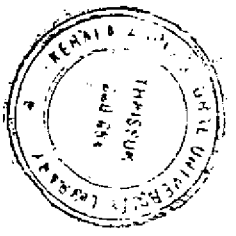
REGULATION OF SEX IN *Garcinia gummigutta*(L.) Robs. THROUGH CONTROLLED POLLINATION AND TOP WORKING



By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF POMOLOGY AND FLORICULTURE

COLLEGE OF HORTICULTURE

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DECLARATION

I hereby declare that the thesis entitled **"Regulation of sex in *Garcinia gummigutta* (L.) Robs. through controlled pollination and top working"** 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 to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

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CERTIFICATE

Certified that this thesis entitled **"Regulation of sex in *Garcinia gummigutta* (L.) Robs. through controlled pollination and top working"** is a record of research work done independently by **Ms. Zahida P.M.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. **Zahida P.M.**, a candidate for the degree of **Master of Science in Horticulture** with major field in Pomology and Floriculture, agree that the thesis entitled **"Regulation of sex in *Garcinia gummigutta* (L.) Robs. through controlled pollination and top working "** may be submitted in partial fulfilment of the requirement for the degree.

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Zahida P. M.

AFFECTIONATELY DEDICATED TO
MY LOVING PARENTS

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INTRODUCTION

INTRODUCTION

Garcinia gummigutta, popularly called as Kodampuli or Malabar Tamarind, belonging to the family Clusiaceae, is an acidic fruit bearing tree. It occupies the backyards of Kerala's homesteads more often as one among the miscellaneous tree crops. It is a native of Malaysia and Western Ghats of Kerala. *Garcinia* consists of about 434 species with more than 30 edible species. It is one of the slow growing and late bearing trees of tropics. *Garcinia gummigutta* is an evergreen androdioecious tall growing tree, with round canopy commonly seen in the Western Ghats of Kerala. It thrives best in evergreen forests of Konkan, stretching southwards to the Kerala coast and Western Ghats upto 180 m in the Nilgiris.

Garcinia fruits have a unique use in Kerala being exclusively preferred in culinary preparations involving fish. Fish flavoured with acidic fruit rinds is a delicacy of Kerala's cuisines. Kodampuli rind is the richest source of (-)-hydroxy citric acid (HCA) and its derivatives are unique, potent metabolic regulators of obesity (Verghese, 1996). Being rich in acids, the rind possesses marked antiseptic properties. Kodampuli is listed as a remedy against rheumatism, rickets and enlargement of spleen and also in veterinary medicine as a rinse for mouth disease in cattle.

Segregation among the seedling progenies is recognized as one of the major problems in the cultivation of *Garcinia*. The seedling progenies segregate into males and females. The only option to overcome this problem is to use vegetatively propagated material. Eventhough vegetative propagation is standardised (Nazeema, 1992), large scale adoption of this method is constrained, due to the non availability of sufficient number of orthotrops, which are required for the production of plants with normal growth habit. So seedlings continue to be the major propagules. Therefore, in this investigation, efforts were taken for identification of the sex of the plant at the seedling stage.

Marked differences between male and female with respect to growth rate, colour of the emerging flushes and the total phenol content were identified in mature trees (Muthulakshmi, 1998). It was also essential to work out whether such relationship exists in the seedling stage as well. Fruit and seed set through different modes of pollination is reported in kodampuli (Sherly, 1994). Relationship, if any, between mode of seed development and sex of the seedlings is also to be investigated.

Garcinia is dioecious and segregation of sex of seedling progenies into productive females and unproductive males is common. This results in fifty per cent unproductive trees. This is another main problem in its cultivation. The sex of the tree can be identified only after 7-8 years of planting. Removal of male trees at that stage causes considerable loss in terms of time and resources to the grower. In this study, efforts were taken to convert male trees into productive female trees by top working.

Chemical growth regulation has been recognized as one of the major research priorities for horticultural science. The recent development of many highly active growth retardants has further enhanced the potential of chemical growth regulation in horticulture with particular reference to fruit production. Paclobutrazol (PBZ) is one of the most important representative of triazole and is available in the market with the trade name cultar.

Garcinia flowers in hot season and fruits ripen in the rains. Post harvest handling is yet another major problem faced by the growers. As the harvest season coincides with monsoon showers, curing and drying of rind is difficult. In this context, if flowering can be advanced to an early date by bioregulator application, harvest can be completed before rains, which would be a great boon to the farmers.

Kerala is blessed with tremendous diversity in populations of *Garcinia*. Commercial cultivation of the crop in India is handicapped due to various reasons mentioned and is a backyard crop even now. The need of the hour is to develop a

definite methodology for identifying the sex of the plant in the early stage of seedling growth and a successful method of vegetative propagation for the conversion of unproductive males to productive females to overcome the problems of dioecy. The problems during post harvest handling of the crop during rains can also be resolved to a great extend if flowering could be advanced to an early date. Thus, a radical change can be brought about in *Garcinia* culture.

In this background, the present study has been carried out with the following objectives.

1. To explore the possibility of regulating sex through controlled pollination.
2. To convert unproductive males to productive females through top working.
3. To advance flowering and fruit set through bioregulator application.
4. To study morphological and physiochemical variations in polyembryonic seedlings.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Regulation of sex through controlled pollination

2.1.1 Sex forms in *Garcinia*

Garcinia gummigutta is reported to be dioecious in nature with male and hermaphrodite flowers seen on separate trees (Chandrarathna, 1948; CSIR, 1956; George, 1988; KAU, 1991 and Nazeema, 1992). George *et al.* (1992) and Sherly (1994) described *Garcinia gummigutta* as androdioecious since male and bisexual flowers occur in separate trees. In *Garcinia mangostana,* also the existence of male and hermaphrodite flowers is recorded (Cobley, 1956; CSIR, 1956; Veeraragavathatham and Balashanmugham, 1989). According to Purseglove (1969), mangosteen is unisexual and dioecious, but only female trees with infertile staminodes exist in Malaya and Jawa.

2.1.2 Flowering pattern and floral biology.

Very little work has been done on flower characters and floral biology of kodampuli. Therefore, the literature pertaining to its related species is also cited here.

2.1.2.1 Kodampuli

Thomas (1965) reported that the flowering season of kodampuli is during February. Other reports show that the flowering period of both male and bisexual trees occur from January to April (Verghese, 1991; Jacob, 1992 and George *et al.*, 1992).

Visual flower bud emergence is noticed in male trees from November onwards and in hermaphrodite from December onwards. Depending on the varying functions of stamen and pistil, six flower types were noticed in male and bisexual

types of *Garcinia gummigutta* with varying fruit setting capacity (Muthulakshmi, 1998).

2.1.2.2 Related species

Two main seasons of flowering, namely March and November, were reported in *Garcinia livingstonei* (Devivedi and Bajpai, 1974) and April-May and October-November in *Garcinia mangostana* (Krishnamurthi *et al.*, 1964). *Garcinia morella* another important species of *Garcinia,* flowers in May (Chandrarathna, 1948) and Kokam (*G. indica*) flowers from November-February (Karnik and Gunjate, 1984). The flowers are structurally hermaphrodite but functionally dioecious in allspice (Sreeja, 1999).

2.1.2.3 Anthesis and anther dehiscence

Anthesis of both male and female flowers of *Garcinia indica* was between 0600 hours and 0800 hours and anther dehiscence occurred 15-20 minutes before anthesis (Karnik and Gunjate, 1984). According to Sherly (1994), anthesis of male and bisexual flowers of *Garcinia gummigutta* started at 1630 hours and continued upto 1830 hours. The peak period of anthesis in hermaphrodite trees was between 1700 hours to 1730 hours when on average 58.2 per cent flowers opened.

2.1.2.4 Stigma receptivity

Stigma receptivity was maximum on the day of anthesis in *Garcinia indica* (Karnik and Gunjate, 1984). Stigma was receptive from 30 hours before anthesis and continued to be in receptive stage for 12 hours after anthesis in *Garcinia gummigutta* (Sherly, 1994).

2.1.2.5 Pollen studies

The exact measurement of the amount of pollen produced per anther is essential to evaluate the worth of a variety more accurately. Pollen production and fertility were higher for staminate flowers in *Garcinia gummigutta*. Pollen production in staminate flowers was about 10 times that of bisexual flowers (Sherly, 1994). In the case of bisexual flower, 6-20 stamens often sterile were found surrounding the ovary, which was 6-10 celled with 6-10 lobbed stigma (George *et al.*, 1992).

Pollen production studies have been reported in fruit crops like guava by Nair *et al.* (1964), in sapota, papaya and pomegranate by Rao and Khader (1962) and in varikka and koozha types of jack by Joseph (1983). In Kokam (*Garcinia indica*) the pollen grain production/ anther was estimated to be 3640 in male and 3603 in hermaphrodite (Karnik and Gunjate, 1984).

2.1.2.6 Pollen viability

The extent of pollen viability is of vital importance in hybridization work. Stanley and Linskens (1974) suggested various methods for testing the viability of pollen grains including both germination and non-germination assays. Stain test involves staining technique, which gives colour to viable pollen and is used as indices of viability. Zirkle (1937) described the method of mounting pollen grains in acetocarmine.

Balasubramanyan (1959) in guava, Singh (1961) and Moti *et al.* (1973) in mango and Nalawadi *et al.* (1977) in sapota had followed acetocarmine test to find out the percentage fertility. Sherly (1994) reported 27.03 per cent fertility for pollen from bisexual flowers.

2.1.3 Fruit set and development

Fruit set and not the flower production was found to have great bearing upon the yield in most of the crops. Inadequate pollination or conditions existing after pollination were reported as one of the main reasons responsible for poor fruit set in mango (Mukherjee, 1953). Mukherjee (1949) and Singh (1954) have reported the ultimate set in mango as one per cent. Factors like high percentage of male flowers, defective pollination, adverse weather conditions and vegetative growth were reported as the causes for low fruit set in mango (Singh, 1964). Fruit development was reported to be parthenocarpic in *Garcinia mangostana*. Fruit ripening in kodampuli is reported during south west monsoon (George, 1988). Jacob (1992) reported that fruits take about 4 months to ripen.

2.1.4 Seed dormancy

Kachru *et al.* (1972) suggested that stratification followed by growth substances treatment GA_3, was most effective in breaking dormancy of grape seeds. Ilyas (1978) showed that dehusked clove seeds started germination 16 days after sowing while normal seeds germinated only after 27 days. Mathew (1979) obtained 75 per cent germination when the seeds of nutmeg were soaked for 24 hours in 200 ppm GA. The stimulatory effect of applied GA on germination of seeds has been widely accepted (Hartmann and Kester, 1989).

Burslem (1989) reported that seed coat inhibited germination in cinnamon seeds possibly by restricting the availability of oxygen. Various seed treatments are reported to have pronounced influence on breaking seed dormancy of different tree species (Ramamoorthy *et al.*, 1989; Bhagat *et al.*, 1992). Corral *et al.* (1990) reported that heat treatments (100°C for 30 minutes) and soaking in distilled water broke dormancy in citrus seeds. *Garcinia gummigutta* seeds exhibit long term dormancy. Several treatments were tried and seeds without seed coat recorded faster and higher percentage of germination (Mathew and George, 1995). In seeds of *Garcinia*

gummigutta (L.) Robs, seed coat removal before sowing advanced germination (Chacko and Pillai, 1997).

2.1.5 Polyembryony

Polyembryony is a phenomenon of occurrence of more than one embryo in a seed resulting in development of more than one seedling from it (Maheswari, 1974).

Polyembryony has been reported for several native tree species like *Tectona grandis* (Dabral, 1977), *Butea monosperma* (Shrivas and Bajpai, 1990) etc. Muthulakshmi (1998) reported 10 per cent polyembryony in kodampuli. Bhanja (2000) reported polyembryony in *Madhuca indica*. He also observed that among the twin seedlings one was smaller but not so weak compared to the other.

2.1.6 Sex determination in kodampuli

2.1.6.1 Influence of sex on morphological characters

Sherly (1994) reported that in kodampuli, the colour of the emerging leaves showed marked differences among male and hermaphrodite trees. Mathew *et al.* (1996) reported that there was significant variation in length and breadth of leaves of male and female kodampuli trees. With respect to leaf characters like length, width, length/width ratio, area and shape of leaves, male and bisexual trees did not show any marked differences (Muthulakshmi, 1998).

