lane 12 – Protein profile of CM 12 genotype
Lane 13 – Protein profile of CM 13 genotype
Lane 14 – Protein profile of CM 14 genotype
Lane 15 – Protein profile of CM 15 genotype
Lane 16 – Protein profile of CM 16 genotype
Lane 17 – Protein profile of CM 17 genotype
Lane 18 – Protein profile of CM 18 genotype
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Lane 34 – Protein profile of CM 34 genotype
Lane 35 – Protein profile of CM 35 genotype
Lane 36 – Protein profile of CM 36 genotype
Lane 37 – Protein profile of CM 37 genotype
Lane 38 – Protein profile of CM 38 genotype
Lane 39 – Protein profile of CM 39 genotype
Lane 40 – Protein profile of CM 40 genotype

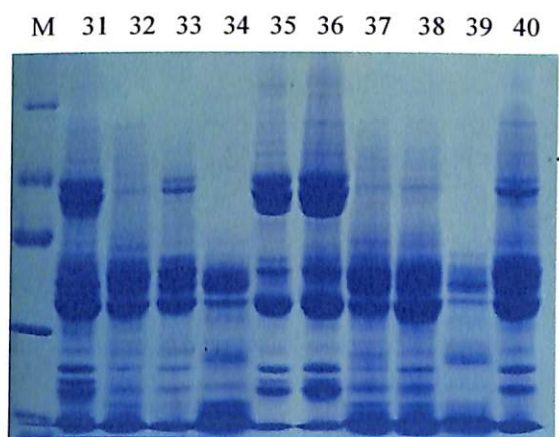
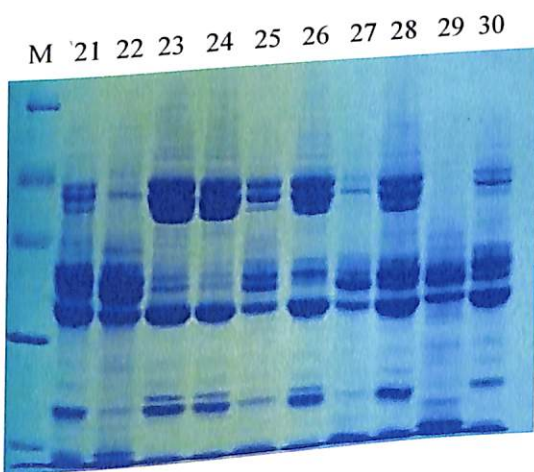
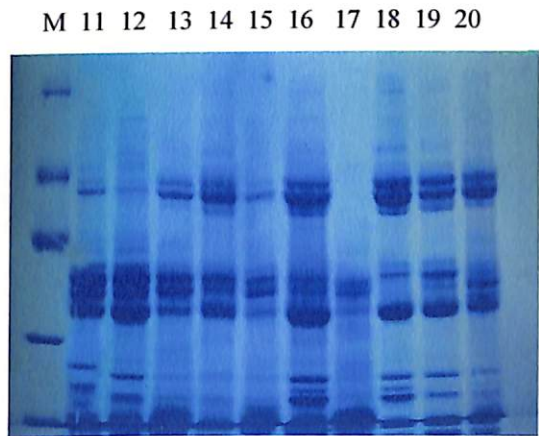
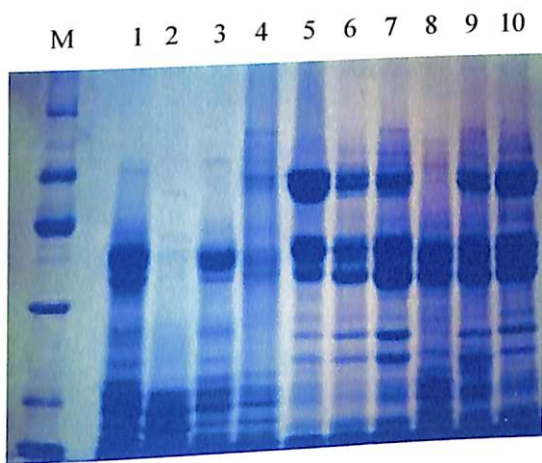


Plate 5. Electrophoretic pattern of protein in forty *Cucumis melo* genotypes

CM 1	1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				</
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Table 18. Similarity matrix for the landraces of melon generated using protein analysis

Table 18. Similarity matrix for the landraces of melon generated using protein analysis

C1 represent CM 1 genotype
C2 represent CM 2 genotype
C3 represent CM 3 genotype
C4 represent CM 4 genotype
C5 represent CM 5 genotype
C6 represent CM 6 genotype
C7 represent CM 7 genotype
C8 represent CM 8 genotype
C9 represent CM 9 genotype
C10 represent CM 10 genotype
C11 represent CM 11 genotype
C12 represent CM 12 genotype
C13 represent CM 13 genotype
C14 represent CM 14 genotype
C15 represent CM 15 genotype
C16 represent CM 16 genotype
C17 represent CM 17 genotype
C18 represent CM 18 genotype
C19 represent CM 19 genotype
C20 represent CM 20 genotype
C21 represent CM 21 genotype
C22 represent CM 22 genotype
C23 represent CM 23 genotype
C24 represent CM 24 genotype
C25 represent CM 25 genotype
C26 represent CM 26 genotype
C27 represent CM 27 genotype
C28 represent CM 28 genotype
C29 represent CM 29 genotype
C30 represent CM 30 genotype
C31 represent CM 31 genotype
C32 represent CM 32 genotype
C33 represent CM 33 genotype
C34 represent CM 34 genotype
C35 represent CM 35 genotype
C36 represent CM 36 genotype
C37 represent CM 37 genotype
C38 represent CM 38 genotype
C39 represent CM 39 genotype
C40 represent CM 40 genotype

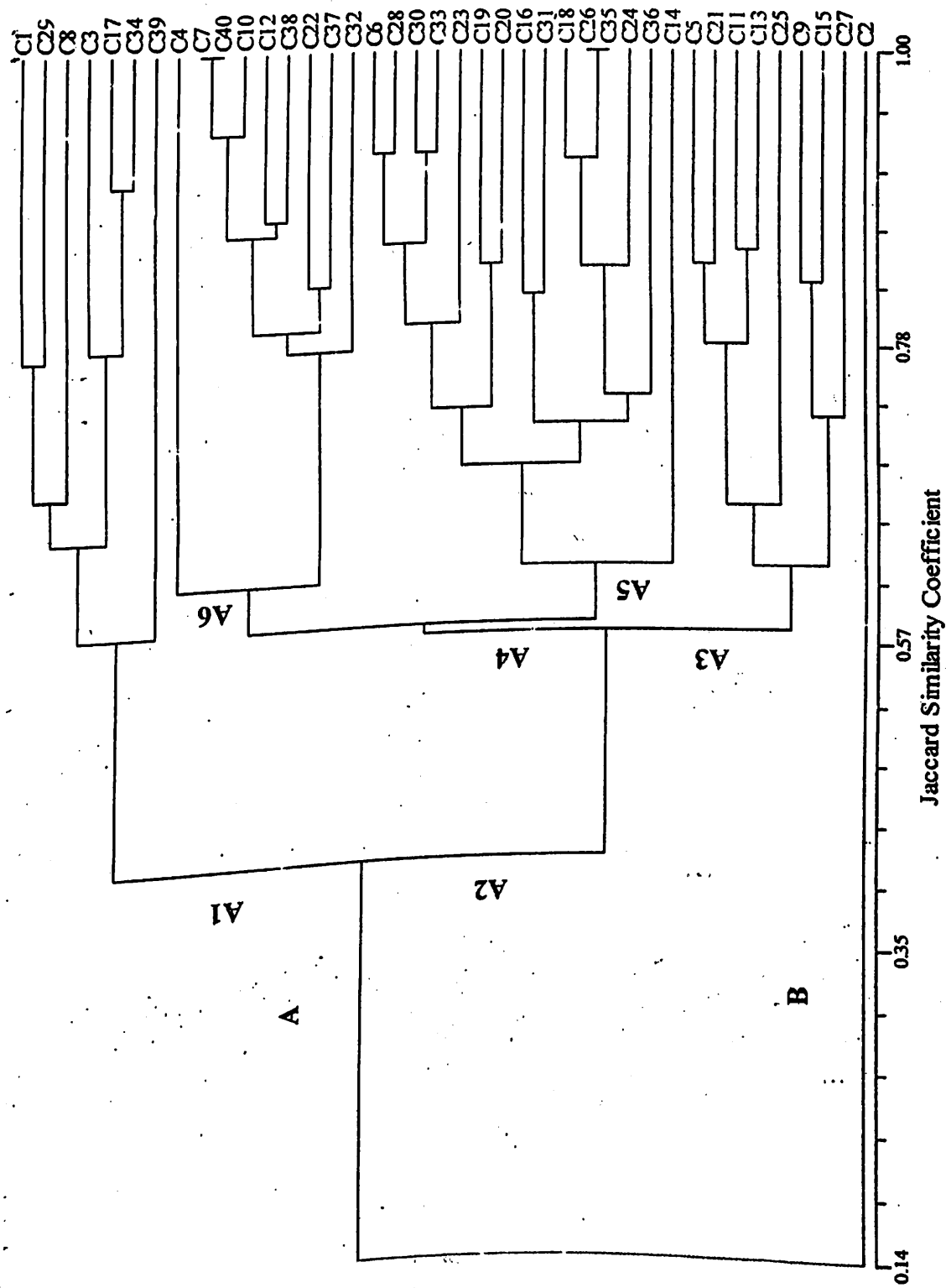


Fig. 5. Dendrogram for forty landraces of melon based on data from protein analysis

Cluster A2 was subdivided into cluster A3 and A4. Cluster A3 consisted of 8 accessions *viz.*, CM 27, CM 15, CM 9, CM 25, CM 13, CM 11, CM 21 and CM 5. Sub cluster A4 was divided into two minor clusters A5 and A6.

Subcluster A5 comprised of 15 accessions *viz.*, CM 14, CM 36, CM 24, CM 35 CM 26, CM 18, CM 31, CM 16, CM 20, CM 19, CM 23, CM 33, CM 30, CM 28 and CM 6. Sub cluster A6 consisted of 9 accessions *viz.*, CM 32, CM 37, CM 22, CM 38, CM 12, CM 10, CM 40, CM 7 and CM 4.

Within subcluster A1, all the accessions showed a similarity ranging from 57.1 to 90.9 per cent. Of these accessions CM 39 was most distinctly related to CM 1, sharing only 57.1 per cent similarity at level.

In sub cluster A3, CM 27 showed a 58.3 per cent similarity with CM 5. Within the subcluster A5, CM 36 was distinctly related with CM 6, sharing only 52.9 per cent similarity. In subcluster A6, CM 10 widely diversified from CM 4, sharing only 55.6 per cent similarity.

In major cluster A, CM 1 showed 37.5 per cent similarity with CM 27. CM 2 was grouped in the other major cluster B. On comparison among the cluster

A and B, it can be seen that CM 1 shows a similarity of only 13.3 per cent with CM 2.

4.3 MOLECULAR CHARACTERIZATION

The study was made to assess the extent of genetic diversity among forty landraces of melon from diverse ecosystems using RAPD markers.

4.3.1 Isolation of Genomic DNA

Etiolated 15-20 days old seedlings were used to extract genomic DNA. DNA was isolated from the various landraces of melon following the protocol standardized by Baudracco-Arnas (1995) and Brown *et al.* (1998).

The DNA yield of forty cucumber accessions ranged from 15 to 6985 $\mu\text{g ml}^{-1}$. The purity of DNA ($\text{OD}_{260}/\text{OD}_{280}$) (Table 19) ranged from 1.08 to 2.03.

Table 19. Quality and quantity of DNA isolated from landraces of melon using modified Baurdracco - Arnas method

S. No.	Sample No.	260nm	280nm	Ratio $\left(\frac{260}{280}\right)$	DNA yield, $\mu\text{g ml}^{-1}$
1.	CM 1	0.003	0.002	1.5	15
2.	CM 2	0.010	0.006	1.67	50
3.	CM 3	0.007	0.004	1.75	35
4.	CM 4	0.013	0.007	1.86	65
5.	CM 5	1.397	1.288	1.08	6985
6.	CM 6	0.009	0.005	1.8	45
7.	CM 7	0.006	0.004	1.5	30
8.	CM 8	0.021	0.012	1.75	105
9.	CM 9	0.008	0.006	1.33	40
10.	CM 10	0.005	0.003	1.67	25
11.	CM 11	0.037	0.020	1.85	185
12.	CM 12	0.038	0.021	1.81	190
13.	CM 13	0.007	0.005	1.4	35
14.	CM 14	0.020	0.012	1.67	100
15.	CM 15	0.019	0.011	1.82	95
16.	CM 16	0.009	0.007	1.29	45
17.	CM 17	0.012	0.009	1.33	60
18.	CM 18	0.032	0.018	1.78	160
19.	CM 19	0.041	0.022	1.86	205
20.	CM 20	0.020	0.013	1.54	100
21.	CM 21	0.035	0.021	1.67	175
22.	CM 22	0.019	0.013	1.46	95
23.	CM 23	0.006	0.004	1.5	30
24.	CM 24	0.031	0.016	1.94	155
25.	CM 25	0.022	0.012	1.83	110
26.	CM 26	0.034	0.019	1.79	170
27.	CM 27	0.006	0.004	1.5	30
28.	CM 28	0.065	0.032	2.03	325
29.	CM 29	0.010	0.006	1.67	50
30.	CM 30	0.009	0.005	1.8	45
31.	CM 31	0.011	0.007	1.57	55
32.	CM 32	0.032	0.019	1.68	160
33.	CM 33	0.018	0.011	1.67	90
34.	CM 34	0.017	0.011	1.54	85
35.	CM 35	0.012	0.007	1.71	60
36.	CM 36	0.015	0.008	1.88	75
37.	CM 37	0.014	0.007	2.00	70
38.	CM 38	0.029	0.019	1.53	145
39.	CM 39	0.022	0.013	1.69	110
40.	CM 40	0.020	0.011	1.82	100

4.3.2 Testing the Quality of DNA

For RAPD profile analysis, the DNA should be free of RNA and protein. Moreover, it needs intact, unsheared DNA sample of sufficient quantity. To access the quality, all the genomic DNA samples were run on 0.9% agarose gel and the gel was stained with ethidium bromide and bands appeared in the gel were documented, using ultraviolet transilluminator.

4.3.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction, standardized for the amplification of the DNA from *Cucumis melo* L. (Staub *et al.*, 2000) was used for forty landraces of melons. The 15 µl reaction mixture consisting of 1.5 µl 10x Taq buffer (10mM Tris-HCl, pH 9.0, 1.50 mM KCl and 0.01% gelatin), 0.05 mM each of dNTPs, 1 unit of Taq DNA polymerase, 5.0 pM of primer and 10ng of DNA gave good amplification. The programme consisted of an initial denaturation at 94°C for 4 minutes, followed by 3 cycles of denaturation at 94°C for 15 sec, annealing at 35°C for 15 sec and extension at 72°C for 75 sec, then 40 cycles of denaturation at 94°C for 15 sec, annealing at 40°C for 15 sec, and extension at 72°C for 75 sec. The synthesis step of the final cycle was extended further by 7 minutes. The amplification products were cooled to 4°C after the reaction.

Forty six decamer primers were screened for their efficiency using the DNA isolated from CM 28 as the representative sample. Out of the 46 decamer primers, 31 yielded amplification products. There was no amplification with the primers like OPA-13, OPA-16, OPA-17, OPB-03, OPB-09, OPB-10, OPB-15, OPB-17, OPB-18, OPAN-05, OPN-08, OPE-07, OPG-17, OPAL-10 and OPL-19. The total number of bands, number of faint bands and the number of intense bands produced by the primers are given in table 20.

The primers amplified 103 bands (2.24 bands per primer) of which 96.12% (99 bands) were polymorphic and four bands (3.88%) were monomorphic. Monomorphic bands were produced by the primers OPA-15, OPB-01, OPB-07 and OPB-19.

Table 20. Primer associated banding patterns with the DNA of CM 28 using 46 primers supplied by the Operon Inc, CA, USA

S. No.	Primers	Total number of bands	Number of intense bands	Number of faint Bands
1.	OPA – 01	6	4	2
2.	OPA – 02	5	3	2
3.	OPA – 03	2	2	0
4.	OPA – 04	5	3	2
5.	OPA – 05	2	0	2
6.	OPA – 06	3	2	1
7.	OPA – 07	4	3	1
8.	OPA – 08	5	4	1
9.	OPA – 09	4	3	1
10.	OPA – 10	4	3	1
11.	OPA – 11	3	2	1
12.	OPA – 12	4	2	2
13.	OPA – 13	0	0	0
14.	OPA – 14	4	3	1
15.	OPA – 15	1	1	0
16.	OPA – 16	0	0	0
17.	OPA – 17	0	0	0
18.	OPA – 18	4	2	2
19.	OPA – 19	2	0	2
20.	OPA – 20	2	1	1
21.	OPB – 01	1	0	1
22.	OPB – 02	2	0	2
23.	OPB – 03	0	0	0
24.	OPB – 04	3	2	1
25.	OPB – 05	3	2	1
26.	OPB – 06	2	1	1
27.	OPB – 07	1	1	0
28.	OPB – 08	4	3	1
29.	OPB – 09	0	0	0
30.	OPB – 10	0	0	0
31.	OPB – 11	2	0	2
32.	OPB – 12	5	4	1
33.	OPB – 13	3	2	1
34.	OPB – 14	4	1	3
35.	OPB – 15	0	0	0
36.	OPB – 16	5	3	2
37.	OPB – 17	0	0	0
38.	OPB – 18	0	0	0
39.	OPB – 19	1	1	0
40.	OPB – 20	7	5	2
41.	OPN – 08	0	0	0
42.	OPE – 07	0	0	0
43.	OPG – 17	0	0	0
44.	OPL – 19	0	0	0
45.	OPAL – 10	0	0	0
46.	OPAN – 05	0	0	0

The maximum number of RAPDs (7 bands) were produced by the primer OPB-20; six RAPDs were produced by the primer OPA-01 and five RAPDs were produced by the primers OPA-02, OPA-04, OPA-08, OPB-12 and OPB-16. OPA-07, OPA-09, OPA-10, OPA-12, OPA-14, OPA-18, OPB-08 and OPB-14 produced four RAPDs. Three RAPDs were produced by the primers OPA-06, OPA-11, OPB-04, OPB-05 and OPB-13. The least number of RAPDs (2 bands) was obtained by the primers like OPA-03, OPA-05, OPA-19, OPA-20, OPB-02, OPB-06 and OPB-11 (Fig.6 and 7).

The highest number of intense bands (5 bands) was produced by OPB-20 primer. OPA-01, OPA-08 and OPB-12 produced four intense bands. Three intense bands were produced by the primers like OPA-02, OPA-07, OPA-09, OPA-10, OPA-14 and OPB-08. OPA-03, OPA-04, OPA-6, OPA-11, OPA-12, OPA-18, OPB-04, OPB-05, OPB-13 and OPB-16 produced two intense bands. The lowest number of intense bands (one band) was obtained by primers like OPA-15, OPA-20, OPB-06, OPB-07, OPB-14 and OPB-19 (Fig. 8 and 9).