2.1.7 Sex determination in other crops

Many scientists have investigated the possibility of using morphological characters as sex markers. Janse (1898) stated that male nutmeg trees had smaller leaves and less horizontal branches. But this difference was not clear and prominent enough in young seedlings and hence it was not possible to determine the sex of the plant in the seedling stage.

Prestoe (1948) reported that sex of nutmeg seedlings, less than 30 cm height, could be identified by observing the leaf form and venation. Flach (1966) observed a slight difference in the size between female and male nutmeg trees.

Lacombe (1980) observed significant differences in the internodal length and growth rate of male and female plants of dioecious hemp (*Cannabis sativa*). He also reported that the sex of the plants could be determined from the age of 15 days based on early vegetative characters. Chen *et al.* (1985) registered 100 per cent correlation between sex and length/width ratio of leaves of jojoba (*Simmondsia chinensis*), a dioecious dessert shrub.

Krishnamoorthy *et al.* (1992) conducted a detailed study of characters in nutmeg, like, leaf shape, size of leaves, venation, colour of new sprouts and days for germination of seeds and concluded that none of these characters can be considered as a marker of sex in nutmeg seedlings.

Thomas (1997) reported that nutmeg male plants had higher length/width ratio of leaves than that of females. Morphological characters viz. stem colour, stem pigmentation and petiole colour in the cultivar Khaeg Dum, from Thailand were useful in identifying female and hermaphrodite seedlings in papaya (Somsri, 1999).

2.1.8 Influence of sex on biochemical characters

2.1.8.1 Phenolics and sex expression

The term phenolics embrace a wide range of plant substances, which possess a common aromatic ring bearing one or more hydroxyl substituents. Phenolics play an important role in plants hormonal balance, disease resistance and protection of injured tissue from infection. Phenolics in high concentration are toxic to plant cells themselves (Tepper and Anderson, 1984). Hence phenolics will be present in plants in small quantities. The number of phenolic compounds present and the content were found to be differing in males and females of some dioecious species.

Studies by Singh and Jindal (1974) indicated that when the leaf extracts of *Carica papaya* were subjected to 10 colourimetric tests specific to phenolics, the sex of the plant could be determined with an accuracy of 86 per cent using FC reagent. Another study by Bhattacharya and Rao (1982) indicated that by application of growth regulator in papaya the males continued to have higher phenol content except for TIBA (50-200 ppm). It was suggested that any treatment that decreases the phenolic content would enhance femaleness. Thomas (1997) reported that leaves of the male nutmeg plants had higher phenol content than female ones. Muthulakshmi (1998) reported that male kodampuli plants had higher phenol content than female ones.

2.1.8.2 Protein and sex expression

Prasad and Iyengar (1982) reported that leaves of female jojoba plants had higher total protein than males. Dutta and Mazumdar (1989) studied the protein content of male and female papaya plants and reported that the protein content was higher in the female trees regardless of plant part examined.

2.1.8.3 Isozyme and sex expression

Isozymes are multiple molecular forms of enzymes that catalyse the same reaction but the deficiency of which leads to changes in physico chemical properties (Market and Moller, 1959). Isozymes are useful aids in deciphering the evolutionary relationship within different groups of plants and animals (Oliver and Zapater, 1985). Analysis of isozyme by polyacrylamide gel electrophoresis (PAGE) has been considered as a unique and powerful technique for ascertaining genetic relationships in plants. Further PAGE provides a tool for species and cultivar identification where morphological and cytological data are inadequate (Wilkinson and Beard, 1972).

Munoz *et al.* (1982) observed the sex of the adult and juvenile plants of papaya could be identified using peroxidase zymogram where male plants had more bands than female. Suganuma and Iwasaki (1983) analysed peroxidase isozyme in the

leaves of date palm electrophoretically and reported that there was difference in the zymogram of male and female plants.

In nutmeg, Thomas (1997) reported that the peroxidase banding pattern was similar for both male and female plants. Muthulakshmi (1998) reported that in kodampuli, peroxidase banding pattern was similar for both male and female trees. For prediction of sex type in papaya seedlings a range of isozymes were investigated. Esterase and peroxidase gave variations in banding patterns among ten cultivars of papaya used. They were able to distinguish male from female plants in the Australian cultivar Richter (Somsri, 1999).

2.2 Alteration of sex through top working

Garcinia gummigutta seems to be androdioecious in nature (George *et al.*, 1992). Being a dioecious plant the seedlings of *Garcinia gummigutta* segregate into female and unproductive male plants in various proportions. Seedlings take a long period of six to seven years for flowering (Kennedy *et al.*, 1999).

Information available on kodampuli pertaining to top working through budding and grafting is limited. So information on other crops, which would substantiate the present study, has been reviewed.

2.2.1 Top working

Top working means the rejuvenation of an established plant, tree, shrub, or vine either by grafting or budding the branches with new scions.

2.2.1.1 Beheading the tree

Singh (1953) reported that inferior trees can be headed back to a height of 1.2 m from the ground during March and the stumps produced can be shield budded in

early June with scions of improved cultivars. Conversion of undesirable trees of apple and pear after making a frame has been reported from India and abroad (Maney, 1939 and Srivastava, 1965 a, b).

Srivastava *et al.* (1975) observed that plum trees headed back within 1.0 to 1.5 m from the main crotch during the dormant season produced a number of new shoots on the stumps during the growing season. In mango inferior trees were headed back to 60-90 cm length and the wounds were coated with some disinfectant like Bordeaux paste immediately (Kanwar and Jawanda, 1963).

Khan *et al.* (1986) reported that beheading the trees 0.75 to 1.00 m above ground level was found to be ideal in cashew from point of view of sprouting and after care of the successful graft and trees. In top working trials of cocoa, plants were cut back at jorquette height and also at 30 cm height from ground level (CCRP, 1988)

Kar *et al.* (1989), in an attempt to top work wild pomegranate with the commercially cultivated pomegranate, trees were cut back to a height of 90 cm from the ground level which could produce enough shoots for budding and grafting.

2.2.1.2 Time taken for sprouting and number of sprouts

Khan *et al.* (1986) from Ullal observed that in cashew, the trees resprouted in 30-40 days after beheading. On an average 50-100 sprouts were recorded on each tree, 45 days after sprouting. Prasad *et al.* (1988) also observed a similar trend and sprouting was observed in 28 to 45 days after beheading. In similar experiments at Vridhachalam, the cashew trees started sprouting in about 25-30 days after beheading (Pugalendhi and Shah, 1991).

2.2.1.3 Grafting and budding in different crops

Mathew (1979) tried different methods of grafting and found that approach grafting gave higher percentage of success (95%) followed by side and wedge grafting in nutmeg. Shanmugasundaram *et al.* (1999) reported that top working could be done by budding or by grafting in nutmeg. After the first flowering excess male plants were cut back at convenient height leaving few branches below. On the orthotropic shoots cleft grafting was done during the end of rainy season. Krishnamoorthy (2000) reported sex conversion in nutmeg. Soft wood grafting was done on one or two erect shoots. All male trees could be converted to productive females by this method.

Rao *et al.* (1957) found that side grafting could be successfully done in cashew with 70 per cent take by placing moist moss above and below the union. Ohler (1979) quoting Pexicoto, indicated that patch budding, side grafting and tip grafting could be applied for top working adult cashew trees but cautioned that performance need to be ascertained. According to Das and Mishra (1985), in cashew, side grafting was more successful than T-budding and they observed that the best months for grafting in order of success (80 to 60 per cent) were January, February, September, July and June. In cashew, Khan *et al.* (1986) from Ullal have reported that for top working, patch budding gave very low (18.3 per cent) success and growth of sprouts was not rapid and so they considered this technique not promising for top working. Studies for the first time on the rejuvenation of unhealthy cashew plantations by top working were initiated at ARS, Ullal, Dakshinakannada Karnataka during 1983 (Khan *et al.*, 1986).

Nazeem *et al.* (1984) tried several methods of propagation in jack, namely, grafting, budding and layering and best success was obtained by air layering ringed shoots. According to Jose and Valsalakumari (1991) epicotyl grafting in jack gave maximum sprouting of 83.3 per cent when compared to soft wood grafting.

In top working of cocoa, budding on new chupon growth gave cent per cent success in almost all seasons (CCRP, 1990). In cocoa, top working trials were carried out by budding on hard trunk. But success rate was very low and even after repeated budding only three out of six plants could be successfully budded. Nagabhushanam and Nair (1988) conducted trials on asexual propagation of cocoa and found that patch budding gave only 35 per cent success.

Srivastava (1962) observed that in guava, both the forkert and patch budding methods gave a complete take and also found that plants propagated by forkert method grew better than those by patch method. Further studies in clonal propagation of guava showed that forkert method of budding gave better results than shield or patch budding (Chandra, 1965). Mukherjee and Majumdar (1983) reported that in guava large seedling can be top worked either by cleft/crown bark grafting. Thus poor quality seedling trees can be converted to superior varieties.

In rubber, double working as crown budding was first tried out in Java in 1926. Possibility of forming a tree with improved rootstock, trunk and crown components was also envisaged by this method (DeVries, 1926). Top working was tried in rubber to reduce the wind damage (Samaranayake *et al.*, 1984).

Singh *et al.* (1973) recommended that in ber beheading should be done during April-May in Punjab and budding could be done during April-July (Pareek, 1983) or July-August (Singh *et al.*, 1973). The scope and usefulness of top working the extensive wild plantations of inferior quality ber has been well recognized (Pareek, 1983).

Bael can be grafted on to a number of related plants such as *Aegle fraeglegabonensis* and *Aeglopsis chevalieri* (Reuther *et al.*, 1967). Old and uneconomic bael tree can be turned into economic and vigorous one by top working (Jauhari and Singh, 1971).

2.3 Regulation of flowering

Chemical growth regulation has been recognized as one of the major priorities for horticultural science. The recent development of many highly active growth retardants has further enhanced the potential of chemical growth regulation in horticulture with particular reference to fruit production (Ram *et al.*, 1996).

2.3.1 Method of application

Single pre-bloom soil application of paclobutrazol was reported beneficial, cheap, easy and produced quick and prominent results as chemical entered into the roots of the tree. Williams *et al.* (1989) studied the effect of a single soil application of paclobutrazol over an extended period in grape vines. Cultar in its present formulation could be effectively applied as foliar sprays or as soil drenches in mango (Voon *et al.*, 1991).

2.3.2 Paclobutrazol and early flowering

Soil drench of 10 g paclobutrazol per tree caused precocious, profuse, axillary and cauliflorous flowering in young bearing mango trees (Kulkarni, 1988). Burondkar and Gunjate (1992) reported that paclobutrazol application increased the number of flowering shoots and led to flowering earlier in the season than normal. Paclobutrzol increased flowering in apple, avocado, apricot, citrus, cranberry, cherry, grape, mango, olive, plum, pear and peach (Ram *et al.*, 1996). In litchi, application of cultar 90 days before expected date of flowering was more effective compared to its application 60 days before expected date of flowering (Ahmad *et al.*, 2000).

2.3.3 Paclobutrazol and yield

In mango, soil drench of 10 g paclobutrazol per tree was reported to increase 16 per cent fruit yield (Kulkarni, 1988). Voon *et al.* (1991) reported that the

increased flowering induced by cultar usually resulted in increased harvests. Burondkar and Gunjate (1992) opined that paclobutrazol could be used effectively for regular and heavy cropping in mango varieties grown in Konkan region of Maharashtra. Paclobutrazol did not affect the fruit yield in the treatment year but significantly increased yield in the following year in apples, peaches, pears and mangoes (Ram *et al.*, 1996).

2.3.4 Paclobutrazol and fruit quality

Hillier and Rudge (1991) opined that cultar could be used primarily as a fruit quality enhancer and as a means of generating regular cropping. Paclobutrazol did not affect TSS and firmness of apple, apricot, banana, cherry, mango, peach, and plum (Ram *et al.*, 1996)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Investigations were undertaken to explore the possibility of regulating sex through controlled pollination and top working and to shift flowering and fruit set to an early date through bioregulator in *Garcinia gummigutta*. The studies were conducted in the trees maintained at the College Orchard, Department of Pomology and Floriculture and at the Vegetable Research Farm, Department of Olericulture, College of Horticulture, Vellanikkara during 1999-2001.

Experiment I

3.1 Regulation of sex through controlled pollination

The experiment was carried out in the College Orchard and Vegetable Research Farm. Flowering female trees were selected.

3.1.1 Pollen studies

Pollen studies with respect to pollen fertility were taken up.

3.1.1.1 Pollen fertility

The pollen for the studies was collected from mature flower buds during the morning hours on the day of opening from dehisced anthers. Well matured unopened buds were selected from male and hermaphrodite trees. Pollen from each bud was collected in acetocarmine - one per cent glycerine mixture on a slide and covered with clean cover slip. The slides were kept undisturbed for 30 minutes to allow the pollen grains to take stain properly before examining it under microscope. Fertility was calculated as the percentage of normal, well stained pollen grains to the total number of pollen grains in each microscopic field. Ten such fields were observed in each slide. The average was worked out and expressed as percentage.

3.1.2 Pollinating agents

To study the agents helping in pollination of kodampuli, the female trees were closely observed during the flowering season. In order to assess the extent of air borne pollen, slides covered with cellophane tape with sticky side exposed were suspended at different sites near the female trees. Slides were collected next morning and examined under microscope for kodampuli pollen and foreign matters.

3.1.3 Pollination studies

To ascertain the role of mode of pollination in regulating sex, fruit set and development, seed germination and seedling variations by open pollination, self pollination, hand pollination or artificial cross pollination and agamospermy were studied. Studies were taken up utilising 100 flowers each for all experiments.

3.1.3.1 Open pollination

Individual bisexual flower buds were selected and tagged before anthesis. These were later examined for fruit set and extent of open pollination was worked out.

3.1.3.2 Self pollination

Individual flowers were selected and covered one day prior to anthesis for preventing any pollen contamination from outside. Later the covers were removed after flower opening and fruit set was recorded.

3.1.3.3 Hand pollination

Flowers were emasculated and covered one day before opening. These flowers were hand pollinated on the next day by dusting the pollen collected from

male flowers on the stigmatic surface. Pollinated flowers were tagged and observed for fruit set.

3.1.3.4 Agamospermy

Individual flowers were emasculated on the day prior to anthesis and covered to prevent any pollen contamination from outside. Fruit set was recorded.

3.1.4 Time taken for set

Time taken for fruit set was observed separately for each mode of pollination.

3.1.5 Fruit set, fruit measurements and fruit drop

3.1.5.1 Fruit set

Percentage of fruit set through open pollination, self pollination, hand pollination and agamospermy was recorded.

3.1.5.2 Fruit measurements

The fruits, which were set by different ways of pollination, were evaluated for morphological characters.

a) Fruit girth: Observations on girth were taken at 20 days interval till harvest. Measurements were taken using a scale and non-elastic twine.
b) Fruit shape: Shape of the fruit was noted during its development.

3.1.5.3 Fruit drop

Fruit drop was recorded separately for different modes of pollination.

3.1.6 Seed Germination

Seeds obtained from different ways of pollination were sown separately for germination.

3.1.6.1 Percentage germination

Percentage germination of seeds in each set was recorded.

3.1.6.2 Treatments to break seed dormancy

Garcinia gummigutta seeds remain dormant for a period of one year. This is a major limiting factor in large-scale propagation of *Garcinia*. Various seed treatments were given to break dormancy. Ten seeds were subjected to each treatment and were replicated thrice. The percentage of germination and time taken for germination were recorded. The different treatments included in the study are given in the Table 1.

3.1.7 Polyembryony

Hundred seeds were selected at random and were sown to study the polyembryony and percentage polyembryony was recorded.

3.1.8 Seedling variations

3.1.8.1 Based on mode of pollination

Twenty five seedlings each were evaluated for the following morphological and physiochemical variations.

Table 1. Seed treatments for breaking dormancy

Treatment No.	Treatments
T_1	Soaking the seeds in boiling water and cooled
T_2	Dip in concentrated sulphuric acid for two minutes
T_3	Dip in concentrated sulphuric acid for three minutes
T_4	Dip in concentrated sulphuric acid for five minutes
T_5	Seeds kept in freezer and sown after 15 days
T_6	Seeds kept in freezer and sown after 30 days
T_7	Seeds kept in freezer and sown after 45 days
T_8	Seeds kept in freezer and sown after 60 days
T_9	Seeds sown in wet sawdust and placed in refrigerator and sown after 15 days
T_{10}	Seeds sown in wet sawdust and placed in refrigerator and sown after 30 days
T_{11}	Seeds sown in wet sawdust and placed in refrigerator and sown after 45 days
T_{12}	Seeds sown in wet sawdust and placed in refrigerator and sown after 60 days
T_{13}	Seeds immersed in cowdung slurry overnight
T_{14}	Seeds immersed in cow's urine overnight
T_{15}	Seeds treated with thiourea 2000 ppm
T_{16}	Seeds treated with thiourea 2500 ppm
T_{17}	Seeds treated with GA_3 500 ppm
T_{18}	Seeds treated with GA_3 1000 ppm
T_{19}	Seed coat removed
T_{20}	Control (seeds with seed coat)

3.1.8.1.1 Growth rate of seedlings

a) Height

Height of seedlings was noted at monthly interval.

b) Number of leaves

Number of leaves was also counted at monthly interval.

c) Length of the leaf

Length of the third leaf from the top was recorded at monthly interval.

d) Width of the leaf

Width of the third leaf from the top was noted at monthly interval.

3.1.8.1.2 Pigmentation

Pigmentation of the flush at the growing point of the seedlings was observed.

3.1.8.1.3 Biochemical characterisation

Biochemical analyses were carried out at Biochemistry Laboratory of College of Horticulture, Vellanikkara. The total phenol content, protein banding pattern and isozyme banding patterns were analysed.

3.1.8.1.3.1 Total phenol content

Seedlings from each set were evaluated for total phenol content by Folin-Ciocalteau method (Sadasivam and Manikam, 1992). The total phenol content of male and female trees was also recorded.

3.1.8.1.3.2 Protein banding pattern

Poly acrylamide gel electrophoresis using Hoefer Mighty Small^{TM} 11 gel system was used for protein and isozyme analysis. Acrylamide monomers were

polymerized with N-N methylene bisacrylamide [CH_2 (NHCONH = CH_2)$_2$ bis] to obtain the gel. Freshly prepared ammonium persulphate acted as catalyst and N, N, N^1, N^1 – tetra methyl ethylene diamine (TEMED) as chain initiator. PAGE was preferred because of its chemical inertness, high resolution, easiness in handling, transparency of the gel and easiness in preparation. Poly acrylamide gel electrophoresis (PAGE) using vertical slab gel was carried out for analysis of protein banding pattern.

Leaf samples were collected from each set. Protein pattern of male and female trees was also recorded.

For preparation of gel, following stock solutions were made.

I. Monomer at different levels such as 7.5 and 10.0 per cent polymerization

	7.5% polymerization	10% polymerization
Acrylamide	29.2 g	40.0 g
Bisacrylamide	0.8 g	1.2 g
Water	100 ml	100 ml

Stored at 4°C in amber coloured bottle

II. 4x Resolving gel buffer (1.5 M tris HCl, pH 8.8)

Tris base – 36.8 g

Adjust pH 8.8 with HCl

Water – 200 ml

III. 4x stacking gel buffer (0.5 M tris HCl, pH 6.8)

Tris base – 6 g

Dissolve in 50 ml water. Adjust pH to 6.8 with HCl. Then made upto 100 ml.

IV. Initiator – 10 per cent Ammonium per sulphate

V. TEMED

VI. 2x treatment buffer (0.125 M tris HCl, pH 6.8, 20% glycerol + dye BPB 1%)

4x stacking gel buffer – 5 ml

Glycerol – 2 g

BPB 1% solution – 1 ml

Water to 10 ml

The buffers and monomers were stored in amber coloured bottles at 0.4°C. Ammonium persulphate solution was prepared fresh each time.

Preparation of gel

Gel column of 7.5 per cent and 10 per cent polymerisation were carried out. The size of the slab gel was 16 cm x 14 cm x 0.01 cm. Three fourth of the slab was filled with resolving gel and kept for half an hour for polymerization. Poured distilled water on the top of the gel solution to prevent drying. After half an hour, poured the water out and applied the stacking gel above the resolving gel. Combs were placed on the top to make the wells. The glass plates were mounted in the polymerisation stand.

The composition of resolving gel and stacking gel is given below.

Resolving gel (10 ml)

Monomer – 2.7 ml

Resolving buffer – 2.5 ml

APS – 0.100 ml

TEMED – 0.010 ml

Water – 4.69 ml

Stacking gel (5 ml)

Monomer – 0.700 ml

Stacking buffer – 1.25 ml

APS – 0.050 ml

TEMED – 0.010 ml

Water – 3 ml

Protein extraction

Protein extract was prepared from the sample by using the extraction buffer mentioned below.

Extraction buffer

Phosphate buffer (pH 7.2, 0.05 M)

Stock solution A – 2.78 g/100 ml NaH_2PO_4

Stock solution B – 5.365 g/100 ml $Na_2H\ PO_4\ 7H_2O$ or

7.17 g/100 ml $Na_2H\ PO_4\ 12H_2O$

28 ml of A and 72 ml of.B were mixed and adjusted the pH to 7.2. It was then made up to 200 ml. The buffer was provided with 0.1 per cent ascorbic acid, 0.1 per cent sodium sulphite, 0.1 per cent cysteine HCl.

Preparation of sample

Fully opened second leaf from the top was utilised for the study. Fresh leaf samples were collected in ice buckets. 3.5 g sample was homogenised in five ml extraction buffer at 4°C. While grinding, about 500 mg insoluble PVP was added to avoid the interference of phenolics. The sample was centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was taken for analysing protein banding pattern.

Sample and dye were mixed in 9:1 proportion. Loading of sample in the electrophoresis apparatus was done without further delay.

Loading the sample

After polymerisation, the gels were transferred to the electrophoresis unit. Sufficient quantity of precooled electrode buffer was taken in the upper and lower tanks. Fifteen micro litre sample was applied to each well with transfer pipette. Upper tank was connected to the cathode and lower one to the anode. Bromophenol blue (0.002%) was added to the upper tank as the tracer dye.

A constant current of 7 mA was applied and maintained till the end of running. Electrophoresis was carried out at about 10°C.

Electrode buffer

Tris base – 1.52 g

Glycine – 7.20 g

Water – 500 ml

Staining of gel

After running, the gels were immersed in the staining solution and placed on the shaker and kept for overnight.

Staining solution

Methanol – 50 ml

Acetic acid – 40 ml

Water – 100 ml

Coomasie brilliant blue – 0.20 g

Destaining of gel

After proper staining, the gels were destained by placing it in destaining solution and the protein bands became visible.

Destaining solution

Methanol – 5 ml

Acetic acid – 4 ml

Water – 100 ml

3.1.8.1.3.3 Isozyme banding pattern

Polyacrylamide gel electrophoresis using vertical slab gel was carried out for isozyme analysis of esterase and peroxidase.

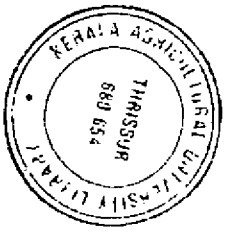
Preparation of sample

The sample extract as prepared for analysing protein banding pattern was used for isozyme studies. But the maturity of the leaf sample taken for the analysis of peroxidase enzyme varied. Mature leaf i.e., third leaf from the top was collected. Loading and running of sample were carried out as that of protein banding.

a) Esterase banding pattern

Staining for esterase

Esterase bands were made visible by incubating the gels for 20 minutes in 150 ml, 0.15 M phosphate buffer (pH 7.2), containing 150 mg fast blue RR salt and 60 mg α-naphthyl acetate dissolved in 5 ml acetone and rinsing them 5 minutes in a 15:4:1 mix of water, methanol and acetic acid. Observations were recorded without delay after draining the solution.



b) Peroxidase banding pattern

Staining for peroxidase

Incubated the gel in the following solution.