Of these primers, the highest number (three) of faint bands was produced by OPA-04, OPB-14 and OPB-16. OPA-01, OPA-02, OPA-05, OPA-12, OPA-18, OPA-19, OPB-2, OPB-11 and OPB-20 produced two faint bands. The least number of faint bands (one band) was obtained by OPA-06, OPA-07, OPA-08, OPA-09, OPA-10, OPA-11, OPA-14, OPA-20, OPB-01, OPB-04, OPB-05, OPB-06, OPB-08, OPB-12 and OPB-13 (Fig. 8 and 9).

For further PCR amplification, seven primers were selected (OPA-01, OPA-02, OPA-04, OPA-08, OPB-12, OPB-16 and OPB-20) based on their performance in DNA amplification and more number of polymorphic bands produced in reaction. Out of seven primers, 4 primers (OPA-01, OPA-08, OPB-12 and OPB-20) were selected for DNA amplification of 40 landraces of melon. These four primers produced the highest number of bands as well as the highest number of intense bands. The PCR reactions were repeated atleast twice in order to check the reproductivity. Data obtained from 4 primers that gave reproducible bands, were used for statistical analysis.

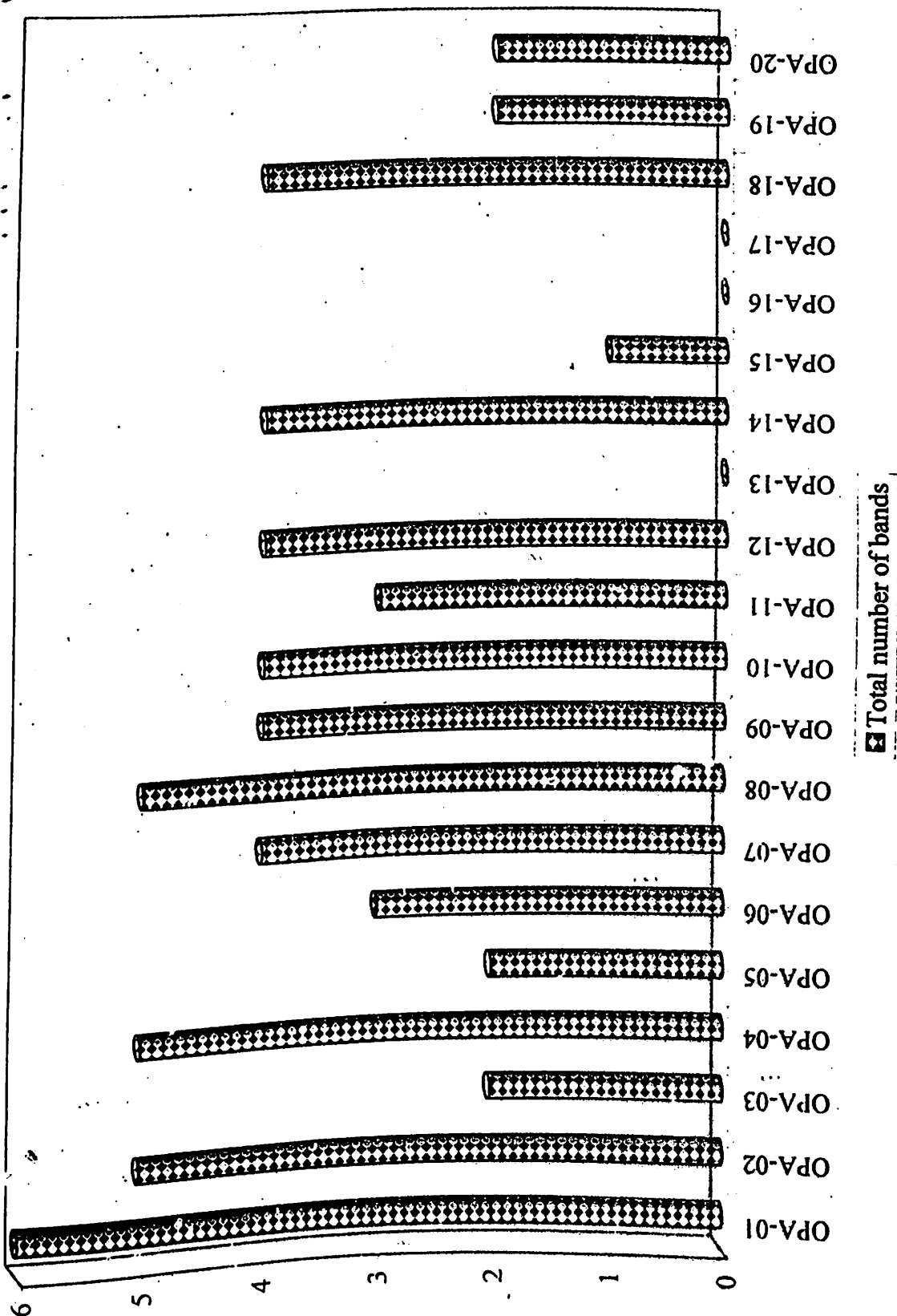


Fig. 6. Amplification profiles (Total number of bands) of the DNA of CM 28 using 20 primers

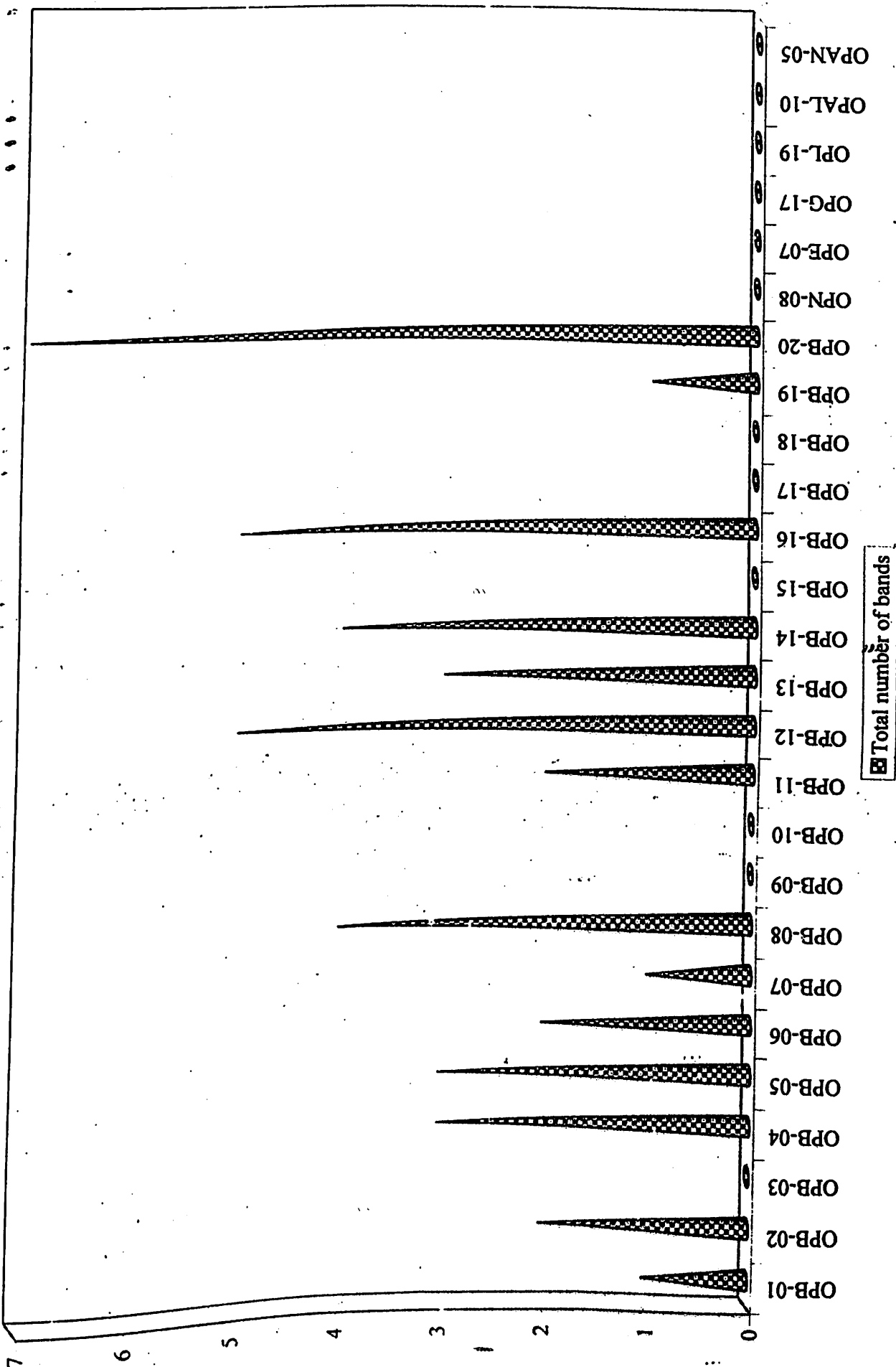
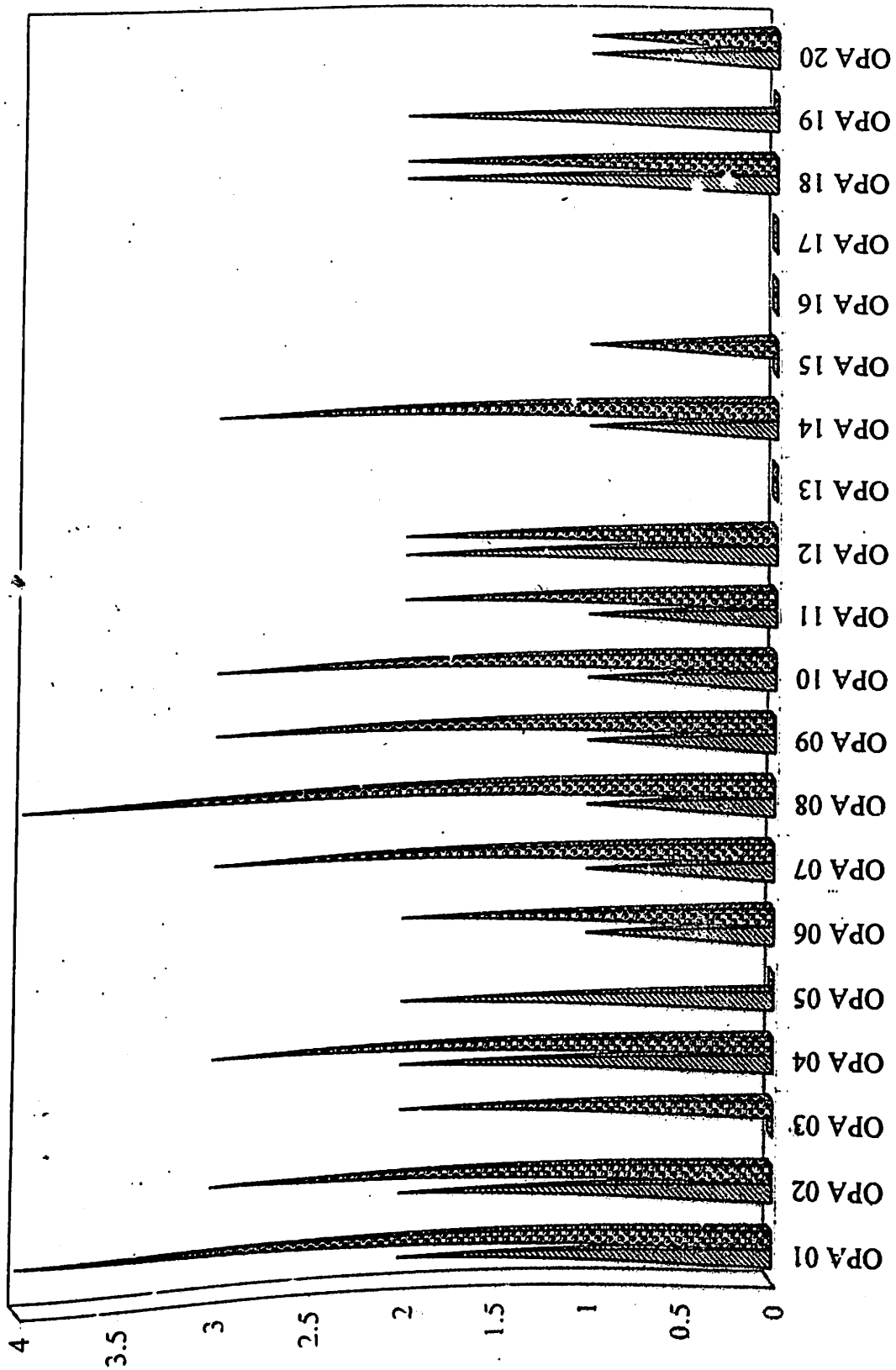


Fig. 7. Amplification profiles (Total number of bands) of the DNA of CM 28 using 26 primers



Number of faint bands Number of intense bands

Fig. 8. Amplification profiles (Number of faint bands and number of intense bands) of the DNA of CM 28 using 20 primers

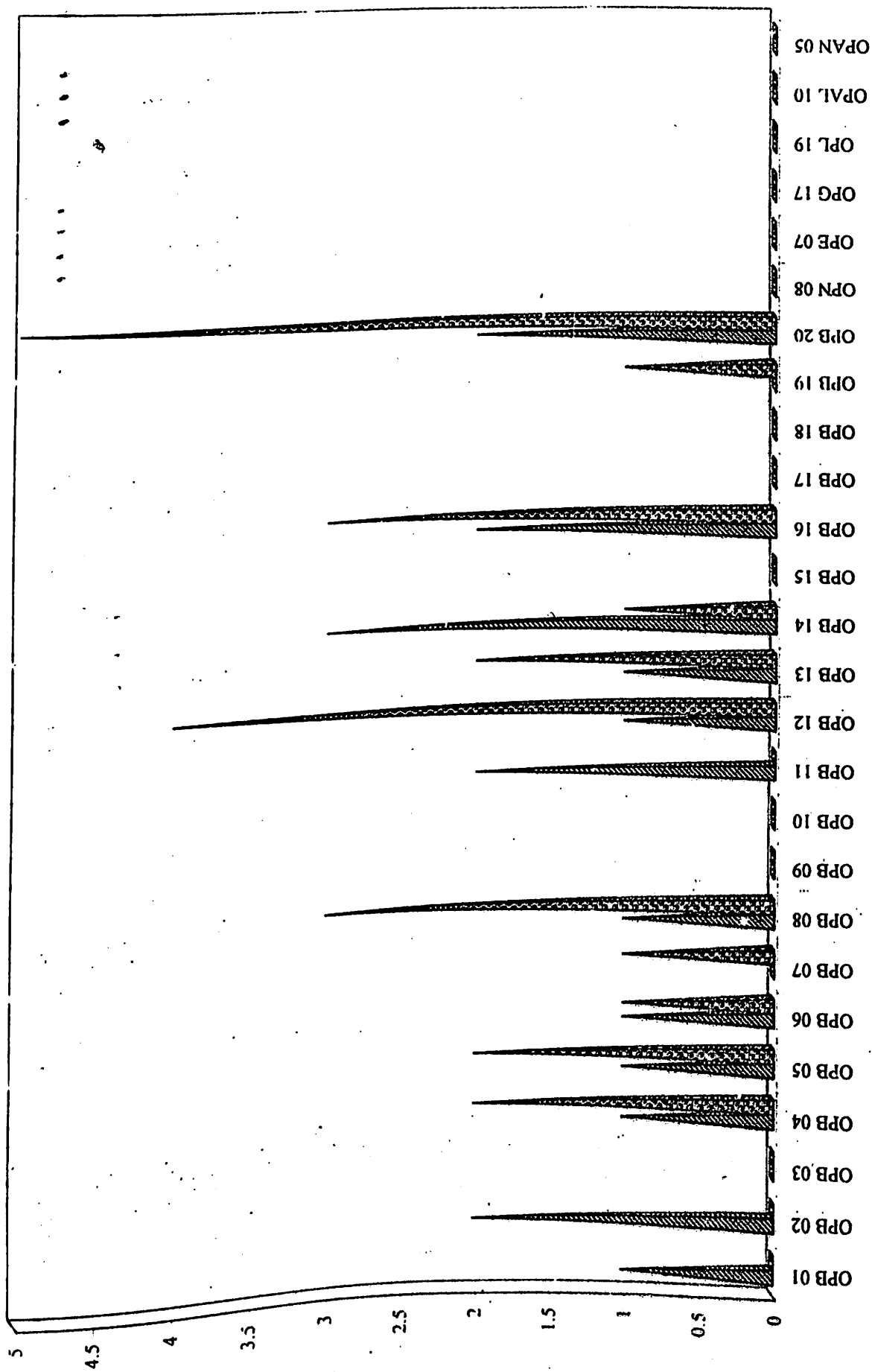


Fig. 9. Amplification profiles (Number of faint bands and number of intense bands) of the DNA of CM 28 using 26 primers

The four primers produced 29 scorable bands with an average of 7.25 bands per primer. The number of bands resolved per amplification was primer dependant and varied from a minimum of 5 and to maximum of 10. The nucleotide sequence of these four primers and number of informative RAPD markers given by each primer are shown in table 21 and Fig. 10.

The primer OPA-01 produced a total of 7 bands with 3 monomorphic bands. CM 1, CM 2, CM 3, CM 7, CM 11, CM 13, CM 15, CM 16, CM 17, CM 19, CM 20, CM 21, CM 22, CM 25, CM 26, CM 27, CM 28, CM 31, CM 32, CM 33, CM 35, CM 36 and CM 38 gave 7 bands each. 6 bands each were obtained in CM 4, CM 5, CM 6, CM 8, CM 9, CM 10, CM 12, CM 14, CM 18, CM 23, CM 24, CM 34, CM 37 and CM 39. CM 29, CM 30 and CM 40 produced 5 bands each (Fig. 11 and Plate 6).

The primer OPA-08 produced a total of 7 bands with 1 monomorphic band for all the clones. 6 bands each were obtained by CM 16, CM 17, CM 19, CM 23, CM 25, CM 27, CM 28, CM 30, CM 32, CM 34, CM 35, CM 36, CM 37, CM 38, CM 39 and CM 40. CM 1, CM 4, CM 15, CM 18, CM 20, CM 22, CM 29, CM 31 and CM 33 gave 5 bands each. 4 bands were produced in CM 6, CM 8, CM 10, CM 11, CM 12 and CM 13. CM 2, CM 3, CM 5, CM 7, CM 9, CM 14, CM 21 and CM 24 gave 3 bands each. CM 26 produced 2 bands (Fig. 12 and Plate 7).

Five scorable bands were obtained after amplification with the primer OPB-12, with one monomorphic band for all the clones. CM 8, CM 10, CM 18, CM 19, CM 20, CM 29, CM 30, CM 31, CM 33, CM 38, CM 39 and CM 40 produced 5 bands each. 4 bands each were obtained by CM 1, CM 4, CM 6, CM 11, CM 12, CM 13, CM 17, CM 21, CM 23, CM 24, CM 25, CM 26, CM 27, CM 28, CM 32, CM 35, CM 36 and CM 37. CM 4, CM 7, CM 14, CM 15, CM 16, CM 22, and CM 34 gave 3 bands each. CM 2 and CM 9 produced 2 bands each (Fig. 13 and Plate 8).