0.2 M acetate buffer – 200 ml (pH 5.6)

Benzidine – 0.20 g

H_2O_2 3% - 0.80 ml

Fresh stain was prepared each time. Acetate buffer and benzidine were mixed, heated for dissolving, cooled, filtered and then H_2O_2 was added (add H_2O_2 just before use). The gels were immersed in staining solution till brown bands appeared and destained in 7 per cent acetic acid. As the bands appeared faded on standing for long time, photographs were taken on the same day of staining.

3.1.8.2 Seedling variations based on pigmentation

3.1.8.2.1 Growth rate of seedlings

The seedlings were grouped based on flush colour and variation in height, number of leaves, length and width of the leaves were recorded.

3.1.8.2.2 Biochemical characterisation

3.1.8.2.2.1 Total phenol content

Total phenol content of the seedlings belonging to different flush colour, green, red and brown were also recorded.

3.1.8.2.2.2 Protein banding pattern

Protein banding pattern of seedlings of each flush colour was analysed.

3.1.8.2.2.3 Isozyme banding pattern

Esterase and peroxidase banding patterns of seedlings of each flush colour were also analysed.

3.1.9 Variations in polyembryonic seedlings

3.1.9.1 Growth rate of seedlings

Growth rate of polyembryonic seedlings like height, number of leaves, length and width of third leaf were recorded at monthly intervals. Twenty seedlings were taken for study.

3.1.9.2 Pigmentation

Pigmentation of the seedlings was also observed.

3.1.9.3 *Biochemical characterization*

3.1.9.3.1 Total phenol content

Variation in total phenol content among the seedlings was recorded.

3.1.9.3.2 *Protein* banding pattern and isozyme banding pattern

Protein banding pattern, peroxidase banding pattern of polyembryonic seedlings were recorded.

3.1.10 Growth pattern of seedlings

The general trend in the growth pattern of seedlings was worked out.

Experiment II

3.2 Alteration of sex through top working

The second part of the experiment was carried out both in the College Orchard and Vegetable Research Farm.

3.2.1 Beheading

Flowering male trees of 10 to 15 years age were selected (Plate 1). They were cut at 1m height leaving one or two small branches below the cut end. Beheading was done during April. The cut portion of the stem was protected from fungal infection by applying Bordeaux paste and covered with poly bag (Plate 2). To protect the tree from excessive sunlight or rain, artificial shade with coconut leaves was given properly. Observations on girth of the trunk were recorded.

3.2.2 Sprouts

Sprouts developed within two weeks and attained graftable size in two months. Number of sprouts, length and girth of the sprouts were recorded for trees subjected to each treatment.

3.2.3 Different methods of top working tried

Cleft grafting or flush grafting, bark grafting and patch budding were tried. Thirteen trees were subjected to each treatment. Three to four graftings or buddings were tried on each tree.

Plate 1. Mature male tree

Plate 2. Beheaded male tree

3.2.3.1 Cleft grafting (flush grafting)

Orthotropic or erect shoots that came up from the stump just below the beheaded portion were ready for cleft grafting two months after beheading. Three to four healthy flushes were retained on four sides. A vertical cut of 3 cm deep was made on the flush. Precured scion from female tree was taken. The thickness of scion shoot was exactly the same as that of erect shoot produced in the beheaded male tree. The scion was inserted into the cleft of male shoot in such a way that the wedge was fixed exactly into the cleft. Grafted portion was tied firmly using a polythene strip ensuring prevention of water into the grafted portion (Plate 3). Grafting was carried out from June-October (2000 and 2001).

Preliminary study was taken up to standardise the age of sprout and age of scion.

3.2.3.1.1 Effect of age of sprout on flush grafting or cleft grafting

Flush grafting was done on sprouts of one month, two months, three months, four months and five months old and grafting success was recorded.

3.2.3.1.2 Effect of age of scion on flush grafting

Grafting was carried out with scions of varying maturity and the percentage success was recorded.

3.2.3.1.3 Influence of season on success of flush grafting

Percentage success of flush grafts, which were grafted at different months, was calculated.

Plate 3. Steps involved in cleft grafting

a. Sprout/flush as rootstock

b. A vertical downward cut for inserting scion

c. Insertion of scion into the stock

d. Securing of scion using polythene strip

3.2.3.2 Bark grafting

Bark grafting was done on the stump of the beheaded trees. Bark was peeled at a distance of 3 cm from top. The precured scion of six to eight months old was inserted in such a way that the deep cut of the scion touches the wood and shallow cut touches the bark. It was then tied firmly with jute thread. The grafted portion was pasted with mud and then covered with a polythene sheet, which was again fastened with jute thread (Plate 4). Three to four graftings were done on each tree. For retention of moisture moss was placed around the grafts during hot sunny days. Bark grafting was carried out from June-October (2000 and 2001).

3.2.3.2.1 Effect of girth of the tree on success of bark grafts

Bark grafting was done on trees of different girths 30cm, 40cm, 50cm, 65cm and 150cm using scion of six to eight months old and grafting success was recorded.

3.2.3.3 Patch budding

Patch budding was carried out in 2 month old flushes. A rectangular piece of bark was removed from the flush. Similar piece of bark with dormant bud was taken from orthotropic shoots of female trees. The scion was placed on the stock and tied firmly with bud exposed using a polythene strip taking care that the bud is not inverted.

3.2.4 Preparation of scion

Healthy scions were collected from orthotropic shoots of female trees. The scions were precured before the detachment from the female trees. The precured scions were given a shallow cut on one side and a deep cut on the other side. Ten cm long cuttings were taken for grafting (Plate 5).

Plate 4. Steps involved in bark grafting

a. Preparation of rootstock – flap of bark opened to receive scion

b. Inserting of scion

c. Tying with jute thread

d. Pasting with mud

e. Wrapping with polythene sheet

Healthy and high yielding female trees were selected for collecting bud wood. The bud wood (scion) was taken from orthotropic shoots with bark thickness that could match more or less the peeled bark of the stock. Bud piece was removed by cutting a rectangular patch and it was lesser in size so that when inserted on the stock, some space was left on four sides.

3.2.5 After care

Frequent watering was given to grafts immediately after grafting. Frequency was reduced after 3 weeks. During periods of severe wind and hot weather, the grafts were protected by providing a temporary shelter on either sides and on the top with plated coconut leaves and hanging water filled pots with provision for dripping of water through the whips fixed in the holes to maintain humidity around the grafts (Plate 6). Refilling of pots was done periodically. During heavy rains, placing a polythene cover over the grafts protected the grafts. The scions after graft take, started bending downwards. To prevent breakage, staking was done. The sprouts arising from below the grafted portion were removed periodically. The polythene strip was removed after 30 to 45 days when the perfect union occurred. When the grafts were heavily infested with leaf hoppers, a spray of Ekalux @ 2 ml/l was given.

3.2.6 Percentage of success

Percentage success of grafting/budding was calculated.

3.2.7 Growth of scion at monthly intervals

Following observations like girth of the scion, height of the scion, number of branches and number of leaves were recorded at monthly intervals.

Plate 5. Precured scion

Plate 6. After care of the grafted shoot

Plate 7. Application of cultar

Experiment III

3.3 Regulation of flowering through bioregulator

Efforts were made to shift flowering to an early date through bioregulator application. The experiment was carried out in the College Orchard and Vegetable Research Farm of College of Horticulture, Vellanikkara.

3.3.1 Selection of trees

Flowering female trees of 15 years of age were selected and three trees were subjected to each treatment.

3.3.2 Measurement of canopy

The canopy measurement of each tree was recorded using a tape. The canopy spread in North-South and East-West directions were taken and the average was recorded.

3.3.3 Rate and method of application

The rate of cultar was calculated based on canopy measurements. Cultar @ 3 ml and 5 ml/metre canopy diameter/spread was drenched. The measured amount of cultar was dissolved in 10 litres of water. Then it was poured into shallow pits dug around the tree at equal intervals (Plate 7). Irrigation was given soon after application and continued for two months. Data on the canopy measurements and amount of cultar applied is given in the Table 2.

Table 2. Rate and amount of application of cultar

Sl.No.	Treatments	Canopy (ml)	Amount of cultar (ml)	Time of application
1	T_1	5.45	16.30	16.9.2000
2	T_2	6.15	30.76	16.9.2000
3	T_3	4.73	14.18	1.10.2000
4	T_4	3.40	17.02	1.10.2000
5	T_5	3.52	10.57	16.10.2000
6	T_6	3.65	18.25	16.10.2000
7	Control	5.65		

T_1 - 3 ml cultar 90 days before flowering

T_2 - 5 ml cultar 90 days before flowering

T_3 - 3 ml cultar 75 days before flowering

T_4 - 5 ml cultar 75 days before flowering

T_5 - 3 ml cultar 60 days before flowering

T_6 - 5 ml cultar 60 days before flowering

3.3.4 Time of application

The measured amount of cultar was drenched 60, 75 and 90 days before the expected date of flowering. The expected date of flowering was noted and the time of application was fixed.

3.3.5 Flowering and fruiting characters

a) Time of flowering

Date of appearance of flowers in 50 per cent of the branches was recorded in each treatment.

b) Percentage fruit set

Percentage fruit set in each tree was calculated. For this 100 flowers were bagged on all the four sides and percentage set was noted.

c) Time taken for maturity

Time taken for the fruits to mature was calculated by counting the number of days from fruit set to harvest.

d) Yield

Yield of each tree subjected to each treatment was recorded.

3.3.6 Fruit measurements at harvest

a) Length and girth of the fruits

Length of 10 fruits from each tree was measured from base to apex using flexible twine and mean was calculated. Girth of ten fruits from each tree was measured and the mean worked out.

b) Number of ridges per fruit

Number of segments of 10 fruits from each tree was counted and the mean worked out.

c) Number of seeds

Number of seeds per fruit was noted for ten fruits from each tree in each treatment and the mean calculated.

3.3.7 Fruit quality

a) Total soluble solids (TSS)

TSS of fresh rind as well as mucilage was found out by using Erma hand refractometer (0-32° Brix) and expressed in degree brix.

b) Total acidity

Acidity of fresh rind was determined by titration with standard sodium hydroxide solution and expressed as percentage of citric acid.

c) Hydroxy citric acid

Hydroxy citric acid was estimated as suggested by Lewis *et al.* (1964).

d) Recovery percentage

One kilogram of fresh fruits was subjected to smoke drying after removing the pulp and seed. The dried fruit rind was weighed and the recovery percentage was measured.

$$\text{Recovery percentage} = \frac{\text{Weight of dried rind}}{\text{Weight of fresh rind}} \times 100$$

3.4 Statistical analysis

Statistical analysis was carried out using Mstat-C Package.

RESULTS

RESULTS

The experiments conducted in the year 1999-2001 under the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara revealed the following results.

Experiment I

Hundred flowers each were subjected to self pollination, hand pollination, open pollination and agamospermy.

4.1 Regulation of sex through controlled pollination

4.1.1 Pollen studies

The data on pollen fertility showed significant difference between male and bisexual flowers (Table 3).

Table 3. Pollen fertility of male and bisexual flowers

Flower type	Acetocarmine stain test		
	Total number of pollen observed	Number of fertile pollen	Percentage fertility
Male flower	2798.33	1942.00	69.0
Bisexual flower	1238.00	270.66	21.8
CD	(462.33)	(94.68)	(41.88)

Acetocarmine stain test showed 69 per cent and 21.8 per cent fertility for pollen from male and bisexual flowers respectively.

4.1.2 Pollinating agents

Air sampling did not give any result with kodampuli pollen. However it was found that many insects were visiting the kodampuli flowers during anthesis period. Insects trapped included ants, honeybees and small beetles. This indicates that pollination in kodampuli is mainly entomophilous.

4.1.3 Pollination studies

Data on percentage fruit set observed under different modes of pollination are presented in Table 4. The data showed that fruit set occurred both under artificial cross pollination or hand pollination and open pollination. Practically no fruit set was observed in self pollination and agamospermy.

Table 4. Fruit set under different modes of pollination

Sl.No.	Treatments	No. of flowers pollinated	Fruit set	Percentage set (%)
1	Self pollination	100	0	0
2	Hand pollination	100	60	60
3	Open pollination	100	35	35
4	Agamospermy	100	0	0

4.1.4 Time taken for fruit set

Data on time taken for fruit set was observed separately for open pollination and hand pollination. No pronounced difference was noted in the time taken for fruit set under different modes of pollination. The average number of days taken for fruit set in artificial cross pollination and open pollination was 2.4 and 2.6 days respectively.

4.1.5 Fruit set, fruit measurements and fruit drop

4.1.5.1 Fruit set

Data on extent of fruit set obtained under different modes of pollination are presented in Table 4. Bisexual flowers, which were bagged for eliminating the chances of cross pollination failed to set any fruit. The fruit set was only 35 per cent under open pollinated conditions. The fruit set increased to 60 per cent when the bisexual flowers were artificially pollinated with pollen from male flowers. Flowers, which were emasculated and bagged, also failed to set fruit.

4.1.5.2 Fruit measurements

a) Fruit girth

Data on fruit growth recorded in terms of girth at different stages of development are presented in Table 5.

Table 5. Increase in fruit girth (cm) under different modes of pollination

Sl.No.	Treatments	Days after set						
		20	40	60	80	100	120	At harvest
1	Hand pollination	4.16	5.58	8.64	10.40	13.16	15.07	16.80
2	Open pollination	3.50	5.46	7.40	9.20	11.20	12.88	14.77

t value – 2.2823

Fruits obtained through hand pollination were generally larger in size when compared to open pollinated fruits throughout the stages of development though statistically much difference was not noticed. Significant difference was noticed only at the final stage of development.

b) Fruit shape

Round fruits and fruits with one side flat could be observed in both open and artificially cross pollinated fruits (Plate 8).

4.1.5.3 Fruit drop

Fruit drop was recorded for open pollination and artificial cross pollination. Chi-square analysis indicated no significant difference between treatments in percentage fruit drop (Table 6).

Table 6. Fruit drop under different modes of pollination

Sl.No.	Treatments	Flowers pollinated	Number of fruits set	Number of fruits dropped	Percentage drop
1	Hand pollination	100	60	36	60
2	Open pollination	100	35	22	63
CD					NS

Data showed 63 per cent drop in fruits which were open pollinated and 60 per cent drop in fruits which were cross pollinated.

4.1.6 Seed germination

4.1.6.1 Percentage germination

Germination of open pollinated seeds was 65 per cent and that of cross pollinated seeds was 70 per cent.

4.1.6.2 Seed dormancy

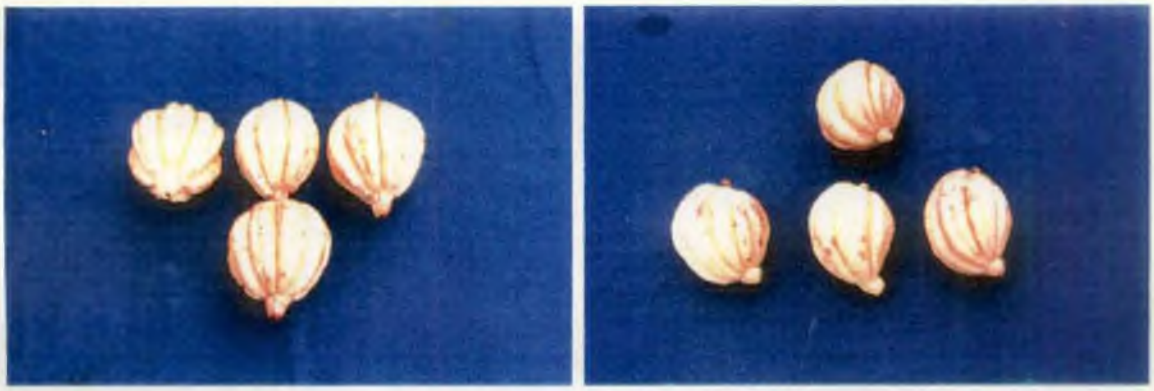
Results on percentage germination and time taken for germination of seeds subjected to various treatments are given in the Table 7.

Table 7. Effect of various seed treatments in breaking seed dormancy

Treatments	Germination (%)	Time taken (months)
T_1	60	12
T_2	50	11
T_3	0	0
T_4	0	0
T_5	55	12
T_6	60	12
T_7	50	12
T_8	50	12
T_9	60	13
T_{10}	50	13
T_{11}	55	13
T_{12}	60	12
T_{13}	60	12
T_{14}	63	13
T_{15}	50	13
T_{16}	55	12
T_{17}	65	13
T_{18}	60	12
T_{19}	60	1
$T\ 20_{(control)}$	70	13

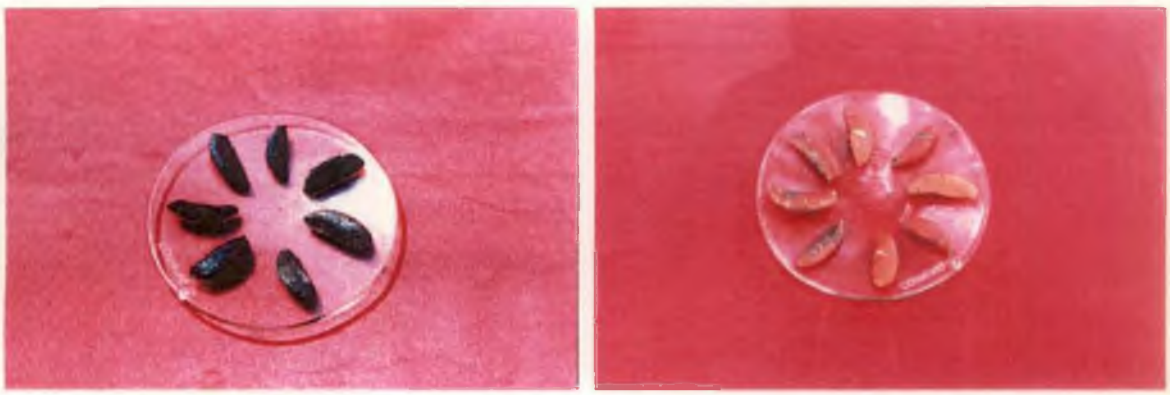
Data indicated that the treatments did not differ much except for the treatment T_{19} (seeds without seed coat) that germinated in one month and recorded 60 per cent germination (Plate 9b). The treatments T_3 and T_4 did not give any germination. The treatments T_1, T_5, T_6, T_7, T_8, T_{12}, T_{13}, T_{16} and T_{18} took one year to germinate whereas the treatments T_{11}, T_{14}, T_{15} and T_{17} germinated only after 13 months. The treatment T_{20} (control) gave 70 per cent germination in 13 months time (Plate 9a).

Plate 8. Fruit shape



a. Round fruits **b.** Fruits with one side flat

Plate 9. Seeds of *Garcinia*



a. With seed coat **b.** Without seed coat

Plate 10. Polyembryony

4.1.7 Polyembryony

Of the hundred seeds selected at random, seven of them showed polyembryony. Percentage polyembryony recorded was seven per cent. In the polyembryonic seeds, generally two seedlings were observed and rarely three (Plate 10).

4.1.8. Seedling variations

The results regarding the morphological and physiochemical variations of seedlings in each treatment are given below.

4.1.8.1 Based on mode of pollination

4.1.8.1.1 Growth rate of seedlings

The difference in growth rate was recorded in open pollinated and artificially cross pollinated seedlings and is shown in Table 8.

a) Height

Analysis of variance indicated that there existed no significant difference between open pollinated and hand pollinated seedlings with regard to height taken at monthly intervals.

b) Number of leaves

Results showed no significant difference in the number of leaves taken at monthly intervals. The mean number of leaves of open pollinated and hand pollinated seedlings during the first month was 6.9 and 5.9 respectively and during fourth month was 9.5 and 8.0 respectively.

Table 8. Growth rate of seedlings based on mode of pollination

Sl No.	Month / Treatments	Growth parameters															
		Height (cm)				No. of leaves				Length (cm)				Width (cm)			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	Hand pollination	6.90	7.68	8.41	9.12	5.90	6.00	6.10	8.00	3.83	3.32	3.57	4.06	2.13	2.04	2.51	2.12
2	Open pollination	5.87	6.26	7.20	8.00	6.95	7.00	7.15	9.50	3.95	4.10	4.18	5.11	1.98	2.08	2.07	2.59
	CD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

c) Length of the leaf

No significant difference was noticed in length of leaf between open pollinated and hand pollinated seedlings.

d) Width of the leaf

Data on the width of the third leaf indicated no significant variation between open pollinated and hand pollinated seedlings.

4.1.8.1.2 Pigmentation

Differences in pigmentation were observed in the seedlings of both the treatments. Both the open pollinated and hand pollinated seedlings varied in flush colour with green, red and brown (Plate 11).

4.1.8.1.3 Biochemical characterisation based on mode of pollination

4.1.8.1.3.1 Total phenol content

a) Male and female trees

The total phenol content of mature male and female trees of known sex was analysed and is given in the Table 9.

Table 9. Total phenol content of male and female trees

Item	Total phenol (mg/100 g)	
	Male	Female
Mean	1685.75	1138.00
Range	1376-2004	1052-1160

Plate 11. Seedlings with varied pigmentation

The average total phenol content of male tree was 1685.75 mg 100 g^{-1} and that of female tree was 1138.00 mg 100 g^{-1}.

b) Seedlings

The total phenol content of the seedlings of each treatment is given in Table 10.

Table 10. Total phenol content of seedlings

Item	Total phenol (mg/100 g)					
	Based on mode of pollination		Based on pigmentation			CD
	Open pollinated	Hand pollinated	Green	Red	Brown	
Mean	1097.2	1207	1240.85	1031.28	1029.42	NS
Range	608-1530	630-1631	812-1452	912-1363	608-1530	NS

No significant difference was noted in the total phenol content of the open pollinated and artificially cross pollinated seedlings. The total phenol content ranged from 608 mg 100 g^{-1} to 1530 mg 100 g^{-1} in open pollinated seedlings and 630 mg 100 g^{-1} to 1631 mg 100 g^{-1} in artificially cross pollinated seedlings.

4.1.8.1.3.2 Protein banding pattern

Different extraction buffer systems were tried for isolating active protein groups from leaf tissue. Tris buffer and phosphate buffer with different constituents were tried. Phosphate buffer, which contained antioxidants like ascorbic acid, L cysteine HCl, sodium sulphite, insoluble polyvinyl pyrolidone to remove phenolic interferences and β mercapto ethanol to break disulphide linkages were used.

a) Protein band of mature male and female trees

Electrophoresis showed the presence of only a single fast moving protein with Rm value 0.38 in female trees whereas in male trees two bands with Rm 0.38 and 0.63 were visible.

b) Protein band of seedlings

Banding pattern of open pollinated and hand pollinated seedlings showed no difference. In both sets, seedlings with two bands were resolved with Rm value 0.63 and 0.38 (Plate 12).

4.1.8.1.3.3 Isozyme banding pattern

I. Esterase banding pattern

a) Esterase band of mature male and female trees

Number of esterase bands resolved for both male and female was three with Rm value 0.17, 0.24 and 0.37. But the intensity of the bands varied. In general the bands of male trees were less prominent compared to the female trees. In the case of female trees band with 0.37 was more prominent.

b) Esterase band of seedlings

The esterase bands were least prominent in both open pollinated and hand pollinated seedlings. A single band with Rm 0.37 was resolved for both open pollinated and hand pollinated seedlings. Thus, no difference existed between open pollinated and artificial cross pollinated seedlings with respect to esterase banding pattern (Plate 13).