The highest number of scorable bands (10 bands) was produced with 3 monomorphic bands by the primer OPB-20. The primer OPB-20 gave the

Table 21. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the landraces of melon used in this study

S. No.	Primer	Sequence	Number of informative RAPD markers
1	OPA-01	CAGGCCCTTC	7
2	OPA-08	GTGACGTAGG	7
3	OPB-12	CCTTGACGCA	5
4	OPB-20	GGACCCTTAC	10

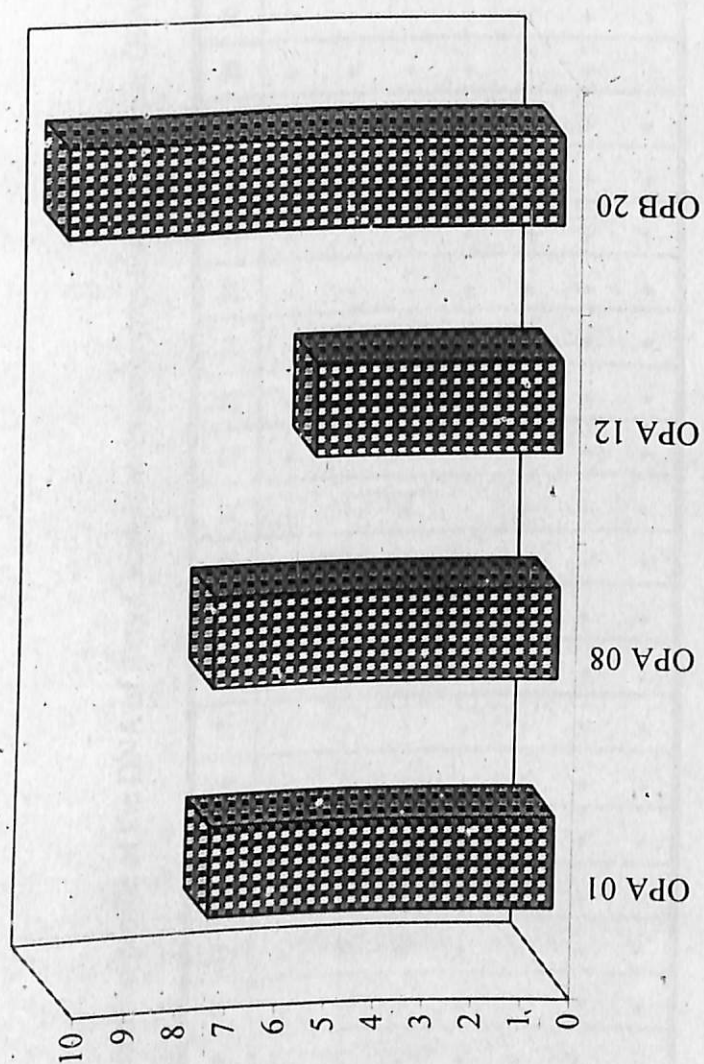


Fig. 10. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the landraces of melon used in the study

Fig. 11. Representation of the amplification profile of the DNA of forty *Cucumis melo* genotypes using the primer OPA-01

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+

‘+’ Represent presence of band; ‘-’ Represent absence of band

Lane 1 – PCR molecular weight marker (US biochemicals)
Lane 2 – Amplification profile of CM 1 genotype
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Lane 38 – Amplification profile of CM 37 genotype
Lane 39 – Amplification profile of CM 38 genotype
Lane 40 – Amplification profile of CM 39 genotype
Lane 41 – Amplification profile of CM 40 genotype

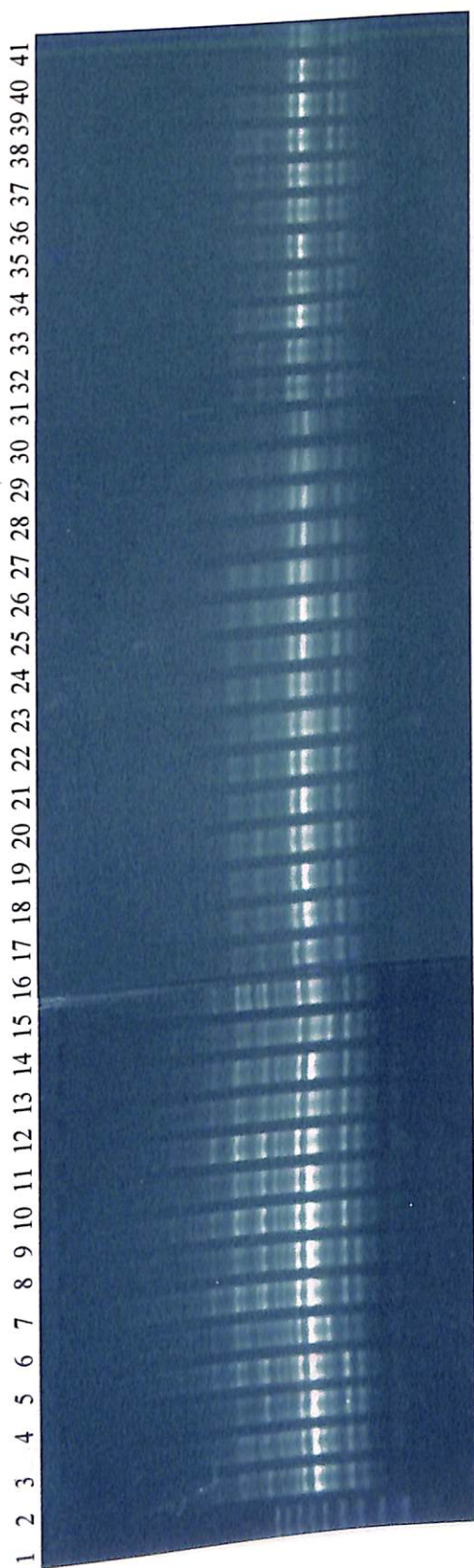


Plate 6. Amplification profiles of the DNA of forty *Cucumis melo* genotypes using the primer OPA-01

Fig. 12. Representation of the amplification profile of the DNA of forty *Cucumis melo* genotypes using the primer OPA-08

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+
+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	

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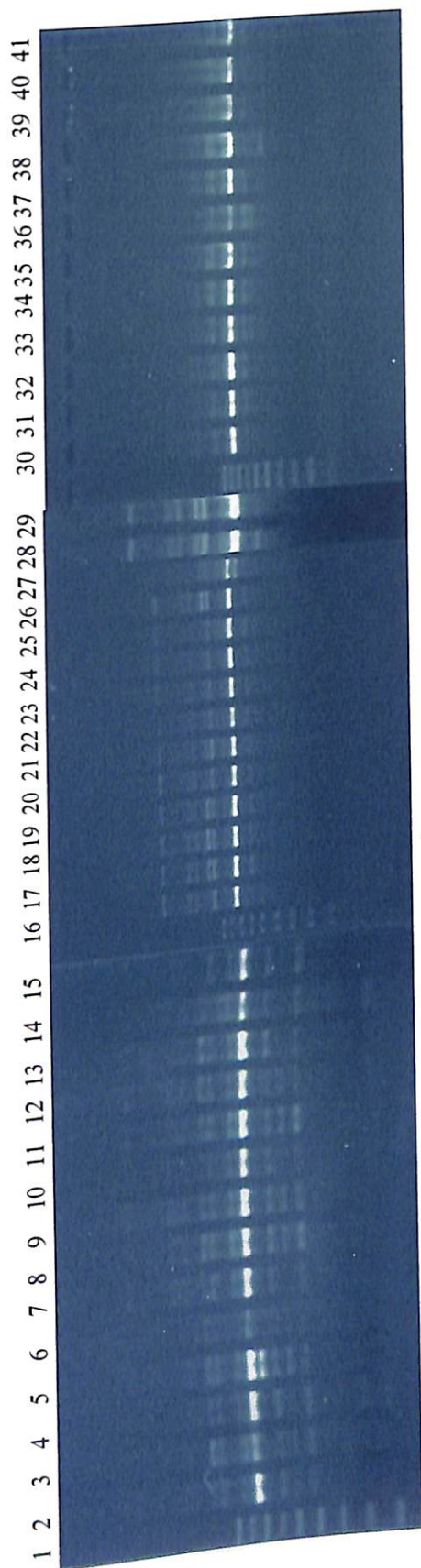


Plate 7. Amplification profiles of the DNA of forty *Cucumis melo* genotypes using the primer OPA-08

Fig. 13. Representation of the amplification profile of the DNA of forty *Cucumis melo* genotypes using the primer OPB-12

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	+	+	+
+	+	-	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+

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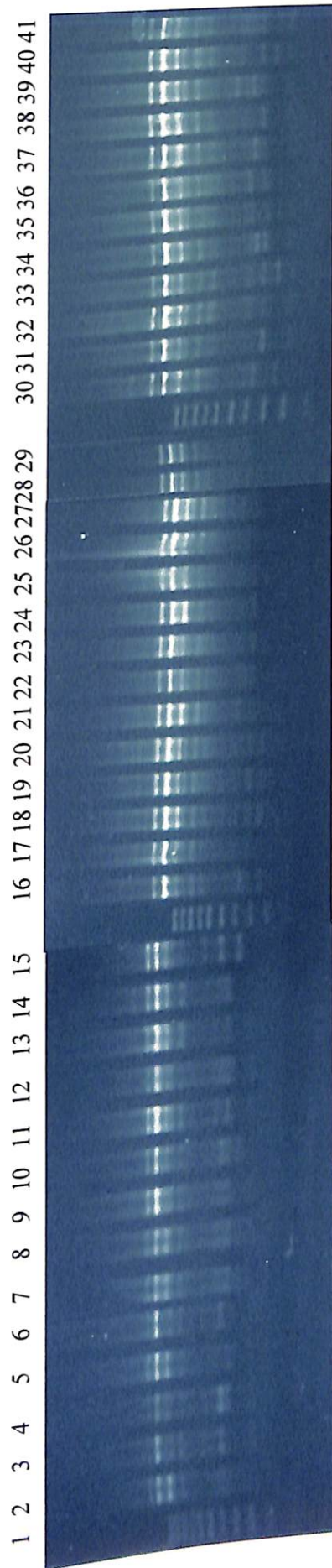


Plate 8. Amplification profiles of the DNA of forty *Cucumis melo* genotypes using the primer OPB-12

highest number of bands 10 in CM 7, CM 8, CM 10, CM 17, CM 19, CM 23, CM 25, CM 27, CM 28, CM 36 and CM 38. CM 4, CM 11, CM 12, CM 15, CM 16, CM 18, CM 20, CM 22, CM 31, CM 32, CM 34, and CM 35 gave 9 bands each when OPB-20 was used for amplification. CM 1, CM 9, CM 14, CM 30 and CM 33 gave 8 bands each. 7 bands were produced by CM 3, CM 6, CM 21, CM 24, CM 26 and CM 40. 6 bands were produced by CM 2, CM 29, CM 37 and CM 39. CM 5 and CM 13, gave 5 bands each (Fig. 14 and Plate 9).

4.3.4 Data Analysis

The banding pattern from RAPD analysis for each primer was scored by visual observation. Reproducible bands were scored for their presence (+) or absence (-) for all the landraces of melon studied. RAPD marker data were subjected to cluster analysis using NTSYS program for all the accessions to estimate similarity indices and genetic relatedness among the accessions. The similarity index (SI) values were computed as a ratio of number of similar bands to the total number in pair wise comparison of the accessions. The SI values obtained for each pair wise of RAPD bands among the forty landraces melon are shown in table 22. Based on the DNA fingerprinting data, the genetic distances were used to construct a dendrogram for the 40 accessions following the UPMGA method using NTSYS program (Rolf, 1997). Option SAHN was performed which resulted in the dendrogram shown in Fig. 15.

It was seen that forty landraces of melon were grouped into two major clusters namely, A and B. The major cluster A was comprised of 38 accessions *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32, CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12, CM 11, CM 24, CM 37, CM 29, CM 4, CM 26, CM 39, CM 40, CM 3, CM 13, CM 14, CM 6 and CM 9.

The major cluster A was divided into two minor clusters namely, A1 and A2. The minor cluster A1 consisted of 36 accessions *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32,

Fig. 14. Representation of the amplification profile of the DNA of forty *Cucumis melo* genotypes using the primer OPB-20

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
+	-	-	+	-	-	+	+	-	+	+	+	-	+	+	+	+	-	+	-	-	+	+	-	+	-	+	+	-	-	+	+	+	+	+	+	-	+	-	-
+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+	+	+	+	+	+	-	+	-	-
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-
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-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+	-	+	-	-	-	+	-	+	+	+
-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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‘+’ Represent presence of band; ‘-’ Represent absence of band

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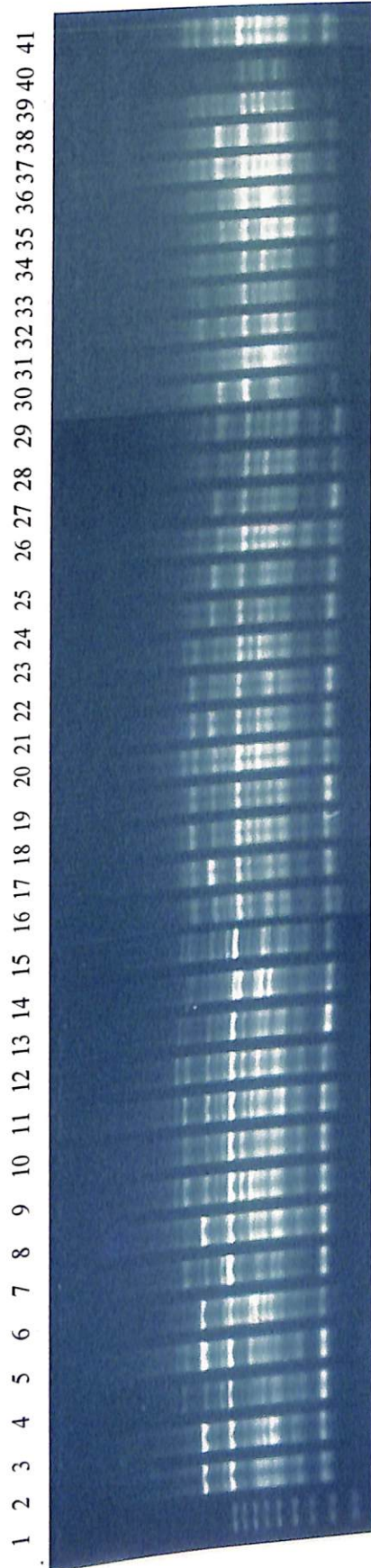


Plate 9. Amplification profiles of the DNA of forty *Cucumis melo* genotypes using the primer OPB-20

X	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8	CM9	CM10	CM11	CM12	CM13	CM14	CM15	CM16	CM17	CM18	CM19	CM20	CM21	CM22	CM23	CM24	CM25	CM26	CM27	CM28	CM29	CM30	CM31	CM32	CM33	CM34	CM35	CM36	CM37	CM38	CM39	CM40		
CM1	1																																									
CM2	0.75	1																																								
CM3	0.833	0.609	1																																							
CM4	0.845	0.68	0.76	1																																						
CM5	0.517	0.75	0.609	0.577	1																																					
CM6	0.63	0.583	0.6	0.571	0.542	1																																				
CM7	0.741	0.577	0.72	0.741	0.538	0.592	1																																			
CM8	0.75	0.535	0.607	0.75	0.5	0.731	0.846	1																																		
CM9	0.854	0.609	0.696	0.654	0.5	0.696	0.826	0.76	1																																	
CM10	0.75	0.536	0.807	0.75	0.5	0.731	0.846	0.854	0.76	1																																
CM11	0.778	0.555	0.692	0.655	0.464	0.692	0.68	0.885	0.79	0.885	1																															
CM12	0.808	0.577	0.654	0.741	0.481	0.72	0.84	0.92	0.826	0.92	0.88	1																														
CM13	0.833	0.727	0.818	0.962	0.609	0.6	0.592	0.607	0.56	0.607	0.63	0.654	1																													
CM14	0.833	0.652	0.818	0.76	0.48	0.667	0.72	0.731	0.696	0.731	0.76	0.792	0.76	1																												
CM15	0.846	0.68	0.692	0.714	0.577	0.78	0.808	0.815	0.792	0.815	0.846	0.88	0.731	0.76	1																											
CM16	0.885	0.72	0.731	0.75	0.615	0.731	0.778	0.786	0.76	0.786	0.815	0.846	0.741	0.731	0.96	1																										
CM17	0.889	0.667	0.741	0.821	0.63	0.678	0.852	0.857	0.704	0.857	0.821	0.852	0.704	0.741	0.889	0.926	1																									
CM18	0.786	0.63	0.704	0.786	0.592	0.769	0.75	0.889	0.731	0.889	0.786	0.815	0.714	0.704	0.786	0.821	0.893	1																								
CM19	0.857	0.843	0.714	0.793	0.607	0.714	0.821	0.893	0.678	0.893	0.857	0.821	0.731	0.714	0.857	0.893	0.964	0.928	1																							
CM20	0.815	0.72	0.8	0.75	0.68	0.731	0.714	0.786	0.63	0.786	0.75	0.714	0.826	0.731	0.75	0.786	0.857	0.889	0.893	1																						
CM21	0.917	0.739	0.826	0.769	0.56	0.68	0.731	0.741	0.708	0.741	0.769	0.8	0.692	0.826	0.84	0.808	0.815	0.778	0.786	0.808	1																					
CM22	0.846	0.68	0.692	0.778	0.64	0.63	0.741	0.75	0.654	0.75	0.714	0.741	0.8	0.692	0.846	0.885	0.889	0.786	0.857	0.815	0.769	1																				
CM23	0.815	0.654	0.731	0.815	0.615	0.731	0.778	0.852	0.692	0.852	0.75	0.646	0.783	0.8	0.815	0.852	0.926	0.889	0.893	0.852	0.741	0.815	1																			
CM24	0.8	0.696	0.708	0.8	0.583	0.64	0.692	0.769	0.667	0.769	0.667	0.76	0.769	0.783	0.731	0.704	0.778	0.808	0.75	0.769	0.869	0.731	0.769	1																		
CM25	0.852	0.692	0.769	0.786	0.654	0.704	0.815	0.821	0.667	0.821	0.786	0.815	0.64	0.769	0.812	0.889	0.963	0.857	0.926	0.889	0.778	0.852	0.961	0.741	1																	
CM26	0.731	0.696	0.708	0.8	0.583	0.518	0.76	0.704	0.667	0.704	0.667	0.692	0.741	0.64	0.667	0.843	0.714	0.678	0.689	0.704	0.792	0.667	0.643	0.826	0.678	1																
CM27	0.889	0.667	0.741	0.821	0.63	0.678	0.852	0.857	0.704	0.857	0.821	0.852	0.741	0.741	0.889	0.926	0.857	0.893	0.964	0.857	0.815	0.889	0.726	0.778	0.853	0.714	1															
CM28	0.889	0.667	0.741	0.821	0.63	0.678	0.852	0.857	0.704	0.857	0.821	0.852	0.708	0.741	0.889	0.926	0.857	0.893	0.964	0.857	0.815	0.889	0.726	0.778	0.863	0.714	0.857	1														
CM29	0.731	0.625	0.64	0.667	0.481	0.708	0.63	0.769	0.667	0.769	0.731	0.76	0.63	0.708	0.731	0.704	0.714	0.808	0.75	0.704	0.782	0.607	0.704	0.826	0.678	0.68	0.714	0.714	1													
CM30	0.714	0.815	0.63	0.714	0.577	0.692	0.678	0.815	0.654	0.815	0.714	0.741	0.769	0.63	0.715	0.75	0.821	0.923	0.857	0.815	0.704	0.714	0.815	0.8	0.786	0.667	0.821	0.821	0.875	1												
CM31	0.852	0.63	0.704	0.786	0.536	0.704	0.815	0.889	0.667	0.889	0.652	0.815	0.704	0.769	0.852	0.821	0.893	0.857	0.926	0.821	0.846	0.786	0.821	0.806	0.857	0.741	0.893	0.893	0.808	0.786	1											
CM32	0.852	0.63	0.704	0.786	0.592	0.643	0.815	0.821	0.667	0.821	0.786	0.815	0.731	0.704	0.852	0.889	0.963	0.857	0.926	0.821	0.778	0.923	0.889	0.741	0.926	0.678	0.963	0.963	0.678	0.786	0.857	1										
CM33	0.815	0.592	0.667	0.57	0.5	0.667	0.778	0.851	0.63	0.852	0.815	0.778	0.833	0.731	0.815	0.785	0.857	0.821	0.893	0.786	0.808	0.815	0.786	0.769	0.821	0.704	0.857	0.857	0.769	0.75	0.961	0.889	1									
CM34	0.846	0.815	0.76	0.846	0.518	0.63	0.808	0.815	0.72	0.815	0.778	0.808	0.769	0.833	0.778	0.815	0.889	0.852	0.857	0.75	0.769	0.778	0.885	0.8	0.852	0.667	0.889	0.889	0.731	0.778	0.852	0.852	0.815	1								
CM35	0.923	0.692	0.769	0.852	0.592	0.643	0.815	0.821	0.667	0.821	0.786	0.815	0.741	0.769	0.812	0.889	0.963	0.857	0.926	0.821	0.846	0.852	0.889	0.806	0.826	0.741	0.963	0.963	0.741	0.786	0.926	0.926	0.889	0.923	1							
CM36	0.689	0.667	0.741	0.821	0.63	0.678	0.852	0.857	0.704	0.857	0.821	0.852	0.75	0.741	0.889	0.926	0.854	0.893	0.864	0.857	0.815	0.889	0.926	0.778	0.963	0.714	0.857	0.857	0.714	0.821	0.893	0.963	0.857	0.889	0.963							