Plate 12. Protein banding pattern

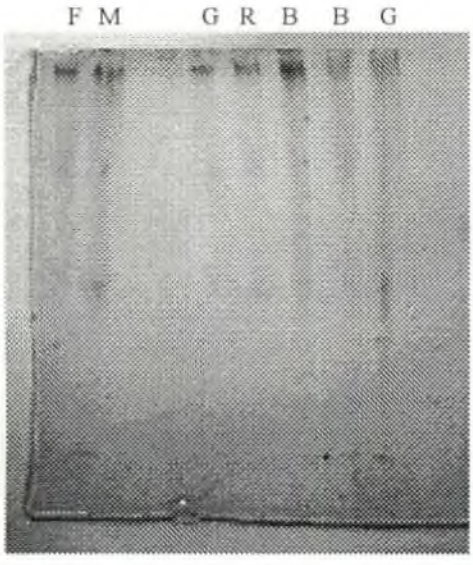


Plate 12a. Open pollinated seedlings

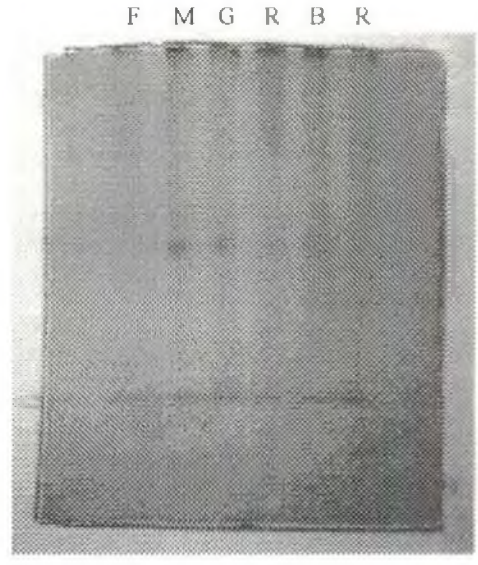


Plate 12b. Hand pollinated seedlings

Plate 13. Esterase banding pattern

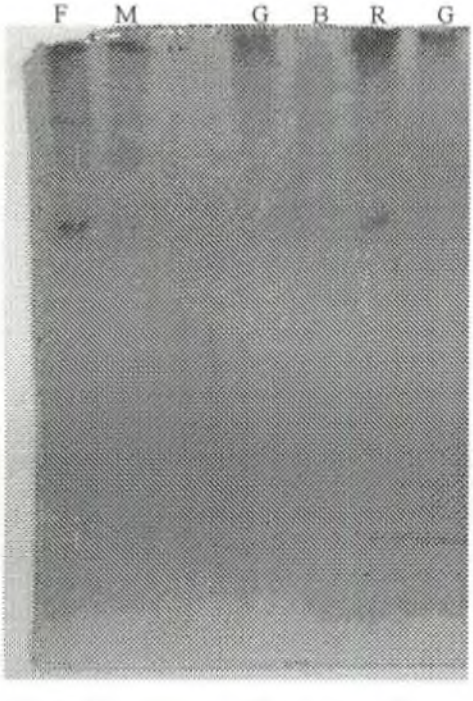


Plate 13a. Open pollinated seedlings

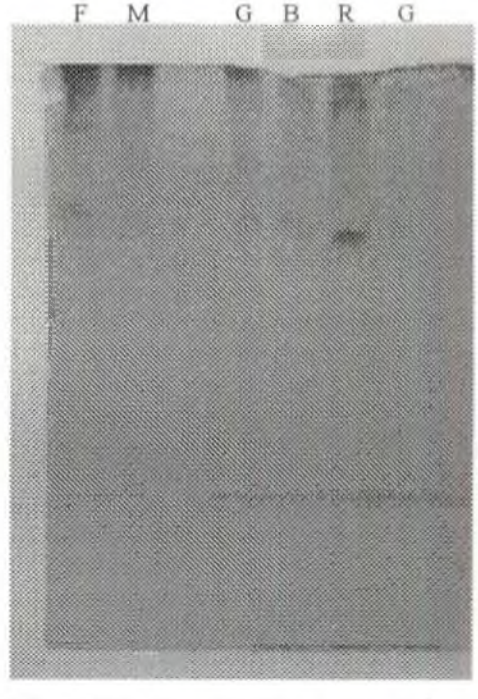


Plate 13b. Hand pollinated seedlings

F = mature female tree
M = mature male tree
G = seedlings with green flush colour
B = seedlings with brown flush colour
R = seedlings with red flush colour

II. Peroxidase banding pattern

a) Peroxidase band of male and female trees

Total number of peroxidase bands resolved for male was two and that for female was three. The Rm value for male was recorded as 0.39 and 0.44 respectively. In the case of female tree it was 0.22, 0.39 and 0.44. The intensity of the bands also varied. Bands of female trees with Rm 0.39 and 0.44 were more intense compared to that of male trees.

b) Peroxidase band of seedlings

The number of bands resolved varied in open pollinated seedlings. Seedlings with 2 bands (Rm 0.39, 0.44) and seedlings with 3 bands (Rm 0.22, 0.39, 0.44) were noticed. Hand pollinated seedlings also showed the same banding pattern. Hence no difference could be noticed on mode of pollination and peroxidase banding pattern (Plate 14).

4.1.8.2 Based on pigmentation of seedlings

The seedlings were grouped on the basis of flush colour. Frequency of occurrence of each pigmentation was noted in a population of 100 seedlings and is given in Table 11

Table 11. Growth parameters of seedlings based on pigmentation

Sl.No.	Treatments (flush colour)	Frequency of occurrence (%)	Growth parameters			
			Height of the seedling (cm)	Number of leaves	Length of the leaf (cm)	Width of the leaf (cm)
1	Green	50	6.757	9.286	5.114	2.071
2	Red	20	6.943	8.429	5.714	2.157
3	Brown	30	7.057	11.143	5.743	2.471
CD			NS	NS	NS	NS

Plate 14. Peroxidase banding pattern

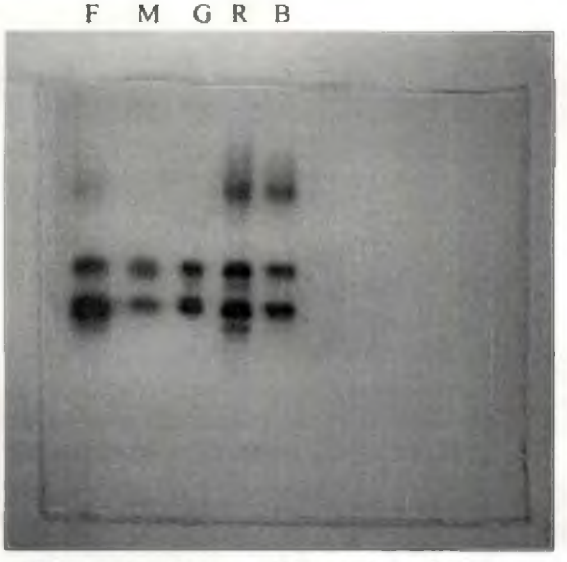


Plate 14a. Open pollinated seedlings

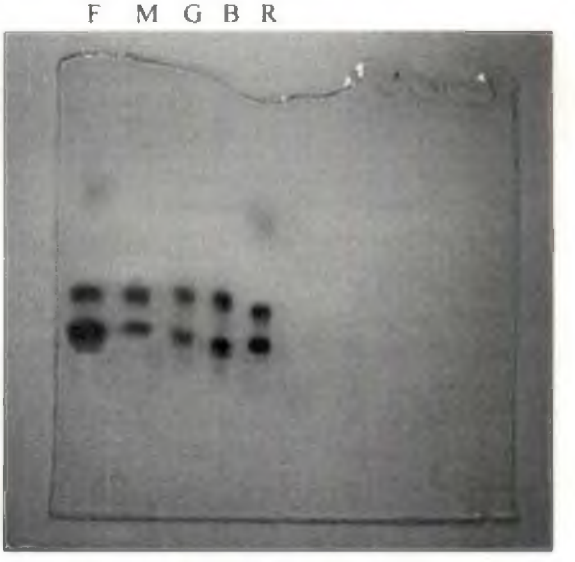


Plate 14b. Hand pollinated seedlings

F = mature female tree
M = mature male tree
G = seedlings with green flush colour
B = seedlings with brown flush colour
R = seedlings with red flush colour

Frequency of green flushed seedling was maximum 50 per cent followed by brown flushed ones (30%) and the minimum (20%) with red flushed seedlings. Regarding the colour of young flush in mature trees, green and red were noticed in male and female trees but red pigmentation was most widely prevalent in female trees.

4.1.8.2.1 Growth rate of seedlings

The seedlings grouped on the basis of flush colour were observed for variation in height, number of leaves and length and breadth of the third leaf from the top. The data is furnished in Table 11. Analysis of variance indicated that the seedlings did not vary significantly in height, number of leaves and length and width of leaf based on flush colour.

4.1.8.2.2 Biochemical characterisation based on pigmentation of seedlings

4.1.8.2.2.1 Total phenol content

Mean values of the total phenol content of seedlings belonging to different flush colour are given in Table 10.

Results showed that 86 per cent of seedlings with green flush colour showed a total phenol content of about 1100 mg 100 g^{-1} and above and in the case of seedlings with red flush colour only 29 per cent seedlings showed a total phenol content of 1000 mg 100 g^{-1} and above. Majority of red flushed seedlings recorded lower values of total phenol. Seedlings with brown coloured flush showed both higher and lower values. The average value of the total phenol content of seedlings with green, red and brown flush colour was 1240.85 mg 100 g^{-1}, 1031.28 mg 100 g^{-1} and 1029.42 mg 100 g^{-1} respectively. Though statistically the difference was non significant, mean values for green seedlings were comparatively on the higher side as compared to red and brown.

4.1.8.2.2.2 Protein banding pattern

The protein banding pattern of seedlings with varied flush colour namely green, brown and red were also analysed. Results showed that seedlings could not be identified based on protein band, as all of them showed two bands each. They could not be correlated with the protein band of mature male and female trees (Plate 12).

4.1.8.2.2.3 Isozyme banding pattern

I. Esterase Banding pattern

In seedlings with green and brown flush, the bands were not visible whereas the seedling with red flush showed a darker band with Rm value 0.37 (Plate 13).

II. Peroxidase banding pattern

Peroxidase bands of the seedlings indicated that the seedlings with red flush colour showed darker bands compared to seedlings with green flush colour. The darker band of seedlings with red flush colour can be compared with that of the female tree. Two bands with Rm 0.39 and 0.44 were intense for seedling with red flush colour (Plate 14).

4.1.9 Variations in polyembryonic seedlings

4.1.9.1 Growth rate of seedlings

Growth rate was measured individually for both the seedlings of a polyembryonic seed and the mean values are depicted in Table 12. Significant difference was noticed in height between two seedlings of a polyembryonic seed (Plate 15). Regarding the number of leaves and length and width of the third leaf from the top no significant variation was noticed between the two seedlings.

Plate 15. Growth rate of polyembryonic seedlings

Plate 16. Pigmentation in polyembryonic seedlings

Table 12. Growth pattern of polyembryonic seedlings

Month / Polyembryonic seedling	Growth parameters															
	Height (cm)				No. of leaves				Length of the leaf (cm)				Width of the leaf (cm)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Seedling 1 (vigorous)	3.93	5.13	5.8	7.8	4.10	5.90	6.90	8.30	2.54	3.36	4.40	5.28	1.57	1.69	1.93	2.61
Seedling 2 (less vigorous)	2.87	4.04	4.38	5.5	3.55	5.05	5.35	7.20	2.21	2.84	3.33	3.47	1.34	1.50	1.68	2.24
CD	0.94	0.94	1.08	1.28	NS	NS	1.43	NS	NS	NS	0.94	NS	NS	NS	NS	NS

4.1.9.2 Pigmentation

Polyembryonic seedlings originating from the single seed showed same pigmentation as well as different pigmentation. Seedlings were either both red, both green or with one red and one green flush colour (Plate 16).

4.1.9.3 Biochemical characterization

The number of seedlings in a polyembryony was two. The seedlings were analysed for biochemical variations.

4.1.9.3.1 Total phenol content

Significant difference was noticed in the total phenol content of the two seedlings (Table 13).

Table 13. Total phenol content of polyembryonic seedlings

Item	Total phenol (mg/100 g)		
	Seedling 1 (Vigorous)	Seedling 2 (Less vigorous)	CD
Mean	858.15	1414.15	(164.48)
Range	462-1401	832-1920	

Vigorous seedlings showed a total phenol content 858.15 mg 100 g^{-1} and less vigorous seedling showed a mean total phenol content of 1414.15 mg 100 g^{-1}. The total phenol content of vigorous seedlings ranged from 462 mg 100 g^{-1} to 1401 mg 100 g^{-1} and that of less vigorous seedlings ranged from 832 mg 100 g^{-1} to 1920 mg 100 g^{-1}. It was also observed that certain polyembryonic seedlings showed more or less the same phenol content. Seedlings with green flush colour showed values towards the higher side and those with red flush colour towards the lower side.