X represent genotypes

Table 22. Similarity matrix for the landraces of melon generated using RAPD primers

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C3 represent CM 3 genotype
C4 represent CM 4 genotype
C5 represent CM 5 genotype
C6 represent CM 6 genotype
C7 represent CM 7 genotype
C8 represent CM 8 genotype
C9 represent CM 9 genotype
C10 represent CM 10 genotype
C11 represent CM 11 genotype
C12 represent CM 12 genotype
C13 represent CM 13 genotype
C14 represent CM 14 genotype
C15 represent CM 15 genotype
C16 represent CM 16 genotype
C17 represent CM 17 genotype
C18 represent CM 18 genotype
C19 represent CM 19 genotype
C20 represent CM 20 genotype
C21 represent CM 21 genotype
C22 represent CM 22 genotype
C23 represent CM 23 genotype
C24 represent CM 24 genotype
C25 represent CM 25 genotype
C26 represent CM 26 genotype
C27 represent CM 27 genotype
C28 represent CM 28 genotype
C29 represent CM 29 genotype
C30 represent CM 30 genotype
C31 represent CM 31 genotype
C32 represent CM 32 genotype
C33 represent CM 33 genotype
C34 represent CM 34 genotype
C35 represent CM 35 genotype
C36 represent CM 36 genotype
C37 represent CM 37 genotype
C38 represent CM 38 genotype
C39 represent CM 39 genotype
C40 represent CM 40 genotype

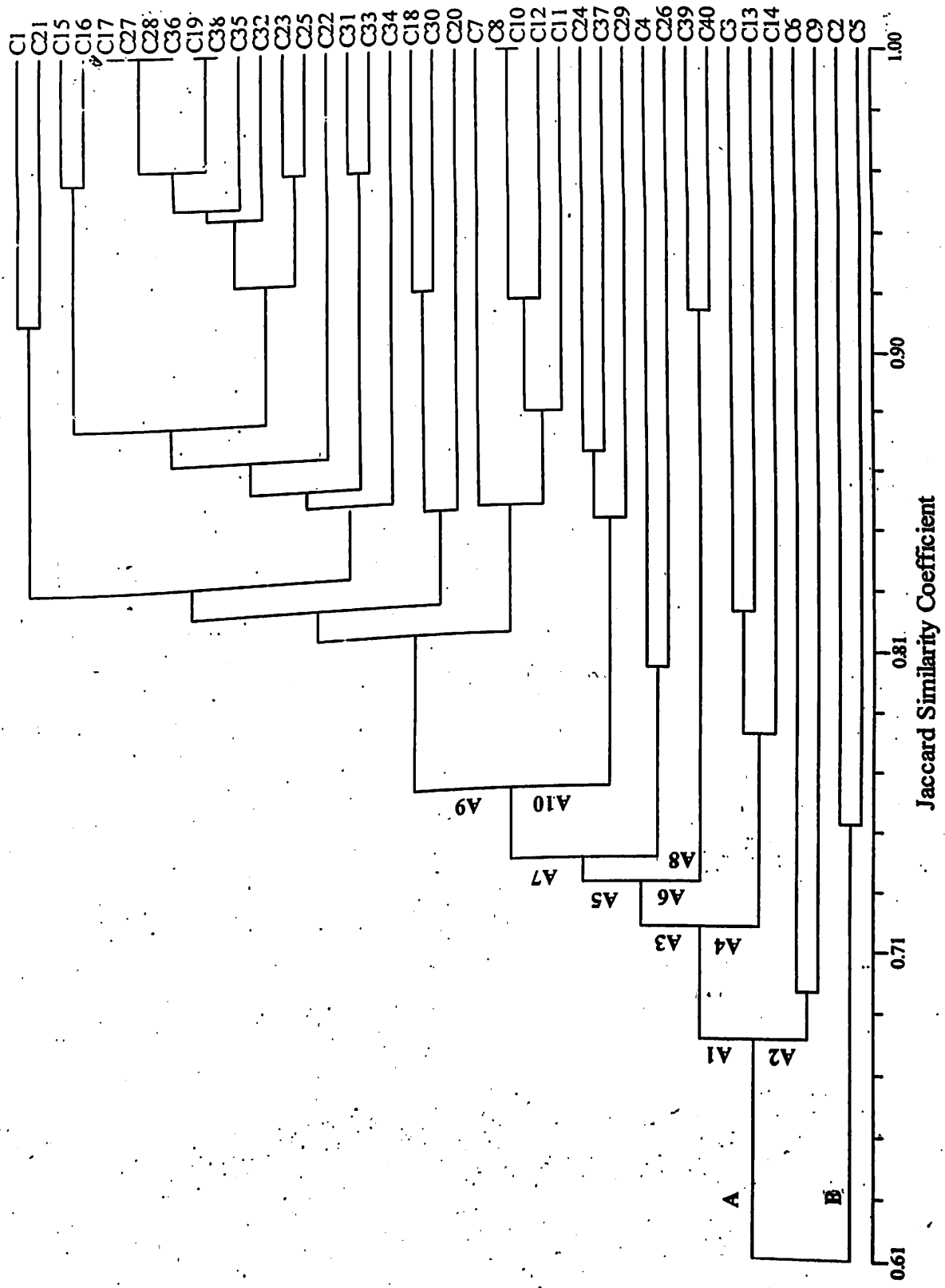


Fig. 15. Dendrogram for forty landrace of melon based on data from RAPD primers

CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12, CM 11, CM 24, CM 37, CM 29, CM 4, CM 26, CM 39, CM 40, CM 3, CM 13 and CM 14, while cluster A2 comprised of CM 6 and CM 9.

Cluster A1 was further divided into two sub clusters namely A3 and A4. Cluster A3 consisted of 33 accessions *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32, CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12, CM 11, CM 24, CM 37, CM 29, CM 4, CM 26, CM 39 and CM 40 and cluster A4 had CM 3, CM 13 and CM 14. Cluster A3 was further grouped into two subclusters namely A5 and A6. Subcluster A5 consisted of 31 accessions *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32, CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12, CM 11, CM 24, CM 37, CM 29, CM 4 and CM 26. CM 39 and CM 40 were representative for subcluster A6.

Cluster A5 was further grouped into two subclusters namely A7 and A8. Subcluster A7 consisted of 29 accessions, *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32, CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12, CM 11, CM 24, CM 37 and CM 29, while sub cluster A8 consisted of CM 4 and CM 26. Cluster A7 was again divided into two subclusters namely A9 and A10. Sub cluster A9 consisted of 26 accessions, *viz.*, CM 1, CM 21, CM 15, CM 16, CM 17, CM 27, CM 28, CM 36, CM 19, CM 38, CM 35, CM 32, CM 23, CM 25, CM 22, CM 31, CM 33, CM 34, CM 18, CM 30, CM 20, CM 7, CM 8, CM 10, CM 12 and CM 11. CM 24, CM 37 and CM 29 were members of the subcluster A10.

In subcluster A2, CM 9 showed a 69.6 per cent similarity with CM 6. Within subcluster A4, CM 14 showed a similarity of only 73.9 and 81.8 per cent, respectively to CM 13 and CM 3. In subcluster A6, CM 40 showed a similarity of 91.7 per cent with CM 39.

In subcluster A8, CM 26 showed a similarity of 80.0 per cent with CM 4. In subcluster A10, CM 29 showed a similarity of 86.9 per cent and 82.6 per cent respectively to CM 37 and CM 24.

Within subcluster A9, all other accessions were grouped together. They showed a similarity ranged from 81.5 to 96.1 per cent. Of these accessions, CM 30 was most distantly related to CM 1, sharing only 71.4 per cent similarity at DNA level.

The accessions in the major cluster A, CM 1 showed a similarity of 65.4 per cent and 63 per cent with CM 9 and CM 6 respectively. The major cluster A, CM 1 showed a similarity of 75 and 80.8 per cent respectively with CM 10 and CM 12. However within the accessions CM 1 showed the least similarity of 65.4 per cent and 63 per cent with CM 9 and CM 6 respectively. CM 2 and CM 5 were grouped in the other major cluster B and showed a similarity of 75% at the molecular level. On comparison among the species, it can be seen that CM 2 shows a similarity of only 57.7 per cent with CM 5.

Based on the SI values, it is seen that all the landraces of melon differ from each other significantly.

Discussion

5. DISCUSSION

Melon (*Cucumis melo* L.) is one of the most popular and traditional vegetable crops of South India. This has been in cultivation throughout the humid tropics with various common names viz., vellari, melon, pickling melon, preserving melon, oriental pickling melon etc.

Fruit yield is the major pathway in the development of superior genotypes and achievement of the goal of self sustenance. The information usually needed for developing high yielding varieties in a particular species pertains to the extent of genetic variability for desirable traits in the available germplasm. In *Cucumis melo*, a large variability is present in the landraces with respect to all the characters (Kalloo *et al.*, 1982). Variability parameters like coefficient of variation, heritability and expected genetic advance, besides degree of association between the various characters and direct effects of yield contributing characters on total fruit yield and genetic divergence are of paramount significance in formulating an appropriate breeding strategy.

The present investigation was carried out using forty genotypes of landraces of melon from diversified origin to elicit information on the quantum of genetic variability, heritability, genetic advance, correlation between yield and yield contributing characters and genetic divergence are discussed hereunder. The study also aimed at characterizing these landraces through protein profile by SDS-PAGE method and through molecular markers by RAPD technique. The results of the study are discussed here

In this investigation, analysis of variance revealed significant differences among the landraces of melon (*Cucumis melo* L.) for all the characters. The presence of considerable variation expressed enough scope for improving the population.

The landrace collected from Ernakulam viz., CM 32 was significantly superior in yield. Average fruit weight, fruits per plant and sex ratio were also more in this accession. The lowest yield was recorded in CM 5. This accession

had low fruits per plant and was late in female flowering. Similar differential performance in yield and yield attributes in melon was reported by Nandpuri *et al.* (1975), Chhonkar *et al.* (1979), Deol *et al.* (1981) and Swamy *et al.* (1985) in many of the local germplasms.

5.1 GENETIC VARIABILITY AND GENETIC DIVERGENCE

5.1.1 Variability Studies

Greater variability ensures better chances of producing new desirable forms. Selection is the fundamental process in the development of superior varieties and it depends on the variability available in the crop. Only the genetic proportion of the total variability contributes to gain under selection. So, knowledge of the genetic variation governing the inheritance of quantitative characters like yield and its components is essential in any crop plant (Allard, 1960).

In the present study, 21 characters of forty landraces of *Cucumis melo* were observed. Estimates of phenotypic coefficient of variation (PCV) were higher than the corresponding values of genotypic coefficient of variation (GCV). Similar results were reported by Kalloo *et al.* (1982) and Vijay (1987) in muskmelon.

The PCV ranged from 3.83 for dry matter content to 49.32 for yield per plant. The highest PCV was observed for yield per plant followed by fruits per plant and average fruit weight. GCV is a better tool to understand variability, as it is free from the environmental components. The GCV helps in comparison and measurement of genetic variability among different characters. The GCV ranged from 3.51 for dry matter content to 44.70 for yield per plant. The highest GCV was observed for yield per plant followed by average fruit weight and fruits per plant. The lowest GCV was observed for dry matter content followed by days to first male flower, days to first female flower and days to first harvest. Rastogi and Aryadeep (1990) observed high PCV and GCV values for yield per plant, fruits per plant, average fruit weight and length of fruit. Since the magnitude of

PCV and GCV were closer in this study, genotype had more contribution than environment. So the selection can be very well done based on phenotypic values.

Lowest GCV was noted for the days to first harvest. A similar result was reported by Deol *et al.* (1981) and Swamy *et al.* (1985). The above result revealed that while selecting for high yielding types of melon, major importance should be given to fruits per plant and average fruit weight.

5.1.2 Heritability and Genetic Advance

Heritability provides information on the degree of inheritance of characters from the parents to the progeny. A good knowledge of heritability is pre-requisite for effective execution of breeding programmes, as it is a measure of success in separating genotypes by selection. Heritability in broad sense expresses the extent to which individual's phenotypes are determined by genotypes. Characters possessing high heritability can be improved directly through selection as they are less affected by the environment. The magnitude of heritability indicates the effectiveness of selection based on phenotypic performance (Johnson *et al.*, 1955). Burton (1953) suggested that heritability along with GCV would provide a clear idea about the amount of genetic advance expected through selection.

In the present study, almost all the characters exhibited high heritability which ranged from 74.04 to 99.04%. The characters included leaf thickness, seeds per fruit, 1000 seed weight, fruit diameter, days to first male flower, fruit girth, fruit length, average fruit weight, days to first harvest, internodal length, days to first female flower, vine length, node number of first female flower, sex ratio, dry matter content, yield per plant, fruits per plant, number of secondary branches and node number of first male flower had high heritable values. Moderate heritability occurred in number of secondary branches (56.86%). Thus high heritability of fruit length is in agreement with the findings of Choudhary *et al.* (1985) and Abusaleha and Dutta (1990) in cucumber. High heritability for average fruit weight by Kalloo and Dixit (1983) and Swamy *et al.* (1985) in

melons and for fruit girth and 1000 seed weight by Mariappan and Pappiah (1990) in cucumber is in agreement with the present study.

Genetic advance indicates the expected genetic progress for a particular trait under a suitable selection system. Higher values of genetic advance as per cent of mean were recorded in yield per plant, average fruit weight and fruits per plant. Similar results were reported by Kalloo and Dixit (1983) for yield per plant, average fruit weight and fruits per plant.

Knowledge of heritability coupled with expected genetic advance of a trait is necessary for assessing the scope of its improvement through selection (Johnson *et al.*, 1955). In the present study, high values of heritability associated with high genetic advance were observed for yield per plant, average fruit weight and fruits per plant. This confirms the earlier findings of Rastogi and Aryadeep (1990) in cucumber, which reveals that variation for these characters is mainly due to action of additive genes and these characters can be improved by selection.

Though heritability was high for leaf thickness, seeds per fruit, days to first male flower and days to first harvest, genetic advance was low in magnitude, indicating the action of non additive genes for expression of these characters. Thus it implies that high heritability is not always an indication of high genetic advance (Johnson *et al.*, 1955).

5.1.3 Correlation Studies

Correlation provides information on the nature and extent of association between characters in a population. The component characters always show interrelationships. When selection pressure is applied on a trait, the population under selection is improved not only for that trait but also for other characters associated with it. This facilitates simultaneous improvement of two or more characters. Therefore, analysis of yield in terms of phenotypic and environmental correlation coefficients of component characters helps in understanding characters that can form the basis of selection.

The present investigation revealed that the yield per plant had significant phenotypic correlation with node number of first male flower, sex ratio, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruit.

Significant genotypic correlation with yield per plant was seen for node number of first female flower, sex ratio, vine length, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruit.

The highly significant phenotypic and genotypic correlation of yield per plant with average fruit weight obtained in the present study is in agreement with the findings of Kalloo *et al.* (1982), Swamy *et al.* (1985), Chacko (1992) and Rakhi (2001).

The association of length of vine was positive and significant at genotypic level with yield per plant. Secondary branches also had a positive association with yield in both correlations. Therefore, it can be concluded that the longer the vine, more will be the number of branches and higher will be the yield as reported by Kalloo *et al.* (1982), Sriramamurthy (2000) and Rakhi (2001).

Positive significant phenotypic and genotypic correlation of internodal length with yield per plant as seen in the present studies was earlier reported by Swamy *et al.* (1985) and Rakhi (2001).

Significant positive correlation of length of fruit with yield in the present study confirms with Choudhary and Mandal (1987), Abusaleha and Dutta (1988) and Rakhi (2001).

In the present investigation, fruits per plant was highly and positively correlated with yield. Similar findings were reported by Swamy *et al.* (1985), Vijay (1987), Lal and Singh (1997), Sriramamurthy (2000) and Rakhi (2001).

Significant positive correlation was observed for seeds per fruit with yield were observed. These observations are in conformity with the result of Sriramamurthy (2000).

Sex ratio had positive significant correlation with yield. The results reported by Saikia *et al.* (1995) in cucumber are in agreement with the present findings.

In the present study, node number of first female flower was seen significantly and negatively correlated with yield. This is in conformity with the findings of Deol *et al.* (1981) and Sriramamurthy (2000).

The results of present study concluded that most important positive characters contributing towards yield per plant at genotypic level were number of secondary branches, average fruit weight and fruits per plant, suggesting that selection procedure applied for increasing these traits will help in eventual increase of yield per plant.

5.1.4 Path Analysis

Yield is dependant on a number of component characters and information on the association of characters with yield and among themselves is essential in any breeding programme. The study of association of characters with yield enables to fix up character which have decisive contributing role in influencing the yield. Path analysis provides information on the association of attributes and their direct and indirect influences on yield depicting importance in selection (Singh and Singh, 1988).

In the present study, fruits per plant and average fruit weight exerted strong and positive direct effect on yield. Direct effects of fruits per plant on yield was also reported by Kalloo *et al.* (1982), Vijay (1987) and Lal and Singh (1997) in muskmelon. Direct effects of average fruit weight on yield was revealed by Nagaprasuna and Rama Rao (1989) and Saikia *et al.* (1995) in cucumber.

Though the direct effects of vine length on yield were low in magnitude, it exerted high and positive indirect effects through fruits per plant. Positive direct effects of vine length on yield were reported by Prasad and Singh (1992) in cucumber. Similarly fruit length also exerted direct positive effect on yield in accordance with the findings of Zhang *et al.* (1995) and Rakhi (2001). Sex ratio

exerted positive direct effects on yield. The results are supported by Solanki and Shah (1989) and Sriramamurthy (2000) in cucumber.

Direct effects of number of secondary branches on yield was low in magnitude, it exerted high and positive indirect effects through fruits per plant and average fruit weight. These results are confirmed by Prasad and Singh (1992). Similarly seeds per fruit exerted low positive direct effects in yield. The present results are in consonance with the finding of Sriramamurthy (2000) in cucumber. Internodal length exerted negative direct effect, even though its total correlation with yield was positive. Its positive indirect effect through fruits per plant could be considered as the cause for this. Similarly node number of first female flower also exerted negative direct effect on yield. This is in agreement with the findings of Saikia *et al.* (1995) in cucumber.

The residual effect observed in the present study was very low (0.0708) indicating almost 93% of the variation in yield per plant was attributable to factors considered in this study.

From the above results of path analysis, it might be concluded that while selecting high yielding types, major emphasis should be given to average fruit weight and fruits per plant with due consideration for vine length, fruit length, sex ratio, number of secondary branches and seeds per fruit.

5.1.5 Cluster Analysis Through Mahalanobis D² Statistics

Assessment of genetic diversity is of much importance in plant breeding research as far as the selection of parents for hybridization is concerned. The economic value of a plant is determined by several characters, which may be correlated. Mahalanobis D² statistics gives a quantitative measure of divergence based on multiple characters.

In the present study, forty landraces of melon were grouped into twenty clusters based on twenty one quantitative characters. Among them, cluster XIV had CM 2 and CM 5 genotypes came under *Cucumis melo* L. (Sweet pleasant and

odour). The clustering pattern did not show any strict parallelism with the geographic origin.

The intra cluster D value was least for cluster II (14.968) and highest for cluster XIX (43.025). The inter cluster distance was maximum (86.943) between cluster XVIII and XX and minimum (22.280) between cluster II and IX. Theoretically, maximum heterosis would be expected in crosses involving parents belonging to these clusters.

The clustering pattern revealed that genetic diversity was not related to geographic diversity which is in line with earlier observations of Owents *et al.* (1983) and Miller and Quisenberry (1986) in cucumber. It appeared that geographic isolation may not be the only determining factor for genetic divergence in the melon genotypes tested. Other factors such as genetic drift and selection in different environments could cause greater genetic diversity than geographical distance.

5.2 Biochemical Characterization

Seed protein variants that migrate at different rates under electrophoresis have been extensively used as molecular genetic marker for characterization of species and cultivars (Bretting and Widrlechner, 1995). Seed proteins have the advantage of being scorable from inviable organs or tissues and the electrophoretic protocol for bulk protein assay is generally simpler than that for isozymes (Cooke, 1984). The experiment was carried out to characterize the forty landraces of melon through seed protein electrophoresis.

The protein yield ranged from 0.858µg/seed (CM 10) to 3.200 µg/seed (CM 20). Totally 20 number of protein bands were produced in the landraces of melon. Maximum 18 bands were produced in CM 7 and CM 40 and the minimum 5 bands in CM 2.

The dendrogram of protein analysis show two major clusters formed namely cluster A and Cluster B. Cluster B has only one dessert genotype CM 2. Major cluster A was further subclustered into four groups *viz.*, A1 (CM 1, CM 29, CM 8,

CM 3, CM 17, CM 34, CM 39), A3 (CM 5, CM 21, CM 11, CM 13, CM 25, CM 9, CM 15, CM 7), A5 (CM 6, CM 28, CM 30, CM 33, CM 23, CM 19, CM 20, CM 16, CM 31, CM 18, CM 26, CM 35, CM 24, CM 36, CM 14) and A6 (CM 4, CM 7, CM 40, CM 10, CM 12, CM 38, CM 22, CM 37, CM 32). A similarity index of 13.3 per cent between CM 1 (cluster A) and CM 2 (cluster B). In D^2 analysis two genotypes (CM 2, CM 5) were in a single cluster, but in protein analysis CM 2 genotype was alone present in separate cluster. CM 5 was combined with other culinary melon.

In the present study, four different clusters were not correlated with D^2 analysis, geographical locations and morphological characters like fruit colour and shape. It might be due to protein deterioration and also environmental effects.

The seed protein profiles indicated that the protein extracted may not have been quite different despite distinct morphological differences necessitating inclusion of other extraction procedure (or) isozymes for characterization of melon studies. Similar results have been reported by Weeden (1983) in bean, Henn *et al.* (1992) in tomato, Upadhyay *et al.* (1998) in bottlegourd, Yadav *et al.* (1998) and Choudhary and Hari Har Ram (2000) in musk melon.

5.3 Molecular Characterization

Morphological and agronomic traits have been extensively used to determine the relationship among plants and varieties (Ortiz *et al.*, 1998). However, morphological markers do not often reflect genetic relationships because of interaction with the environment, epistasis and the largely unknown genetic control of the traits (Smith and Smith, 1989). In contrast, molecular markers are not influenced by environment or developmental stage of a plant, making them ideal for genetic relationship studies. Random amplified polymorphic DNA (RAPD) is one of the useful molecular markers for identifying varieties at the genotypic level. It can help overcome the complication arising from morpho-anatomical characterization. RAPD analysis is used for assessing variation in plants (Newbury and Ford-Lloyd, 1993) for molecular

characterization. RAPD analysis has been successfully employed to analyse *Cucumis melo* germplasm (Staub *et al.*, 2000).

In the present study an attempt was made to determine the extent of genetic diversity in 40 landraces of melon based on RAPD markers, making use of arbitrary primers to amplify random DNA sequence in the genome.

Isolation of genomic DNA of melon was done using modified Williams *et al.* (1990) method standardized by Baudracco-Arnas (1995) and Brown *et al.* (1998) for *Cucumis melo* germplasm. Tissues from young tender leaves were found to yield good quality DNA.

The DNA yield of forty cucumber accessions ranged from 15 to 6985 $\mu\text{g ml}^{-1}$. The purity of DNA ($\text{OD}_{260}/\text{OD}_{280}$) ranged from 1.08 to 2.03.

To identify promising primers for RAPD analysis, forty six decamer primers (Operon Inc., USA) of kit A, kit B, OPAN-5, OPN-8, OPE-7, OPG-17, OPAL-10 and OPL-19 were screened using the DNA of CM 28. The procedure standardized by Staub *et al.* (2000) for *Cucumis melo* germplasm was tried for amplification. Thirty one primers, out of the 46 decamer primers yielded amplification products. The total number of bands, ranged from 1.0-7.0. The primers like OPA-13, OPA-16, OPA-17, OPB-03, OPB-09, OPB-10, OPB-15, OPB-17, OPB-18, OPAN-5, OPN-8, OPE-7, OPG-17, OPAL-10 and OPL-19 did not yield any bands. This indicated that there is no sequence complementary to these primers in the DNA of CM 28 germplasm.

A total of 103 bands (average:2.24 bands per primer) were generated by the thirty one primers of which 96.12 per cent (99 bands) were polymorphic and four bands were monomorphic. Seven primers showed high level of polymorphism. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA (Prasannalatha *et al.*, 1999). There is high probability for the amplified fragments to contain repeated sequences.

For further study, four promising primers for RAPD analysis were identified, based on the number of polymorphic bands obtained. They were OPA-01, OPA-08, OPB-12 and OPB-20. These promising primers were used for amplifying DNA from the forty *Cucumis melo* germplasms. Eleven RAPD primers have been identified for amplification of DNA in determining interspecific variation in *Cucumis* (Staub *et al.*, 1997); sixty four primers in forty six accessions of *Cucumis melo* (Staub *et al.*, 2000) and forty three random primers in thirty-two lines of melon belonging to seven varietal types (Garcia *et al.*, 1998). A study in tea (Liang chen and Yamaguchi, 2002) indicated that a minimum of 15 decamer primers (102 polymorphic bands) were necessary to differentiate the related species. However, Bhat *et al.* (1995) suggested that the number of polymorphisms might be more important than the number of primers for the generation of a stable phenogram. They also suggested that the number of polymorphisms required to generate a stable phenetic analysis would vary with the plant material under investigation and the sequences that are amplified.

The four primers used in this analysis yielded twenty nine scorable bands with an average of 7.25 bands per primer. The number of bands resolved per amplification was primer dependant and varied from 5 to 10.

At the similarity index of 75 per cent between CM 2 and CM 5 genotypes formed a major cluster B. In D^2 analysis based on morphological traits, these two accessions were together in one cluster. In the present study, CM 2 and CM 5 genotypes were under muskmelon group. Remaining genotypes formed a major cluster A. These genotypes came under culinary melon. In this cluster A genotypes were again grouped into six different subclusters *viz.*, A2(CM 6, CM 9), A4(CM 13, CM 14, CM 3), A6 (CM 40, CM 39), A8(CM 26, CM 4), A10(CM 29, CM 37, CM 24) and A9 (CM 11, CM 12, CM 10, CM 8, CM 7, CM 20, CM 30, CM 18, CM 34, CM 33, CM 31, CM 22, CM 25, CM 23, CM 35, CM 38, CM 19, CM 36, CM 28, CM 21, CM 17, CM 16, CM 15, CM 1, CM 21). A similarity index of 65.4 per cent between CM 1 and CM 9 genotypes occurred within the cluster A.

In this study, six different clusters were not in accordance with geographical location, D^2 analysis and morphological traits like fruit colour and shape. Genotypes used in this study, had narrow genetic variation and the number of RAPD primers were not sufficient for accurate result. Owing to narrow genetic base, Mo-Suk *et al.* (1989) revealed that greater number of primers to explain interspecific relationship of melon. This was also confirmed by Staub *et al.* (1997). This suggests that more number of primers may be used for further investigation to obtain clarity in results.

The study revealed that RAPD markers are readily detectable to analyse the variations present among the *Cucumis* genotypes. RAPD markers can easily differentiate *Cucumis melo* cultivars, even closely related ones. The advantages of the RAPD techniques including its speed, low DNA template requirements and technical simplicity, make it a convenient tool for detecting genetic variation within the germplasm. Polymorphism obtained in the present study will be useful in fingerprinting and in determining genetic diversity among the *Cucumis melo* L. genotypes. For future studies on analysis of melon genotypes, broad genetic base genotypes and greater number of RAPD primers are to be included for accurate results.

The forty landraces of melon by comparing different methods like morphological, biochemical and molecular characterization of germplasm. Among these methods, molecular characterization (RAPD markers) gave better results in the comparison of genotypes which is also agreeable with morphological characterization.

Summary

6. SUMMARY

The present study “Morphological, biochemical and molecular characterization in landraces of melon (*Cucumis melo* L.)” was conducted at the Department of Olericulture and Centre for Plant Molecular Biology, College of Agriculture, Vellayani during the period 1999-2002. Forty diverse landraces of melon were collected from Kerala and Tamilnadu regions. The genotypes were evaluated during the summer season of 2000-2001. For this programme, morphological characters like days to first male flower, node number of first male flower, days to first female flower, node number of first female flower, sex ratio, vine length, number of primary branches, number of secondary branches, internodal length, leaf petiole length, leaf thickness, days to first harvest, fruit length, fruit girth, fruit diameter, average fruit weight, dry matter content, fruits per plant, seeds per fruit, 1000 seed weight, yield per plant and fruit shape and colour were used. The objectives of the study were to characterize the landraces of melon through morphological method like D^2 analysis, biochemical method like SDS-PAGE and molecular method like RAPD technique and to compare these three methods. The salient results of the study are summarized.

Morphological studies showed significant differences among the forty accessions for all the twenty two characters. Yield per vine was maximum in CM 32 genotype collected from Manjapra. CM 36 was the earliest female flowering genotype.

Genetic analysis indicated maximum phenotypic and genotypic coefficient of variation (PCV and GCV) for yield per plant followed by average fruit weight and fruits per vine. Dry matter content, days to first female flower, days to first male flower and days to first harvest had low PCV and GCV.

High heritability was observed for all the characters except number of primary branches.

Expected genetic advance as percentage of mean was maximum for yield per plant, average fruit weight, fruits per plant, fruit length, 1000 seed weight,

fruit diameter, sex ratio, vine length, fruit girth, leaf petiole length, number of secondary branch, node number of first female flower, leaf thickness and node number of first male flower.

High heritability coupled with genetic gain was observed in yield per plant followed by average fruit weight and fruits per plant.

At genotypic level, yield per plant was significant and positively correlated to sex ratio, vine length, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruit. Node number of first female flower had significant and negatively correlated with yield per plant.

In D^2 analysis, the forty landraces of melon were grouped into twenty clusters. Maximum number of genotypes was in cluster I and minimum in cluster XX. The dessert melon genotypes CM 2 and CM 5 came under cluster XIV. The slicing and culinary melon genotypes CM 4 and CM 38 came under cluster XVI. The intra cluster D value was highest for cluster XIX. Inter cluster D value was maximum between cluster XVIII and XX. The dessert types formed a separate cluster and the slicing type melon fell along with culinary melon genotypes. D^2 analysis results revealed that genetic diversity was not related to geographic diversity and morphological characters like fruit shape and colour. The D^2 analysis gave precise result to differentiation of culinary melon from dessert melon.

The protein yield extracted from the forty genotypes of *Cucumis melo* seeds ranged from 0.858 (CM 10) to 3.200 $\mu\text{g seed}^{-1}$ (CM 20). Totally, 20 different bands were produced in melon genotypes. Maximum of 18 bands occurred in CM 7 and CM 40. Least number of 5 bands was present in CM 2.

The dendrogram showed two major clusters viz., cluster A and cluster B. Cluster B had only one dessert genotype, CM 2. Cluster A was further grouped into A1, A3 and A5 and A6 sub clusters. In subcluster A6, the slicing melon (CM 4) fell along with culinary melon genotypes. A similarity index within major cluster A showed 37.5 per cent between CM 1 and CM 27. Similarly index between CM 2 (cluster B) and CM 1 (cluster A) showed 13.3 per cent. Seed

protein results were not in line with D^2 analysis, geographical location and morphological characters like fruit shape and colour.

DNA was isolated from the forty genotypes of *Cucumis melo*. The DNA yield of forty melon accessions ranged from 15 to 6985 $\mu\text{g ml}^{-1}$. The purity of DNA ($\text{OD}_{260}/\text{OD}_{280}$) ranged from 1.08 to 2.03. Each DNA sample was subjected to RAPD analysis. Out of forty six decamer primers, 31 yielded amplification products. One hundred and three bands were generated using forty six decamer primers (Operon Inc. CA, USA). Of these 99 bands (96.12%) were polymorphic. Four bands (3.88%) were monomorphic, which accounted to an average of 2.24 bands per primer. Seven primers showed high level of polymorphism. Finally, four promising primers viz., OPA-01, OPA-08, OPB-12 and OPB-20 produced reproducible banding pattern on at least two runs. These primers yielded twenty nine scorable bands with an average of 7.25 bands per primer.

The dendrogram expressed two major clusters viz., cluster A and B. Cluster B had the dessert genotypes of CM 2 and CM 5. Cluster A was further grouped into A2, A4, A6, A8, A9 and A10 subclusters. The subcluster A8 had slicing genotype CM 4 along with other culinary genotypes. A similarity index obtained within the cluster B showed 75 per cent between CM 2 and CM 5 genotypes. A similarity index within the cluster A showed 65.4 per cent between CM 1 and CM 9 genotypes. For the slicing genotype, RAPD results agree with D^2 and protein analysis results. Considering the dessert genotype, RAPD result was in line with D^2 analysis result. RAPD analysis result does not correlate with geographical location and morphological characters like fruit shape and colour.

By characterizing all the forty genotypes of *Cucumis melo* using morphological (D^2 analysis), biochemical (SDS-PAGE method) and molecular method (RAPD marker analysis), the last method (RAPD analysis) gave a perfect differentiation of dessert melon from culinary melon which is also in line with morphological characterization.