4.1.9.3.2 Protein banding pattern and isozyme banding pattern

Protein banding pattern of the twin seedlings were same with Rm value 0.38 and 0.63 and no variation was noticed between the two seedlings of a single seed (Plate 17a).

Variation was noticed with respect to number and intensity of peroxidase bands between two seedlings of a polyembryonic seed. In majority of the polyembryonic seedlings number of bands differed between the two seedlings. The bands resolved for the less vigorous seedling was less intense for majority of the seedlings than the other one (Plate 17b).

4.1.10 Growth pattern of seedlings

The general trend in growth pattern of seedlings was analysed. A continuous seedling growth was observed throughout the year. However the increment in height recorded varied from month to month (Table 14).

Table 14. Increment in height of seedlings

Phases of growth (month)	Average increment in height (cm)
1	0.395 (0.915)^{c}
2	0.550 (0.999)^{bc}
3	0.490 (0.976)^{bc}
4	2.630 (1.747)^{a}
5	2.310 (1.616)^{a}
6	2.410 (1.619)^{a}
7	1.190 (1.235)^{b}
8	1.280 (1.221)^{b}
9	2.185 (2.340)^{a}
10	2.340 (2.270)^{a}
11	2.270 (2.270)^{a}
12	2.650 (2.650)^{a}
CD	(0.277)

The values in parenthesis indicate the transformed values. Transformation $\sqrt{x+0.5}$
Values having any common superscript are not significantly different from one another.

Plate 17. Protein and peroxidase banding pattern of polyembryonic seedlings

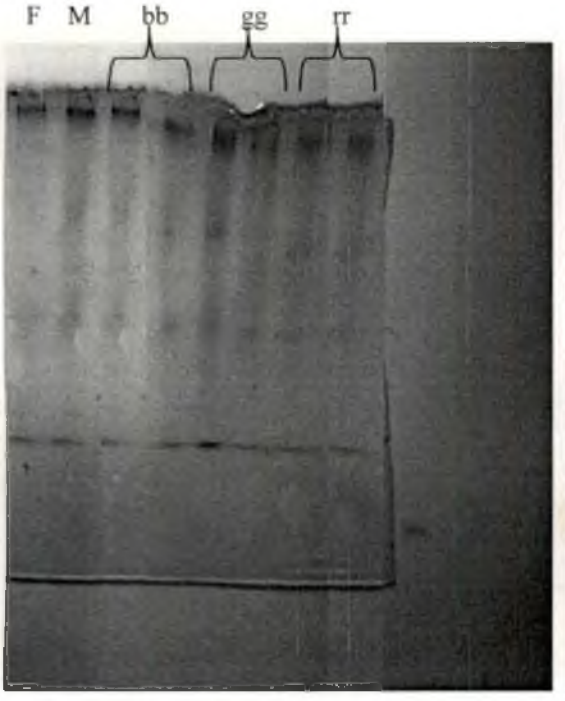


Plate 17a. Protein banding pattern

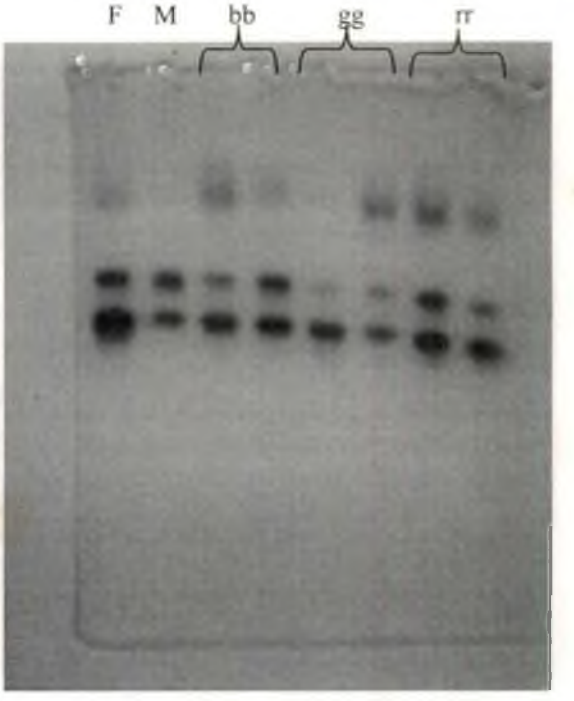
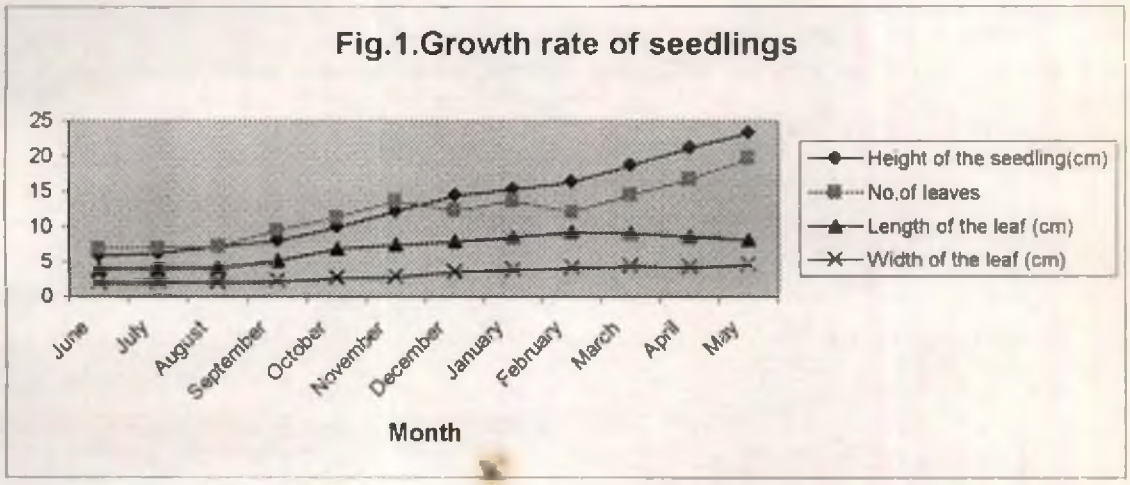


Plate 17b. Peroxidase banding pattern

F = mature female tree M = mature male tree
gg = polyembryonic seedling with green flush colour bb = polyembryonic seedling with brown flush colour
rr = polyembryonic seedling with red flush colour

Statistical analysis of data showed that the mean extension of growth differed significantly among the months.Mean monthly extension of growth was found to be significantly higher during November, December, January, April, May and June. A continuous increase in leaf number was also recorded.No much difference was observed in length and width of the third leaf from the top,during the seedling growth (Fig.1.).



Experiment-II

4.2 Alteration of sex through top working

For conversion of unproductive males to productive females top working was carried out through grafting or budding on beheaded male trees.

4.2.1 Beheading

The male trees were beheaded during April.The observation on girth of the tree, number of sprouts, length of sprouts and girth of sprouts were recorded (Table15 and 16). The girth of 13 trees on which grafting was done ranged from 30 cm to 150 cm.The number of sprouts , one month after beheading ranged from 1 to

Table 15. Characters of beheaded trees used for bark grafting

Tree No.	Girth of the beheaded tree (cm)	Number of sprouts	Height of sprouts (cm)		Girth of sprouts (cm)	
			Minimum	Maximum	Minimum	Maximum
1	30	-	-	-	-	-
2	30	-	-	-	-	-
3	30	1	25		2.5	
4	41	8	2	27	1.8	3.5
5	53	6	9	13	1.5	2.5
6	54	5	0.5	2.0	1.0	1.5
7	65	5	8	28	2.8	4.0
8	150	15	10	30	1.0	2.0
9	40	8	3	23	1.5	4.0
10	50	14	10	35	2.0	2.9
11	41	4	1	10	2.0	2.5
12	34	3	6	10	2.0	3.5
13	35	6	10	32	2.0	3.0

Table 16. Characters of beheaded trees two months after beheading used for flush grafting

Tree No.	Girth of the beheaded tree (cm)	Number of sprouts	Height of sprouts (cm)		Girth of sprouts (cm)	
			Minimum	Maximum	Minimum	Maximum
1	56	6	5	25	0.8	1.2
2	50	6	20	30	1.5	2.0
3	30	2	26	30	2.0	2.2
4	38	5	15	20	1.5	2.0
5	40	4	15	20	1.5	2.0
6	38	3	30	40	1.0	1.5
7	70	4	23	30	1.5	2.0
8	65	5	20	30	1.5	2.0
9	100	15	10	30	0.8	2.0
10	70	6	15	30	1.5	2.5
11	30	5	7	16	2.2	3.0
12	30	2	5	9	2.4	2.5
13	56	11	1	20	2.0	3.0

15, height of the sprouts ranged from 0.5 cm to 35 cm and girth of the sprouts ranged from 1 cm to 4 cm. In another set of 13 trees two months after beheading, the number of sprouts ranged from 2 to 15, height of sprouts from 1cm to 40cm and girth of the sprouts ranged from 0.8cm to 3 cm and they were utilized for flush grafting. Root sucker production was observed in the beheaded trees in this experiment (Plate 18). In the normal case root suckers are not seen around a grown up tree of *Garcinia*.

4.2.2 Girth of the tree and number of sprouts

The influence of girth of the tree on number of sprouts is given by the correlation coefficient 0.757 (Table 17). There is a positive correlation between girth and number of sprouts. Trees with more girth produced more number of sprouts and trees with less girth produced less sprouts.

4.2.3 Effect of age of sprout on flush grafting

Data regarding the influence of age of sprout used as rootstock, on success of flush grafts is given in the Table 18. Observations indicated that two month old sprouts were the best for flush grafting, giving 80 per cent success followed by 3 months and 4 months old flushes with 70 per cent and 30 per cent success respectively. Too tender flushes (one month) and too old flushes (5 months) were not suitable for flush grafting. It was also observed that the girth of the stock should be almost same as that of the scion for a successful graft union. Therefore shoots, which had a minimum length of 20 cm and maximum of 40 cm, were selected for flush grafting.

4.2.4 Effect of type of scion on flush grafting

Relation between type of scion and grafting success is given in Table 19.

Table 17. Effect of girth of the tree on number of sprouts

Sl.No.	Girth (cm)	No. of sprouts
1	56	6
2	50	6
3	30	2
4	38	5
5	40	4
6	38	3
7	70	4
8	65	5
9	100	15
10	70	6
11	30	5
12	30	2
13	56	11

Correlation coefficient-0.757

Table 18. Effect of age of sprout on flush grafting

Sl.No.	Age of new flush	No. of flushes grafted	Initial success	Percentage
1	1 month	10	0	0
2	2 months	10	8	80
3	3 months	10	7	70
4	4 months	10	3	30
5	5 months	10	0	0
CD				(9.488)

Table 19. Effect of type of scion on flush grafting

Sl.No.	Type of scion	Age	No. of shoots grafted	Success	Percentage
1	Light green	1-2 months	16	0	0
2	Dark green	3-4 months	16	4	25
3	Light brown	6-8 months	16	12	75
4	Dark brown	12-16 months	16	8	50
CD					(7.81)

Data indicated that type of scion and the grafting success showed significant variation. Matured scion (light brown) six to eight months old was found to be the best giving 75 per cent success followed by 12-16 months old scion (dark brown) and three to four months old scion (dark green) with 50 per cent and 25 per cent success respectively. Newly emerging light green scion of one to two months maturity started wilting soon after grafting. Matured scions were collected from orthotropic shoots after precuring which gave an erect growth habit whereas plagiotrops showed a drooping or spreading habit (Plate 19).

4.2.5 Influence of season on success of flush grafting

Results showed that season of grafting had a pronounced effect on graft take. Observations regarding the relation between seasons and grafting success is furnished in Table 20.