References

REFERENCES

- Abusaleha and Dutta, O.P. 1988. Interrelationship of yield components in cucumber. *Veg. Sci.* 15: 79-85
- Abusaleha and Dutta, O.P. 1990. Studies on variability, heritability and scope of improvement in cucumber. *Haryana J. Hort. Sci.* 19:349-352
- Akashi, Y., Fukuda, N., Wako, T., Masuda, M. and Kato, K. 2002. Genetic variation and phylogenetic relationships in East and South Asian melons, *Cucumis melo* L. based on the analysis of five isozymes. *Euphytica* 125: 385-396
- Allard, R.W. 1960. Principles of plant breeding, John Wiley and Sons, Inc., New York. p.89-98
- Al-Zahim, M.A., Ford-Lloyd, B.V. and Newbury, H.J. 1999. Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and cytological analysis. *Pl. Cell Rep.* 18: 473-477
- Angelica, V.V., Montarroyos, De Lima, M.A.G., Santos, E.O.D and Defranea, J.G.E. 2003. Isozyme analysis of an active cassava germplasm bank collection. *Euphytica* 130: 101-106
- Anolles, C. G., Bassam, G.B. and Gresshoff, P.J. 1991. DNA amplification on fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology* 9: 553-557
- Anolles, C.G. and Trigiano, R.N. 1997. Nucleic acid markers in agricultural biotechnology. *Agric. Biotech. News Inf.* 9: 235-242
- Archak, S., Gaikwad, A.B., Gautam, D., Rao, E.V.V.B., Swamy, K.R.M. and Karihaloo, J.L. 2003. DNA fingerprinting of Indian Cashew (*Anacardium occidentale* L.) varieties using RAPD and ISSR techniques. *Euphytica* 130: 397-404
- Archak, S., Karihaloo, J.L. and Jain, A. 2002. RAPD markers reveal narrowing genetic base of Indian tomato cultivars. *Curr. Sci.* 82: 1139-1143
- Arnan, G., Lallemand, J. and Bourgoïn, M. 2003. Fast and reliable strawberry cultivars identification using inter simple sequence repeat (ISSR) amplification. *Euphytica*, 129: 69-79
- *Artes, F., Escriche, A.J., Martinez, J.A., Marin, J.G. 1993. Quality factors in four varieties of melon. (*Cucumis melo* L.). *J. Food Qual.* 16: 17

- Asante, I.K. and Offei, S.K. 2003. RAPD – based genetic diversity study of fifty cassava (*Manihot esculenta* Crantz) genotypes. *Euphytica* 131: 113-119
- Astley, D. 1992. Preservation of genetic diversity and accession integrity. *Fld Crops Res.* 219: 205-224
- Babu, H.T.P. 2000. RAPD analysis to assess the genetic stability in tissue culture derived black pepper (*Piper nigrum* L.) plants. M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur, p. 89
- Banno, K., Yifei, L., Ishikawa, H., Nakano, S. and Nobatake, S. 2000. Isozymes and RAPD markers to identify the parent-hood of Japanese pear 'Kuratsuki'. *J. Jpn. Soc. hort. Sci.* 69: 208-213
- Baird, F., Cooper, B.S., Waugh, R., De Maine, M. and Powel, W. 1992. Molecular characterization of inter and intraspecific somatic hybrids of potato using random amplified polymorphic DNA (RAPD) markers. *Mol. Gen. Genet.* 233: 469-475
- Banerjee, N.S., Manoj, P. and Das, M.R. 1999. Male-sex associated RAPD markers in *Piper longum* L. *Curr. Sci.* 77: 693-695
- Baudracco–Arnas, S. 1995. A simple and inexpensive method for DNA extraction from *Cucumis melo* L. *Cucurbit Genet. Coop. Rep.* 18: 50-51
- Baudracco-Arnas, S. and Pitrat, M. 1996. A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, Isozyme, disease resistance and morphological markers. *Theor. Appl. Genet.* 93: 57-64
- Bautista, R., Crespillo, R., Canovas, F.M. and Gonzalo Claros, M. 2003. Identification of olive-tree cultivars with SCAR markers. *Euphytica* 129: 33-41
- Bhat, K.V., Bhat, S.R., Chandel, K.P.S., Lakhanpaul, S. and Ali, S. 1995. DNA fingerprinting of *Musa* cultivars with oligonucleotide probes specific for simple repeats motifs. *Genet. Anal. Tech. Appl.* 45:51-57
- Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* 72:248
- Bretting, P.K. and Widrlechner, M.P. 1995. Genetic markers and horticultural germplasm management. *HortScience.* 30: 1349-1356
- Brown, C.R., Yang, C.P., Mojtahedi, H., Santo, G.S. Masuelli, R. 1996. RFLP analysis of resistance to Columbia rootknot nematode derived from solanum bulb castanum in a BC₂ population. *Theor. Appl. Genet.* 92: 572-576

- Brown, P.T.H., Lang, F.D., Kranz, E. and Lorz, H. 1993. Analysis of single protoplasts and regenerated plants by PCR and RAPD technology. *Mol. Gen. Genet.* 237: 311-317
- Brown, R.N., Myers, J.E., Hutton, M. and Paul Miller, C. 1998. A simple protocol for isolating DNA from fresh Cucurbita leaves. *Cucurbit Genet. Coop. Rep.* 21: 46-47
- Burton, G.W. and Dewane, E.M., 1953. Estimating heritability in tall fescue (*Festuca circunelinaical*) from replicated clonal material. *Agron. J.* 45:478-481.
- Caetano-Anolles, G., Bassam, B.J. and Greshoff, P.M. 1991. DNA amplification fingerprinting: a strategy for genome analysis. *Pl. Mol. Biol. Rep.* 9: 294-307
- *Cerne, M. 1984. Growth and development of pickling cucumber (*Cucumis sativus* L.) as a function of weather conditions and the use of plastics. *Poljoprivredna – Znanstvena smotra.* 67: 451-466
- Cerne, M. 1989. Correlation between some parameters of vegetative and generative developments of less grown pickling cucumber. *Acta Hort.* 242:335-338
- Chacko, E. 1992. Evaluation of dessert type of musk melon (*Cucumis melo* L.) for southern region of Kerala. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p.35-80
- Chakraparti, A., Santha, I.M. and Mehta, S.L. 1999. Molecular characterization of low ODAP somaclones of *Lathyrus sativus*. *J. Pl. Biochem. Biotech.* 8: 25-29
- Chen, X.H., Cao, P.S., Xu, Q. and Dong, G. 1994. Genetic correlation and path coefficient analysis of parthenocarpic yield components of cucumber. In: *Advances in Horticulture* (Ed. Meng, L.Y.) p.249-251
- Chhonkar, V.S., Singh, D.N. and Singh, R.L. 1979. Genetic variability and correlation studies in muskmelon. *Indian J. agric. Sci.* 49: 361-363
- Choudhary, H. and Hari Har Ram 2000. Characterization of indigenous muskmelon germplasm lines based on SDS-PAGE of seed proteins. *Veg. Sci.* 27: 35-38.
- Choudhary, M.L. and Mandal, G. 1987. Correlation and path analysis in cucumber (*Cucumis sativus* L.). *Haryana J. Hort. Sci.* 16:269-273

- Choudhary, M.L., Joshi, S. and Amar Singh. 1985. Genetic studies in cucumber (*Cucumis sativus* L.). *Prog. Hort.* 17:236-240
- Christophani, M., Machado, M.A. and Grattapaglia, D. 1999. Genetic linkage mapping of citrus Sunki Hort and *Poncirus trifoliata* (L.) and mapping of citrus tristeza virus resistance gene. *Euphytica* 109: 25-32
- Chunwongse, J., Chunwongse, C., Black, L. and Hunson, P. 2002. Molecular mapping of the Ph-3 gene for late blight resistance in tomato. *J. hort. Sci. Biotech.* 77: 281-286
- Cooke, R.J. 1984. The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5:59-72
- Cooke, R.J. 1994. The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5: 59-72
- Corazza-Nunes, M.J., Machado, M.A., Nunes, W.M.C., Cristofani, M. and Targon, M.L.P.N. 2002. Assessment of genetic variability in grapefruits (*Citrus paradisi* Macf.) and pummelos (*C. maxima* (Burm.) Merr.) using RAPD and SSR markers. *Euphytica* 126: 169-176
- Cortes, D.F., Reilly, K., Okogbenin, E., Beeching, J.R., Iglesias, C. and Tohme, J. 2002. Mapping wound – response genes involved in post-harvest physiological deterioration (PPD) of cassava (*Manihot esculenta* Crantz). *Euphytica* 128: 47-53
- Crouch, J.H., Crouch, H.K., Constandt, H., Gysel, A.V., Breyne, P., Montagu, M.V., Jarret, R.L. and Ortiz, R. 1999. Comparison of PCR-based molecular marker analysis of *Musa* breeding populations. *Mol. Breed.* 5: 233-244
- Damasco, O.P., Adkins, S.W., Godwin, I.D. and Smith, M.K. 1998. Use of a SCAR-based marker for early detection of dwarf off-types in micropropagated Cavendish bananas. *Acta Hort.* 461: 157-164
- Damasco, O.P., Graham, C.G., Henry, J.R., Adkins, S.W., Smith, M.K. and Godwin, I.D. 1996. Random amplified polymorphic DNA (RAPD) detection of dwarf off types in micropropagated Cavendish (*Musa* spp. AAA) bananas. *Pl. Cell Rep.* 16: 118-123
- Damin-Poleg, Y., Reis, N., Tzuri, G. and Katzir, N. 2001. Development and characterization of microsatellite markers in Cucumis. *Theor. Appl. Genet.* 102: 61-72

- Debener, T. and Mattiesch, L. 1999. Construction of a genetic linkage map for roses using RAPD and AFLP markers. *Theor. Appl. Genet.* 99: 891-899
- Debener, T., Bartels, C. and Mattiesch, L. 1996. RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. *Mol. Breed* 2: 321-327
- Demeke, T., Adams, R.P. and Chibbar, R. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. *Theor. Appl. Genet.* 84: 990-994
- Deol, S.S., Nandpuri, K.S. and Sukhija, B.S. 1981. Genetic variability and correlation studies in muskmelon (*Cucumis melon* L.). *Punjab Veg. Gr.* 15: 18-26
- Deol, S.S., Nandpuri, K.S. and Sukhija, B.S. 1974. Genetic variability and correlation studies in musk melon (*Cucumis melo* L.). *J. Res. (PAN)*. 11: 18-26
- Deputy, J., Ming, R., Ma, H., Liu, Z., Fitch, M., Wang, M., Manshardt, R. and Stiles, J. 2002. Molecular markers for sex determination in papaya (*Carica papaya* L.). *Theor. Appl. Genet.* 106: 107-111
- Deverna, J.W. and Alpert, K.B. 1990. RFLP technology. Horticultural Biotechnology: *Royal Horticultural Biotechnology Symposium, November 7-9, 1989*. (eds. Bennett, A.B. and Neil, S.D.O), University of California, Wiley-Liss, New York, pp. 247-250
- Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis components of crested wheat grass seed production. *Agron. J.* 51:515-518
- Dhanaraj, A.L., Bhaskara Rao, E.V.V., Swamy, K.R.M., Bhatt, M.G., Theertha Prasad, D. and Sondur, S.N. 2002. Using RAPDs to assess the diversity in Indian cashew (*Anacardium occidentale* L.) germplasm. *J. hort. Sci. Biotech.* 77: 41-47
- *Duke, J.A. and Ayensu, E.S. 1985. Medicinal plants of China. Vol.1. Reference publications, Algonac, Michigam. p.362
- Duneman, F., Kahuan, R. and Schmidt, H. 1994. Genetic relationship in *Malus* evaluated by RAPD fingerprinting of cultivars and wild species. *Pl. Breed.* 113: 150-159
- Duran, Y., Rohde, W., Kullaya, A., Goikoetxea, P. and Ritter, E. 1997. Molecular analysis of East African tall coconut genotypes by DNA marker technology. *J. Genet. Breed.* 51: 279-288

- Egashira, H., Ishihara, H., Takashiua, T. and Imanishi, S. 2000. Genetic diversity of the peruvianum – Complex' (*Lycopersicon peruvianum* (L.) Mill and *L. Chilense* Dun.) revealed by RAPD analysis. *Euphytica* 116:23-31
- Ekoskit, K., Thompson, P.G. and Watson, C.F. 1997. Identifying a randomly amplified polymorphic DNA (RAPD) marker linked to a gene for root-knot Nematode Resistance in sweet potato. *J. Am. Soc. Hort. Sci.* 122: 818-821
- *Elkner, K., Krysiak, J. 1984. An appraisal of physical and chemical characteristics of fruits of several determinate melon varieties. *Biuletyn – Warzywniczy*, 27: 33-48
- El-Tarras, A., Hossni, Y.A., Elbanna, A.A. and Shehata, S. H. M. 2001. Identifying strawberry cultivars using protein pattern (PAGE) and random amplified polymorphic DNA (RAPD) markers. *Egypt. J. Hort.* 28: 15-25
- Epplen, J.T., Ammer, H. and Epplen, C. 1991. Oligonucleotide fingerprinting using simple repeat motifs: a convenient, ubiquitously applicable method to detect hypervariability for multiple purposes. *DNA finger printing: Approaches and Applications* (eds. Burke, T., Dolf, G., Jeffreys, A.J. and Wolff, R.). Birkhauser, Basel, pp. 50-69
- Erlich, H.A., Gelfand, D. and Srinsky, G. 1991. Recent advances in the polymerase chain reaction. *Science* 252: 1643-1651
- Faure, S., Noyer, J.L., Horry, J.P., Bakry, F., Lanaud, C. and Gonzalez, D. 1993. A molecular marker based linkage map of diploid bananas. (*Musa acuminata*). *Theor. Appl. Genet.* 87: 517-526
- Fazio, G., Chung, S.M. and Staub, J. 2003. Comparative analysis of response to phenotypic and marker assisted selection for multiple lateral branching in cucumber (*Cucumis sativus* L.). *Theor. Appl. Genet.* 107: 875-883
- Fazio, G., Staub, J.E. and Stevens, M.R. 2003a. Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. *Theor. Appl. Genet.* 107: 864-874
- Fazio, G., Stevens, M.R. and Scott, J.W. 1999. Identification of RAPD markers linked to fusarium crown and root rot resistance (Frl) in tomato. *Euphytica* 105: 205-210
- Galvan, M.Z., Bornet, B., Balatti, P.A. and Branchard, M. 2003. Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica* 132:297-307

- Garcia Lampasona, S., Martinez, L. and Burba, J.L. 2003. Genetic diversity among selected Argentinean garlic clones (*Allium sativum* L.) using AFLP (Amplified Fragment Length Polymorphism). *Euphytica* 132: 115-119
- Garcia, E., Jamilena, M., Alvarez, J.I., Amedo, T., Oliver, J.L. and Lozana, R. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. *Theor. Appl. Genet.* 96: 878-885
- Garcia-Mas, J., Oliver, M., Gomez-Paniagua, H. and De Vincente, M.C. 2000. Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. *Theor. Appl. Genet.* 101: 860-864
- Gaulao, L., Cabrita, L., Oliveira, C.M. and Leitao, M.J. 2001. Comparing RAPD and AFLP analysis in discrimination and estimation of genetic similarities among apple cultivars. *Euphytica* 119: 259-270
- Gayathri, K. 1997. Genetic variability and heterosis in cucumber (*Cucumis sativus* L.) M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p. 41-92
- Giancola, S., Marcucci Poltri, S., Lacaze, P. and Hopp, H.E. 2002. Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica* 127: 95-113
- Gopalakrishnan, T.R., Gopalakrishnan, P.K. and Peter, K.V. 1980. Variability, heritability and correlation among some polygenic characters in pumpkin. *Indian J. agric. Sci.* 50(12): 925-930
- Gopalakrishnan, P.K., Joseph, S., Gopalakrishnan, T.R. and Peter, K.V. 1983. 'Mudikkode Local', a high yielding oriental pickling melon variety. *Indian J. Hort.* 27: 28
- Gregova, E., Zavodna, M. and Kraic, J. 1999. Identification and molecular characterization of durum wheats (*Triticum durum* Desf.) and comparison of identity of samples maintained in active and work collections. *Pl. Genet. Resour. Newsl.* 119:37-40
- *Grubben, G.J.H. 1977. Tropical vegetable and their genetic resources. IBPGR, Rome. p.197
- Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B. 1996. Microsatellites in plants-A new class of molecular markers. *Curr. Sci.* 70: 45-53
- Gurudeep, K., Lal, T., Nandpuri, K.S. and Sharma, S. 1977. Varietal-cum seasonal variation in certain physio-chemical constituents in muskmelon. *Indian J. agric. Sci.* 47: 284-287

- Hammerschlag, F.A., Hashmi, G., Huettel, R., Werner, D. and Ritchie, P. 1996. *Invitro* selection and *invitro* screening for disease resistance in fruit trees. *HortScience*. 31: 695-696
- Haribabu, K. 1985. Correlation studies in cucumber (*Cucumis sativus* L.) *South Indian Hort*. 33: 129-130
- Hashmi, G., Huettel, R., Meyor, R., Krusberg, L. and Hammerschlag, F. 1997. RAPD analysis of somaclonal variants derived from embryo callus cultures of peach. *Pl. Cell Rep*. 16: 624-627
- Hemant Kumar, N.V., Narayanswamy, P., Theerthaprasad, D., Mukunda, G.K. and Sondur, S.N. 2001. Estimation of genetic diversity of commercial mango (*Mangifera indica* L.) cultivars using RAPD markers. *J. hort. Sci. Biotech*. 76: 529-33
- Hen, G., Neitz AWH and Louw AI. 1992. Identification of tomato cultivars (*Lycopersicon esculentum*) by polyacrylamide isoelectric focusing. *Euphytica* 62:77-82
- Herrera, R., Cares, V., Wilkinson, M.J. and Caligari, P.D.S. 2002. Characterisation of genetic variation between *Vitis vinifera* cultivars from Central Chile using RAPD and inter simple sequence repeats (ISSRs) markers. *Euphytica* 124: 139-145
- Hills, D.M. and Moritz, C. 1990. *Molecular Systematics*. Sinauer Association, Sunderland, Mass, p. 490
- Hong, D.J., Shuang, S.H. and Qin, Z.X. 1999. Rapid identification of tomato somaclonal variation with RAPD. *J. trop. Subtrop. Bot*. 7: 308-312
- Horejsi, T., Box, J. and Staub, J.E. 1999. Efficiency of RAPD to SCAR marker conversion and their comparative PCR sensitivity in cucumber. *J. Am. Soc. hort. Sci*. 124: 128-135
- Horejsi, T., Staub, J.E. and Thomas, C. 2000. Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.). *Euphytica* 115: 105-113
- Horoki, T., Terabayasni, S. and Asohira, T. 1986. Morphological and biochemical classification of spring flowering gladiolus and the relationship between major cultivars. *J. Jpn. Soc. hort. Sci*. 55:326-331
- Hurtado, M.A., Westman, A., Beck, E., Abbott, G.A., Llacer, G. and Badenes, M.L. 2002. Genetic diversity in apricot cultivars based on AFLP markers. *Euphytica* 127: 297-301

- Imam, M.K., Abobaker, M.A. and Yacoub, H.M. 1977. Inheritance of some characters in cucumbers II some quantitative characters. *Libyan J. Agric.* 6: 115-125
- Jain, J.P. 1982. Statistical Technique in Quantitative Genetics. Tata Mc Graw-Hill Co., New Delhi. p.281
- Jeffrey, A.J., Wilson, V. and Thein, S.L. 1985. DNA fingerprinting in plants. *Nature* 314: 67-73
- Johnson, H.W., Robinson, H.P. and Comstock, R.E. 1955. Estimate of genetic and environmental variability in soybean. *Agron. J.* 44:314-318
- Johnson, H.W., Robinson, H.P. and Comstock, R.N. 1955a. Estimates of genetic and environmental variability in soybean. *Agron. J.* 47: 311-348
- Joshi, S., Joshi, M.C., Singh, B. and Vishnoi, A.K. 1981. Genotypic and phenotypic variability in cucumber (*Cucumis sativus* L.). *Veg. Sci.* 8:114-119
- Joshi, S.P., Prabhakar, K.R. and Vidhya, S.G. 1999. Molecular markers in plant genome analysis. *Curr. Sci.* 77: 230-240
- Jun, J.H., Chung, K.H., Jeong, S.B. and Lee, H.J. 2002. Identification of RAPD and SCAR markers linked to the flesh adhesion gene F in peach (*Prunus persica* (L.) Batsch. *J. hort. Sci. Biotech.* 77:598-603
- Kaemmer, D., Afza, A., Weising, K., Kahl, G. and Novak, F. J. 1993. Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.). *Biotechnology* 10: 1030-1035
- Kahangi, E.M., Lawton, M.A. and Kumar, C.A.C.Y. 2002. RAPD profiling of some banana varieties selected by small scale farmers in Kenya. *J. hort. Sci. Biotech.* 77: 393-398
- Kallo, G. and Dixit, J. 1983. Genetic components for yield and its contributing traits in muskmelon (*Cucumis melo*). *Haryana J. Hort. Sci.* 12:218-220.
- Kaloo, G., Dixit, J. and Sidhu, A.S. 1979. Path coefficient analysis in muskmelon (*Cucumis melo* L.). *Indian J. Hort.* 39: 243-246
- Kaloo, G., Dixit, J. and Sidhu, A.S. 1982. Studies on genetic variability and character association in muskmelon (*Cucumis melo* L.). *Indian J. Hort.* 38:79-85
- Katzir, N., Damin-Poleg, Y., Tzuri, G., Karchi, Z., Lavi, U. and Cregan, P.B. 1996. Length polymorphism and homologies of microsatellites in several cucurbitaceae species. *Theor. Appl. Genet.* 93: 1282-1290

- KAU. 1996. Package of Practices Recommendations – “Crops 96”. Directorate of Extension, Kerala Agricultural University, Thrissur, p.169-171
- Kaundun, S.S., Zyvoloup, A. and Park, Y.G. 2000. Evaluation of the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica* 115: 7-16
- Kennard, W.C., Poetter, K., Dijkhuizen, A., Meglic, V., Staub, J. and Havey, M. 1994. Linkages among RFLP, RAPD, isozyme, wide crosses of cucumber. *Theor. Appl. Genet.* 89:42-48
- Knavel, D.E. 1991. Productivity and growth of short internode muskmelon plants at various spacing or densities. *J. Am. Soc. hort. Sci.* 116:926-929
- Knerr, L.D. and Staub, J.E. 1992. Inheritance and linkage relationships of isozymes loci in cucumber (*Cucumis sativus* L.). *Theor. Appl. Genet.* 84:217-224
- Krishna Prasad, V.S.R. and Singh, D.P. 1991. Studies on genotypic performance and their interactions with the environment in cucumber (*Cucumis sativus* L.). *Indian J. Hort.* 48: 346-350
- Krishna Prasad, V.S.R., Pichaimuthu, M. and Dutta, O.P. 2002. Adaptive responses and diversity pattern in watermelon. *Indian J. Hort.* 59:298-306
- Kuginuki, Y., Ajisaka, H., Yie, M., Yoshikawa, H., Hida, K.I. and Hirai, M. 1997. RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. *Euphytica* 98: 149-154
- Kuhns, L.J. and Fretz, T.A. 1978. Distinguishing rose cultivars by polyacrylamide gel electrophoresis 11. Isozyme variation among cultivars. *J. Am. Soc. hort. Sci.* 103: 409-416
- *Kuo, H.J., Hsiao, C.H., Chang, W.N., 1988. Studies on flower type transfer and the correlation between flower type and fruit shape in melon (*Cucumis melo* L.). *J. Agric. Res. China* 37: 142-150
- *Ladizinsky, G. and Hymovitz, T. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.* 54:145-151
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assemble of the head bacteriophage T₄. *Nature* 5259:680-685
- Lal, T. and Singh, S. 1997. Genetic variability and selection indices in melon (*Cucumis melo* L.). *Veg. Sci.* 24: 111-117

- Laltaprasad, Gautam, N.C. and Singh, S.P. 1988. Studies on genetic variability and heritability of some important quantitative characters association in watermelon (*Citrullus lanatus* (Thumb.) Mansf.). *Veg. Sci.* 15: 86-94
- Landry, B.S., Li, R.Q., Cheung, W.Y. and Granger, R.L. 1994. Phylogeny analysis of 25 apple rootstocks using RAPD markers and tactical gene tagging. *Theor. Appl. Genet.* 89:847-852
- Lashermes, P., Tronslot, P., Anthony, F., Combes, M.C. and Charrier, A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. *Euphytica* 87: 59-64
- Lemos, E.G.M., Silva, C.L.S.P. and Zaiden, H.A. 2002. Identification of sex in *Carica papaya* L. using RAPD markers. *Euphytica* 127: 179-184
- Lenka, D. and Mishra, B. 1973. Path coefficient analysis of yield in rice varieties. *Indian J. agric. Sci.* 43: 376-379
- Levi, A. and Rowland, L.J. 1997. Identifying blueberry cultivars and evaluating their genetic relationships using RAPD and SSRs anchored primers. *J. Am. Soc. hort. Sci.* 122: 74-78
- Liang Chen and Yamaguchi, S. 2002. Genetic diversity and phylogeny of tea plant (*Camellia sinensis*) and its related species and varieties in the section. *Thea* genus *Camellia* determine by randomly amplified polymorphic DNA analysis. *J. hort. Sci. Biotech.* 77: 729-732
- Lim, S.H., Teng, P.C.P., Lee, Y.H. and Goh, C.J. 1999. RAPD analysis of some species in the genus *Vanda*. *Ann. Bot.* 83: 193-196
- Litt, M. and Luty, J.A. 1989. A hypervariable microsatellite revealed by *invitro* amplification of a dinucleotide repeat within cardiac muscle antigen. *Am. J. Human Genet.* 44: 397-401
- Lopez-Sese, A.I. and Staub, J.E. 2001. Frequency of RAPD polymorphisms in melon (*Cucumis melo* L.) germplasm in different geographic regions. *Cucurbit Genet. Coop. Rep.* 24:76-79
- Lopez-Sese, A.I., Staub, J., Katzir, N. and Guillaumon, M.L.G. 2002. Estimation of between and within accession variation in selected Spanish melon germplasm using RAPD and SSR markers to assess strategies for large collection evaluation. *Euphytica* 127:41-51
- *Lubis, S.H.A., Sastrapradja, D. and Sastrapradja, S. 1977. Variation in seed protein content of *Vigna unguiculata* (L.) Walp. *Annales Bogorienses.* 6:125-132

- *Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proc. Natl. Acad. Sci. India*. 2: 49-55
- Margoliash, E., Fitch, W.M. 1968. Evolutionary variability of cytochrome C primary structures. *Ann. NY Acad. Sci.* 54:145-151
- Mariappan, S. and Pappiah, C.M. 1990. Genetic studies in cucumber (*C. sativus* L.). *South Indian Hort.* 38:70-74
- Martin, C., Uberhuaga, E. and Perez, C. 2002. Application of RAPD markers in the characterization of chrysanthemum varieties and the assessment of somaclonal variation. *Euphytica* 127: 247-253
- Martinez – Gomez, P., Arulsekhar, S., Potter, D. and Gradziel, T.M. 2003. An extended interspecific gene pool available to peach and almond breeding as characterized using simple sequence repeat (SSR) markers. *Euphytica* 131: 313-322
- Mc Collum, T.G., Cantliffe, D.J. and Parks, H.S. 1987. Flowering, fruitset and fruit development in birdsnest type muskmelons. *J. Am. Soc. hort. Sci.* 112: 161-164
- *Mc Daniel, R.G. 1970. Electrophoretic characterization of proteins in *Hordeum*. *J. Heredity* 61:243-247
- Meglic, V.V., Horejsi, T.F., Mc Creight, J.D. and Staub, J.E. 1994. Genetic diversity and inheritance and linkage of isozyme loci in melon (*Cucumis melo* L.). *HortScience*. 29: 449
- *Meng, Z., Xiaofen, N., Wen, C.H. 1999. Genetic path analysis of early yield in cucumber. *Cucurbit Genet. Coop. Rep.* 22: 3-4
- Merrenijk, Gam-Van, Bino, R.J. and Sums, L.C.J.M. 1986. Characterization of cytoplasmic male sterility in petunia hybrida. 1. Location, Composition activity of extracts, *Euphytica* 35:77-78
- Messequer, R. and Arnas, P. 1985. Electrophoretic identification of carnation cultivars. *HortScience*. 20: 372-373
- Miller, J.C. 1985. Genetic studies on time of flowering as components of earliness of cucumber. *HortScience*. 10: 319-321
- Miller, J.C. and Quisenberry, E.J. 1986. Inheritance of time of flowering and its relationship to crop maturity in cucumber. *J. Am. Soc. hort. Sci.* 101:497-501
- Miller, J.C. and Tanksley, S.D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.* 80: 437-448

- Mneney, E.E., Mantell, S.H. and Bennett, M. 2001. Use of random amplified polymorphic DNA (RAPD) markers to reveal genetic diversity within and between populations of cashew (*Anacardium occidentale* L.). *J. hort. Sci. Biotech.* 76: 375-83
- More, T.A., Mishra, J.P., Seshadri, V.S., Doshi, S.P., Sharma, J.C. 1987. Association of fruit shape with flesh area and flesh proportion in muskmelon. *Ann. Agric. Res.* 8: 237-242
- Moretzsohn, M.C., Ferreira, M.A., Amaral, Z.P.S., Coelho, P.J.A., Grattapaglia, D. and Ferreira, M.E. 2002. Genetic diversity of Brazilian oil palm (*Elaeis oleifera* H.B.K.) germplasm collected in the Amazon Forest. *Euphytica* 124: 35-45
- Moretzsohn, M.C., Nunes, C.D.M., Ferreira, M.E. and Grattapaglia, D. 2000. RAPD linkage mapping of shell thickness locus in oil palm. *Theor. Appl. Genet.* 100: 63-70
- Mo-Suk, Y., Im-Sung, H., Go-Gawn, D., Ann-Chong, M., Dim-Doo, H., Mo-Suk, Y. and Im, S.H. 1999. RAPD analysis of genetic diversity of melon species. *Korean J. hort. Sci. Tech.* 16:21-24
- Munthali, M.T., Newbury, H.J. and Ford-Lloyd, B.V. 1996. The detection of somaclonal variants of beet using RAPD. *Pl. Cell. Rep.* 15:474-478
- Murthy, B.R. and Arunachalam. 1966. The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet.* 26A (Symp. No.): 188-198
- Myers, G.O., Falokun, C.A. and Young, N.D. 1996. RFLP mapping of an aphid resistance gene in cowpea (*Vigna unguiculata* L. Walp). *Euphytica* 91:181-187
- Nagaprasuna, R. and Rama Rao, M. 1988. Variability studies in cucumber (*Cucumis sativus* L.). *South Indian Hort.* 36: 237-241
- Nagaprasuna, R. and Rama Rao, M. 1989. Correlation studies and path coefficient analysis in the segregating population of cucumber *Cucumis* sp.). *South Indian Hort.* 37: 212-214
- Nair, N.V., Selvi, A., Sreenivasan, T.V. and Pushpalatha, K.N. 2002. Molecular diversity in Indian sugarcane cultivars as revealed by randomly amplified DNA polymorphisms. *Euphytica* 127: 219-225
- *Nakamura, H., Ischiuchi, D., 1985. Evaluation of local varieties of oriental pickling melon as a useful vegetable in summer season in Okinawa. *Japan agric. Res.* 19:145-150

- Nandpuri, K.S. 1989. Muskmelon (*Cucumis melo* Lin.). *Indian J. Hort.* 33:38-39
- Nandpuri, K.S. and Lal, T. 1978. Varietal response to date of planting in muskmelon. *Veg. Sci.* 5: 8-14
- Nandpuri, K.S., Lal, T. and Singh, S. 1976. Vegetative and reproductive behaviour of muskmelon as studied under diverse conditions. *Indian J. Hort.* 33: 246-251
- Nandpuri, K.S., Singh, S. and Lal, T. 1975. Germplasm scrutiny for the improvement of some economic characters in muskmelon (*Cucumis melo* L.). *J. Res. Punjab agric. Univ.* 12: 252-257
- Natarella, N.J. and Sink, K.C. 1975. Electrophoretic analysis of proteins and peroxidases of selected petunia species and cultivars. *Bot. Gaz.* 132:20-26
- *Naudin, C. 1859. Review des. Cucurbitaceae cultivars on Museum. *Ann. Sci. Natl. Ser. Bot.* 12:79-164
- Nayar, A.S. 2001. Molecular evaluation of genomic stability of banana plants developed by *in vitro* clonal propagation. M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur, p.68
- Nerson, H., Paris, H.S., Edelstein, M., Burgen, Y. and Karchi, Z. 1988. Breeding pickling melon for a concentrated yield. *HortScience.* 23: 136-138
- Newbury, H.J. and Ford-Lloyd, B.V. 1993. The use of RAPD for assessing variation in plants. *Pl. growth regulation.* 12:43-51
- Nkongolo, K.K. 2003. Genetic characterization of Malawian Cowpea (*Vigna unguiculata* (L.) Walp) landraces: diversity and gene flow among accessions. *Euphytica* 129: 219-228
- Norton, J.D., Cospers, R.D., Smith, D.A., Rymal, K.S. 1985. 'Aurora' muskmelon, *HortScience.* 20: 955-956
- Obara-Okeyo, P. and Kako, S. 1998. Genetic diversity and identification of Cymbidium cultivars as measured by random amplified polymorphic DNA (RAPD) markers. *Euphytica* 99: 95-101
- *Oghiakhe, S., Cook, R.J., Jackai, L.E.N. 1993. The characterization of cultivars of cowpeas *Vigna unguiculata* (L.) Walp and *Vigna vexillata* Benth. By electrophoresis of seed proteins. *Trop. Agric.* 70:190-192
- Ohmori, T., Murata, M. and Motoyoshi, F. 1995. Molecular characterization of RAPD and SCAR markers linked to the TM-1 locus in tomato. *Theor Appl. Genet.* 92:151-156
- Ohmori, T., Murata, M. and Motoyoshi, F. 1996. Identification of RAPD markers linked to the Tm-2 locus in tomato. *Theor. Appl. Genet.* 90:307-311

- Ortiz, R., Madsen, S. and Vuylsteke, D. 1998. Classification of African plantain landraces and banana cultivars using a phenotypic distance index of quantitative descriptors. *Theor. Appl. Genet.* 96: 904-911
- Owents, K.W., Bliss, F.A. and Peterson, C.E. 1983. Genetic variation with in and between two cucumber populations derived via the inbred back-cross line method. *J. Am. Soc. hort. Sci.* 110: 437-440
- Pandita, M.L., Dahiya, M.S. and Vashistha, R.N. 1990. Correlation and path coefficient in roundmelon. *Research and development reporter.* 7:106-110
- Paran, I. and Michelmore, R.W. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet.* 85: 985-993
- Parent, J.G. and Page, D. 1998. Identification of raspberry cultivars by sequence characterized amplified region DNA analysis. *HortScience.* 33: 140-142
- Paris, H.S., Yonash, N., Portnoy, V., Mozes-Danbe, N., Tzuri, G. and Katzir, N. 2003. Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theor. Appl. Genet.* 106: 971-978
- Park, S.O., Coyne, D.P., Dorson, A. and Jung, G. 1998. Identifying randomly amplified polymorphic DNA (RAPD) markers linked to major genes for common Bacterial Blight Resistance in Tepary Bean. *J. Am. Soc. hort. Sci.* 123: 278-282
- Patil, R.B. and Bhapkar, D.G. 1987. Genetics divergence among 49 cowpea strains. *J. Maharashtra agric. Univ.* 3:283-285
- Pattanayak, D., Chakrabarti, S.K. and Naik, P.S. 2002. Genetic diversity of late blight resistant and susceptible Indian potato cultivars revealed by RAPD markers. *Euphytica* 128: 183-189
- Perin, C., Hagen, L., De Conto, V., Katzir, N., Damin-Poleg, Y., Portnoy, V., Baudracco-Arnas, S., Chadoecy, J., Dogimont, C. and Pitrat, M. 2002. A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor. Appl. Genet.* 104:1017-1034
- Poleg, Y.D., Tadmor, Y., Tzuri, G., Reis, N., Hirschberg, J. and Katzir, N. 2002. Construction of a genetic map of melon with molecular markers and horticultural traits and localization of genes associated with ZYMV resistance. *Euphytica* 125: 373-384
- Prakash, D.P., Narayanswamy, P. and Suresh N. S. 2002. Analysis of molecular diversity in guava using RAPD markers. *J. hort. Sci. Biotech.* 77:287-293
- Prasad, V.S.R.K. and Singh, D.P. 1992. Estimates of heritability, genetic advance and association between yield and its components in cucumber (*Cucumis sativus* L.). *Indian J. Hort.* 49: 62-69

- Prasannalatha, C.H., Kaur, P. and Bhalla, J.K. 1999. Molecular characterization of somaclonal variants in pigeon pea. *Curr. Sci.* 76: 693-695
- Prasanna, M.N. and Rao, M.R. 1989. Correlation studies and path coefficient analysis in the segregating population of cucumber. *South Indian Hort.* 37: 212-214
- *Prudek, M. and Wolf, J. 1985. Combining ability and phenotypic stability for yield components in field – grown salad cucumbers. *Acta Universitatis – Agriculture – Brno.* 33: 91-98
- Rajendran, P.C. and Thamburaj, S. 1993. Interassociation of characters in watermelon. *Madras Agric. J.* 80: 207-209
- Rajput, J.C., Palve, S.B. and Jamadagni, B.M. 1991. Correlation and pathanalysis studies in cucumber (*Cucumis sativus* L.). *Maharashtra J. Hort.* 5:52-55
- Rakhi, R. 2001. Collection and characterization of Landraces of culinary Melon (*Cucumis melo* L.) in Kerala M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p.42-79
- Ramayya, D.A. and Azeemoddin, G. 1983. Oil from melon seeds. *Indian Express* (English Daily) 31st October. 1983
- Rana, R.S. 1993. Genetic Resources in Vegetable Crops. In *Advances in Horticulture Vol.5. Vegetable Crops Part I* (1993) Ed: K.L. Chadha and G.Kaloo, Malhotra Publishing House, New Delhi. P. 27-43
- Rao, C.R. 1952. *Advanced Statistical Methods in Biometric Research.* John Wiley and Sons. Inc., New York. P.390
- Rao, E.S., Verma, V.K. and Munshi, A.D. 2003. Breeding potential of cucumber (*Cucumis sativus* L.) genotypes using D² analysis. *Indian J. Hort.* 60:53-58
- Rao, R., Vaglio, M. Del, Paino D Urzo, M. and Monti, L. 1992. Identification of *Vigna spp.* through specific seed storage polypeptides. *Euphytica* 62:39-43
- Rastogi, K.B. and Aryadeep. 1990. Variability studies in cucumber (*Cucumis sativus* L.). *Veg. Sci.* 17:224-226
- Ravishankar, K.V., Anand, L. and Dinesh, M.R. 2000. Assessment of genetic relatedness among mango cultivars of India using RAPD markers. *J. hort. Sci. Biotech.* 75: 198-210
- Roberto, A., Young and Kelly, J.D. 1996. RAPD markers flanking the Are gene for anthracnose resistance in common bean. *J. Am. Soc. hort. Sci.* 121:37-41

- Robinson, R.W., Munger, H.M., Whitaker, T.W. and Bohn, G. 1976. Genes of the cucurbitaceae. *HortScience*. 11: 554-568
- Rolf, F.J. 1997. NTSYS-pc: Numerical taxonomy and multi variate analysis system. Exeter Software, New York, USA
- Saikia, J., Shadeque, A. and Bora, G.C. 1995. Genetic studies in cucumber. *Haryana J. Hort. Sci.* 24: 73-76
- Saikia, J., Shadeque, A. and Bora, G.C. 1995a. Studies on correlation and path coefficient analysis in cucumber (*Cucumis sativus* L.). *Haryana J. Hort. Sci.* 24: 126-130
- *Salk, A. 1982. Studies of correlations of some characters associated with yield in melons. *Ege-University-Ziraat-Fatultesi-Dergisi*. 19:19-26
- Sandbrink, J.M., Van Ooijen, J.W., Purimahua, C.C., Vriclink, M., Verkerk, R., Zabel, P. and Lindhout, I. 1995. Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using RFLPs. *Theor. Appl. Genet.* 90:444-450
- Sanwen, H., Baoxi, Z., Milbourne, Cardle, L., Guimei, Y. and Jiazhen, G. 2001. Development of pepper SSR markers from sequence databases. *Euphytica* 117: 163-167
- Satyanarayana, N. 1991. Genetical studies in cucumber (*Cucumis sativus* L.) Ph.D. (Hort.) thesis, University of Agricultural Science, Bangalore. p.56-65
- Schnell, R.J., Knight, R.J. and Schaffer, B. 1993. Genetic relationships among *Mangifera* spp. based on RAPD markers. *Acta Hort.* 341: 86-96
- Serrano, B., Gomez – Aparishi, J. and Hormaza, J.I. (2002). Molecular finger printing of prunus rootstocks using SSRs. *J. hort. Sci. Biotech.* 77: 368-372
- Seshadri, V.S. and Chatterjee, S.S. 1996. The history and adaptation of some introduced vegetable crops in India. *Veg. Sci.* 23: 114-139
- Seshadri, V.S. and More, T.A. 1996. Some considerations of the diversifications of muskmelons in India. *Paper presented at Eucarpia cucurbitacea 1996 Malaga Spain*, May. 1996, Spain. p.106
- *Sheng, L.J. and Staub, J.E. 1999. Analysis of genetic correlation for several major characters of botanical varieties and cultivars in cucumber. *China vegetables*. 5:16-19
- Shibu, M.P., Ravishankar, K.V., Anand, L., Ganeshiah, K.N. and Shanker, R.V. 2000. Identification of specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). *Pl. Genet. Resour. Newsl.* 12: 59-61

- Shibukumar, V.N., 1995. Variability studies in watermelon (*Citrullus lanatus* (Thumb) Mansf.) M.Sc. Hort. Thesis. Kerala Agricultural University, Thrissur, p.45-60.
- Shigyo, M., Miyazaki, T. and Tashiro, Y. 2002. Development of randomly amplified polymorphic DNA markers in cultivated and wild species of sections Cepa and Phyllodolon in Allium. *J. hort. Sci. Biotech.* 77:373-377
- Shigyo, M., Tashiro, Y., IssHiki, S. and Miyazaki, S. 1995. Genetic analyses of glutamate dehydrogenase isozymes in cultivated and wild species of section Cepa in Allium. *J. Jpn. Soc. hort. Sci.* 64: 121-127
- Shimada, T., Hayame, H., Haji, T., Yamaguchi, M. and Yoshida, M. 1999. Genetic diversity of plum characterized by random amplified polymorphic DNA (RAPD) analysis. *Euphytica* 109: 143-147
- Silberstein, L., Kovalski, I., Huang, R.G., Anagnostu, K., John, M.M.K. and Perl-Treves, R. 1999. Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. *HortScience.* 79:101-111
- Simi, S. 2001. Molecular characterization of banana (*Musa* AAB plantain subgroup) clones. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p. 72
- *Singh, B.B. and Ntare, B.R. 1985. Development of improved cowpea varieties in Africa. Singh S.R. and Rachie, K.O. (eds.). Cowpea Research, Production and Utilization. John Wiley and Sons, New York. p.105-115
- Singh, D. and Nandpuri, K.S. 1978. A note on correlation studies in muskmelon. *Indian J. Hort.* 35: 52-53
- Singh, K.P. Choudhary, M.L., Anandakumar, P. and Suchitra. 2002. Characterization of *invitro* induced mutants of carnation by means of electrophoretic protein analysis. *Indian J. Hort. Sci.* 59:427-430
- Singh, M.J., Randhawa, K.S. and Tarsen Lal. 1989. Genetic analysis for maturity and plant characteristic in muskmelon. *Veg. Sci.* 16: 181-184
- Singh, N.K. and Singh, R.K. 1988. Correlation and path coefficient analysis in watermelon (*Citrullus lanatus* (Thumb.) Mansf.). *Veg. Sci.* 15:95-100
- Smith, J. S. C. and Smith, O. S. 1989. The description and assessment of distance between inbred lines of Maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. *Maydica* 34: 151-161
- Solanki, S.S. and Seth, J.M. 1980. Studies of genetic variability in cucumber (*Cucumis sativus* L.). *Prog. Hort.* 12: 43-49

- Solanki, S.S. and Shah, A. 1989. Path analysis of fruit yield components in cucumber. *Prog. Hort.* 21: 322-324
- Solanki, S.S. and Shah, A. 1992. Path analysis of fruit yield components in cucumber. *Prog. Hort.* 21: 322-324
- Somkuwar, R.S., More, T.A. and Mehra, R.B. 1997. Correlation and path coefficient analysis in muskmelon (*Cucumis melo*). *Indian J. Hort. Sci.* 54: 312-316
- Somri, S. 1998. Improvement of papaya (*Carica papaya* L.) for south eastern Queensland-Investigation of sex type and fruit quality. Ph.D. thesis, The University of Galton College, Queensland, p. 125
- Souframanien, J., Manjaya, J.G., Krishna, T.G. and Pawar, S.E. 2003. Random amplified polymorphic DNA analyses of cytoplasmic male sterile and male fertile pigeonpea (*Cajanus cajan* (L.) Millsp.). *Euphytica* 129:293-299
- Sriramamurthy, N. 2000. Genetic variability and correlation studies in cucumber (*Cucumis sativus* L.). M.Sc. (Agri.) Horti. Thesis, Annamalai University, Annamalaiagar. P.40-89
- Srivastava, V.K. and Srivastava, L.S. 1976. Genetic parameters, correlation coefficient and path coefficient analysis in bitter melon. *Indian J. Hort. Sci.* 33: 66-70
- Staub, J., Bacher, J. and Poetter, K. 1996. Sources of potential Errors in the Application of Random Amplified polymorphic DNAs in Cucumber. *HortScience*. 31: 262-266
- Staub, J.E. 2001. Inheritance of RAPD markers in melon (*Cucumis melo* L.). *Cucurbit Genet. Coop. Rep.* 24: 56-60
- Staub, J.E., Box, J., Meglic, V., Horejsi, T.F. and Mc Creight, J.D. 1997. Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in cucumbers. *Genet. Resour. Crop Evol.* 44: 257-269
- Staub, J.E., Danin-Poleg, Y., Fazio, G., Horejsi, T., Reis, N. and Katzir, N. 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeat markers. *Euphytica* 115:225-241
- Staub, J.E., Meglic, V. and Mc Creight, J.D. 1998. Inheritance and linkage relationships of melon (*Cucumis melo* L.) isozymes. *J. Am. Soc. hort. Sci.* 123: 264-272
- Stedje, B. and Bukenya-Ziraba, R. 2003. RAPD variation in *Solanum anguiri* Lam. And *S. aethiopicum* L. (Solanaceae) in Uganda. *Euphytica* 131: 293-297

- Stepansky, A., Kovalski, I. and Perl-Treves, R. 1999. Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Pl. Syst. Evol.* 217:313-332
- Stevens, M.R., Lamb, E.M. and Rhoads, D.D. 1995. Mapping the SW-5 locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. *Theor. Appl. Genet.* 90:451-456
- Stiles, J.I., Lemme, C., Sondur, S., Morshidi, M. and Manshardt, R.M. 1993. Using random amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars. *Theor. Appl. Genet.* 85: 697-701
- Stockinger, E.J., Mulnix, C.A., Long, C.M., Brettin, T.S. and Lezzoni, A.I. 1996. A linkage map of sweet cherry based on RAPD analysis of a microspore derived callus culture population. *J. Heredity* 87: 214-218
- Sujatha, V.S., Seshadri, V.S. Srivastava, K.N. and More, T.A. 1991. Isozyme variation in muskmelon (*Cucumis melo* L.). *Indian J. Genet. Pl. Breed.* 51: 438-444
- Swamy, K.R.M., Dutta, O.P., Ramachander, P.R. and Wahi, S.D. 1985. Variability studies in muskmelon (*Cucumis melo* L.). *Madras Agric. J.* 72: 1-5
- Thottappilly, G., Mignouna, H.D. and Omitogun, O.G. 2000. The use of DNA markers for rapid improvement of crops in Africa. *Afr. Crop Sci. J.* 8: 99-108
- Torjek, O., Bucherna, N., Kiss, E., Homoki, H., Korpelova, Z.F., Bocsa, I., Nagy, I. and Heszky, L.E. 2002. Novel male specific molecular markers (MADC5, MADC6) in hemp. *Euphytica* 127:209-218
- Truksa, M. and Prochazka, S. 1996. Potential use of RAPD markers in verification of cucumber hybrids. *Rostlinna Vyroba* 42: 241-244
- Tyrka, M., Dziadczyk, P. and Hortynski, J.A. 2002. Simplified AFLP procedure as a tool for identification of strawberry cultivars and advanced breeding lines. *Euphytica* 125: 273-280
- Upadhyay, R.K., Hari Har Ram and Singh, D.K. 1998. Seed protein electrophoresis in the indigenous cultivars of Bottle ground (*Lagenaria siceraria* (MOL) STANDL). *Veg. Sci.* 25:11-13
- Urrea, C.A. and Miklas, P.N. 1996. A codominant randomly amplified polymorphic DNA (RAPD) marker useful for indirect selection of Bean Golden Mosaic Virus Resistance in common bean. *J. Am. Soc. hort. Sci.* 121: 1035-1039

- *Valliamcourt, R.E., Wedden, W.F. and Barnard, J. 1993. Isozyme diversity in the cowpea species complex. *Crop. Sci.* 33: 606-613
- Van He Usden, A.W., Van Ooijen, J.W., Vrielink, Van Ginkel, R., Verbeek, W.H.J., Wietsma, W.A. and Kik, C. (2000a). A genetic map of an interspecific cross in *Allium* based on Amplified Fragment Length Polymorphism (AFLP) markers. *Theor. Appl. Genet.* 100: 118-26
- Varghese, Y.A., Knaak, C., Sethuraj, M.R. and Ecke, W. 1997. Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brassiliensis*. *Pl. Breed.* 116: 47-52
- Vendisson, S., Bailliene, F. and Audran, J.C. 1999. Use of RAPD markers to detect chimerism in synthetic grape chimera (*Vitis vinifera* L.). *Vitis* 38: 93-95
- Venkatachalam, P. and Jayabalan, N. 1992. Analysis of leaf proteins in gamma rays induced mutants of *Zinnia*. *Crop Improv.* 19:97-99
- Vidal, J.R., Coarer, M. and Defontaine, A. 1999. Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers. *Euphytica* 109: 161-172
- Vijay, O.P. 1987. Genetic variability, correlation and path analysis in muskmelon. *Indian J. Hort.* 44: 233-238
- Vinterhalter, D.V. and Jances, D.J. 1986. The use of peroxidase polymorphism in the identification of Malling Merton apple rootstocks. *J. Hort. Sci.* 61:147-152.
- Vos, P., Hogers, R., Blecker, M., Lee, V.T., Hornes, M., Frijters, A., Pot, J., Pecleman, J., Kliper, M. and Zabeau, M. 1995. AFLP-A new technique for DNA fingerprinting. *Nucl. Acids Res.* 23: 4407-4417
- Walther, R., Illam, A., Lerser, A., Durdevani, A. and Khayat, E. 1997. Analysis of somaclonal variation in the tissue cultured banana plants (*Musa AAA* cv. Grand Nain). *Proceedings of International Symposium on Importance Varieties for Production of Quality Wine. January 12-15, 1997.*(eds. Altman, A. and Ziv, M.), University of California, Davis, Wiley-Liss, New York, pp.379-383
- Wang, G., Castiglione, S., Zhang, J., Fu, R., Ma, J., Li, W., Sun, Y. and Sala, F. 1994. Hybrid rice (*Oryza sativa* L.) identification and parentage determination by RAPD fingerprinting. *Pl. Cell. Rep.* 14: 112-115
- Wang, Y.H., Thomas, C.E. and Dean, R.A. 1997. A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. *Theor. Appl. Genet.* 95: 791-798

- Waugh, R. and Powell, W. 1992. Using RAPD markers for crop improvement. *Trends Biotech.* 10: 186-191
- Weber, J.L. and May, P.E. 1989. Abundant class of human polymorphism which can be typed using the polymerase chain reaction. *Am.J. Human Genet.* 44: 388-396
- Wechter, W.P., Whitehead, M.P., Thomas, C.E. and Dean, R.A. 1995. Identification of a randomly amplified polymorphic DNA marker linked to the FOM2 fusarium with resistance gene in Muskmelon MR-1. *J. Mol. Pl. Pathol.* 85: 1245-1249
- Weeden N.E. 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. *Euphytica* 33:199-203
- *Wehner, T.C. and Cramer, C.S. 1996. Ten cycles of recurrent selection for fruit yield, earliness and quality in 3 slicing cucumber populations. *J. Am. Soc. hort Sci.* 121:362-366
- Weising, K., Nybom, H., Wolff, K. and Meyer, W. 1995. *DNA fingerprinting in plants and fungi*. CRC Press, Boca Raton, Florida, p. 120
- Welsh, J. and Mc Clelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* 18: 7213-7218
- Wilde, J., Waugh, R. and Powell, W. 1992. Genetic fingerprinting of Theobrome clones using randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* 83: 871-877
- Williams, J.G.K., Kubelik, A.R., Livak, R.J., Rafalski, J.A. and Tingsey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535
- Wing, R.A., Zhang, H.B. and Tanksley, S.D. 1994. Map based cloning in crop plants, tomato as a model system. *Mol. Gen. Genet.* 242: 681-688
- Wright, S. 1954. The interpretation of multivariate systems: 6. Statistics and Mathematics in biology (eds. Kempthorne, O., Bancroft, T.A. Gowen, J.W. and Lush, J.L.) State University Press. Iowa. p.11-33
- Xu, Y., Clark, M.S. and Pehu, E. 1993. Use of RAPD markers to screen somatic hybrids between *Solanum tuberosum* and *S. brevidens*. *Pl. Cell Rep.* 12: 107-109
- *Yaaska, V. 1984. Isoenzymes of superoxide dismutase in seedlings of pulses. Elstii Nsu Tead Uste Akadeemia Toimetised, *Biologia.* 33: 42-49
- Yadav, R.K., Harihar Ram and Anil Kumar. 1998. Electrophoretic characterization of indigenous germplasm lines of muskmelon. *Veg. Sci.* 25: 8-10

- Yan, Y., Hsam, S.L.K., Yu, J, Jiang, Y. and Zeller, F.J. 2003. Allelic variation of the HMW glutenin subunits in *Aegilops tanschii* accessions detected by sodium dodecyl sulphate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis. *Euphytica* 130: 377-385
- Ying, M., Dreyer, F., Lai, D. and Jung, C. 2003. Molecular markers for genic male sterility in chinese cabbage. *Euphytica* 132: 227-234.
- Zhang, M., Xiaofen, W. and Wen, C.H. 1999. Genetic path analysis of early yield in cucumber. *Curcubit Genet. Coop. Rep.* 22:3-4
- Zheng, X.Y., Wolff, D.W., Baudracco-Arnas, S. and Pitrat, M. 1999. Development and utility of cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLPs) linked to the Fom-2 fusarium wilt resistance gene in melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 99:453-463
- *Zope, J.S., Pahulkar, K.S. and Mate, G.B. 1992. Electrophoretic analysis of seed globulins of black gram mutant. *New Agrst.* 3:65-66

* Original not seen

**MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR
CHARACTERIZATION IN LANDRACES OF MELON
(*Cucumis melo* L.)**

KANDASAMY .R

**Abstract of the
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**Department of Olericulture
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695 522**

8. ABSTRACT

The present investigation on 'Morphological, biochemical and molecular characterization in landraces of melon (*Cucumis melo* L.)' was carried out at the Department of Olericulture and Centre for Plant Molecular Biology, College of Agriculture, Vellayani during 1999-2002. Forty genotypes of *Cucumis melo* were collected from Kerala and Tamilnadu regions. Twenty two morphological characters viz., days to first male flower, node number of first male flower, days to first female flower, node number of first female flower, sex ratio, vine length, number of primary branches, number of secondary branches, internodal length, leaf petiole length, leaf thickness, days to fruit harvest, fruit length, fruit girth, fruit diameter, average fruit weight, dry matter content, fruits per plant, seeds per fruit, 1000 seed weight, yield per plant and fruit shape and colour were used in this study. The genetic parameters, correlation and path coefficients were studied and the genetic divergence worked out using D^2 statistics. The genotypes were also characterized by biochemical method (SDS-PAGE) and molecular techniques (RAPD analysis).

Morphological studies showed significant differences among the forty genotypes for all the twenty one characters. CM 32 collected from Manjapra was the top yielder. The highest PCV and GCV were observed in yield per plant followed by average fruit weight and fruits per plant. High heritability coupled with high genetic gain was obtained for yield per plant followed by average fruit weight and fruits per plant.

Sex ratio, vine length, number of secondary branches, internodal length, fruit length, average fruit weight, fruits per plant and seeds per fruits were positively correlated with yield per plant, whereas node number of first female flower was negatively correlated with yield per plant. Path analysis revealed average fruit weight and fruits per plant as primary contributions to yield.

In D^2 analysis, the forty genotypes were grouped into twenty clusters. Maximum genotypes were in cluster I and minimum in cluster XX. CM 2 and

CM 5 (dessert melon) genotypes came under cluster XIV. CM 4 and CM 38 (slicing and culinary melon) genotypes came under cluster XVI. The dessert types formed a separate cluster and the slicing type melon fell along with culinary melon genotypes. The D^2 analysis differentiated culinary types of melon from dessert types.

Total soluble protein yield extracted from the forty genotypes of *Cucumis melo* seeds ranged from 0.858 to 3.200 $\mu\text{g seed}^{-1}$. Maximum of 18 bands were observed in CM 7 and CM 40. Least number of 5 bands was present in CM 2. The dendrogram, showed two major clusters viz., cluster A and cluster B. Cluster B had only one dessert genotype, CM 2. Cluster A had all other culinary melon genotypes including slicing type CM 4. Cluster A was further grouped into four subclusters. A similarity index within major cluster A showed 37.5 per cent between CM 1 and CM 27. A similarity index of 13.3 per cent present between CM 2 (cluster B) and CM 1 (cluster A). Seed protein results were not correlated with D^2 analysis, geographical location and morphological characters like fruit shape and colour.

DNA isolated from the forty genotypes of *Cucumis melo* were subjected to RAPD analysis. Out of the 46 decamer primers screened for RAPD analysis, 31 could produce amplification. Totally 103 bands (average 2.24 bands per primer) were generated by thirty one primers, of which 96.12 per cent (99 bands) were polymorphic. Four bands (3.88 %) were monomorphic. Seven primers showed high level of polymorphism. Finally, four best promising primers viz., OPA-01, OPA-08, OPB-12 and OPB-20 were used for RAPD analysis of 40 *Cucumis melo* genotypes. These primers yielded 29 scorable bands with an average of 7.25 bands per primer. Dendrogram expressed two major clusters were formed viz., cluster A and cluster B. Cluster B had CM 2 and CM 5 of dessert genotypes. Similarity index was 75 per cent between CM 2 and CM 5 genotypes. Cluster A was again subclustered into six groups. CM 4 (slicing melon) was grouped along with culinary melon genotypes. D^2 analysis and protein results of slicing types were in line with RAPD results. Considering all genotypes RAPD analysis does

not agree with geographical location and morphological characters like fruit shape and colour.

In the present study, characterization of forty genotypes of *Cucumis melo* using morphological, biochemical and molecular basis (RAPD marker analysis), the last method gave a perfect differentiation of dessert melon from culinary melon which is also agreeable with morphological characterization.