Table 20. Influence of season on success of flush grafting

Sl.No.	Month of grafting	No. of trees grafted	No. of shoots grafted	Initial success	Percentage	Time taken for sprouting (Days)	
						Without watering	With watering
1	June	2	8	5	63	36	18
2	July	2	8	6	75	35	16
3	August	2	7	5	71	35	20
4	September	2	6	2	33	38	18
5	October	2	8	6	75	38	21
6	November	2	8	3	38	57	40
CD					(11.07)		

Plate 18. Root sucker production in *Garcinia*

Plate 19. Growth habits of top worked scion

a. Erect

b. Horizontal

c. Spreading

d. Drooping

Grafting done during July and October gave highest percentage of success (75%) followed by August (71%) and June (63%). Data also showed that grafting done during September and November gave very low success. The presence of moisture around the grafted shoot also played an important role in grafting success. Retaining high amount of humidity around the grafted shoot could considerably reduce wilting of the grafted scion and helped the scions to sprout faster. On watering, the scion started sprouting in two to three weeks whereas in the absence of watering it took 35 to 50 days.

4.2.6 Effect of girth of the tree on bark grafting

Statistical analysis indicated that girth of the tree has little influence on the success of the grafts. The data showing the relation between girths and grafting success is furnished in the Table 21.

Table 21. Effect of girth of the tree on bark grafting

Sl.No.	Girth (cm)	Number of trees grafted	Number of graftings done	Initial success	Percentage
1	30	3	9	6	66
2	40	3	9	7	77
3	50	3	12	9	75
4	65	1	4	2	66
5	150	1	4	2	66
CD					NS

Bark grafting was carried out in trees of different girths viz. 30 cm, 40 cm, 50 cm, 65 cm and 150 cm. The percentage success recorded for each was 66, 77, 75, 66 and 66 respectively.

4.2.7 Success of grafting or budding

Percentage success of grafting and budding is given in the Table 22.

Table 22. Percentage success of grafting and budding

Sl. No.	Treatments	Number of trees grafted/ budded	Total number of grafting/ budding done	Initial success (After one month)	Percentage	Established (after three months)	
						Number	Percentage
1	Bark Grafting	13	38	27	71	10	33
2	Flush grafting	13	52	39	75	15	38
3	Patch budding	13	26	0	0	0	0

The table shows that the initial success of the grafts was very high compared to the establishment percentage. Initial success of the bark grafts and flush grafts recorded after one month was 71 per cent and 75 per cent respectively. After three months establishment recorded was 33 per cent and 38 per cent respectively for bark and flush grafts. Patch budding was a failure. No significant difference was noticed in the percentage success of bark grafts and flush grafts.

4.2.8 Growth of grafted scion at monthly intervals

Growth of flush grafted and bark grafted scion was recorded at monthly intervals and is furnished in the Table 23. The data indicated that no significant variation existed in growth pattern till the fifth month. Thereafter, slight differences were noticed in all the characters studied (Fig.2). It was seen that the performance of the bark grafted scion was slightly superior to that of the flush grafted one (Plate 20a and 20b).

4.2.9 Top worked tree after one year of grafting

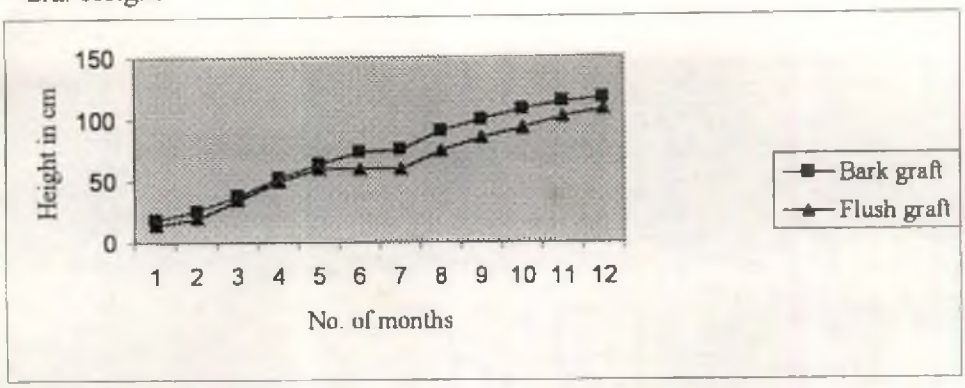
Plate 20 a shows the appearance of a top worked tree after one year of grafting. After one year, the graft had an average height of 117 cm with 27 numbers of branches. The average girth of the graft was 7.9 cm.

Table 23. Growth of grafted scion at monthly intervals

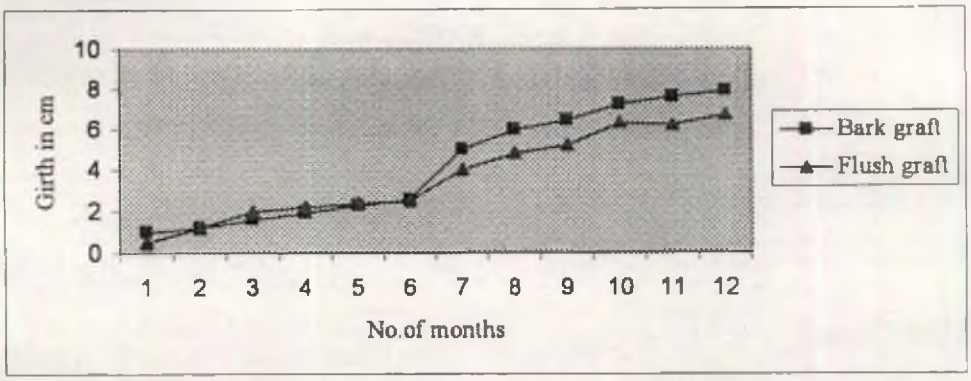
Month / Treatments	Height (cm)				Number of branches				Number of leaves				Girth (cm)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Bark grafting	18.57	25.85	38.14	52.80	1.57	5.14	8.28	12.16	10.80	20.85	32.00	57.50	1.00	1.2	1.6	1.95
Flush grafting	10.00	17.00	25.75	36.25	1.75	2.50	3.00	3.00	12.50	21.25	28.5	33.75	0.95	1.2	1.7	1.9
t- statistic	NS	NS	NS	NS	NS	NS	2.35	3.50	NS	NS	NS	2.29	NS	NS	NS	NS

Fig.2.Growth of top worked scion at monthly intervals

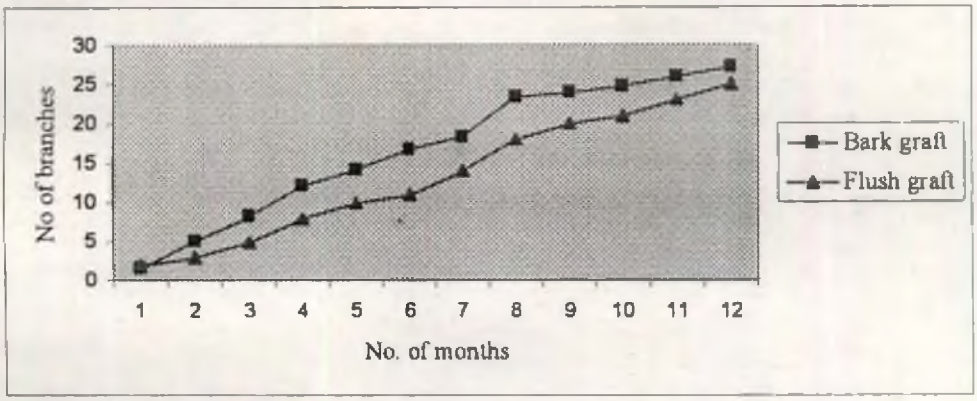
2.a. Height



2.b. Girth



2.c. Number of branches



2.d. Number of leaves

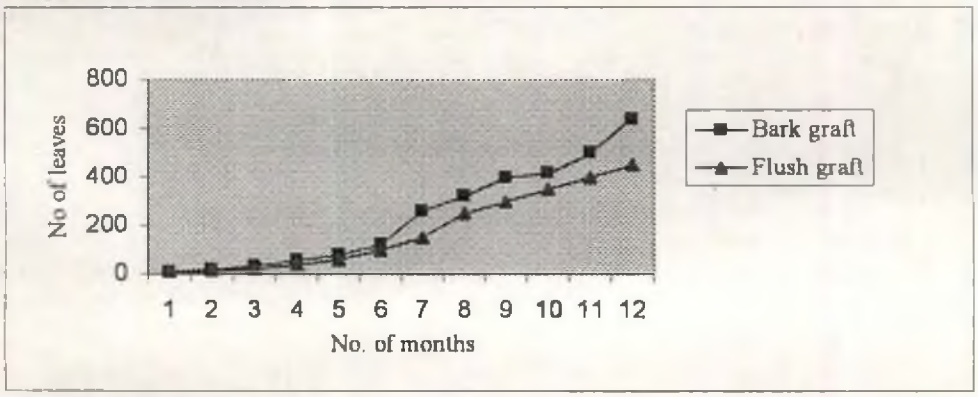


Plate 20a. Growth of top worked scion (bark grafted)

2 months

4 months

6 months

8 months

10 months

12 months

Plate 20b. Growth of top worked scion (flush grafted)

1 month

2 months

3 months

5 months

7 months

Experiment-III

4.3 Regulation of flowering through bioregulator

For regulation of flowering in kodampuli, cultar @ three ml and five ml per metre canopy diameter/spread was drenched 60, 75 and 90 days before the expected date of flowering.

4.3.1 Effect of cultar on flowering and fruiting characters

Time of flowering, percentage fruit set, time taken for maturity and yield were recorded from trees which were treated with cultar. The data recorded are given in the Table 24.

Table 24. Effect of cultar on flowering and fruiting

Sl.No.	Treatments	Number of days for flowering	Percentage fruit set	Time taken for maturity (days)	Yield (kg)
1	T_1	-10	66.00	123.33	20.50
2	T_2	+20	75.00	117.66	22.60
3	T_3	+7	55.00	126.33	13.33
4	T_4	+10	48.33	126.00	10.30
5	T_5	No flowering	-	-	-
6	T_6	No flowering	-	-	-
7	Control	*	68.33	118.33	22.00
CD			(10.05)	NS	(4.53)

'+' early compared to control
'-' late compared to control
*date of flowering of control –18.2.2001

a) Time of flowering

Time of flowering was noted for trees, which were subjected to different treatments. It was observed that treatments T_2 (five ml cultar 90 days before flowering) showed 20 days early flowering T_4 (5 ml cultar 75 days before flowering) showed 10 days early flowering and T_3 (3 ml cultar 75 days before flowering) showed seven days early flowering. Treatment T_1 started flowering ten days late compared to control. Treatments T_5 (three ml cultar 60 days before flowering) and T_6 (five ml cultar 60 days before flowering) did not flower at all.

b) Percentage fruit set

Significant difference was noted between the treatments in percentage fruit set. Analysis of variance indicated that among the treatments, the percentage fruit set was maximum for treatment T_2 (75%) that showed early flowering followed by T_1 (three ml cultar 90 days before flowering) which gave a percentage fruit set of 66. Low fruit set was recorded by T_3 (55 per cent) whereas T_4 recorded the lowest fruit set of 48.33 per cent. T_5 and T_6 did not give any fruit set at all.

c) Time taken for maturity

Regarding the time taken for maturity no significant variations were noted among the treatments. The treatment T_2 took minimum time of 117.66 days and was very close to the control (118.33 days). The treatment T_1 took 123 days whereas T_3 and T_4 took almost the same time of 126.33 and 126.00 days respectively.

d) Yield

Treatments T_1 and T_2 did not show any significant increase in yield compared to control. T_2 recorded an yield of 22.6 kg which was very close to the control (22 kg). T_3 and T_4 recorded very low values of yield (13.33 kg and 10.3 kg

respectively) and were significantly lower than the control (22 kg). T_5 and T_6 did not give any yield at all.

4.3.2 Effect of cultar on fruit measurements

Observations on fruit measurements are given in the Table 25.

Table 25. Effect of cultar on fruit measurements

Sl.No.	Treatments	Length of the fruit (cm)	Girth of the fruit (cm)	Number of segments	Number of seeds
1	T_1	8.53	16.92	8.40	4.26
2	T_2	8.38	16.56	7.66	4.63
3	T_3	8.76	15.93	8.50	4.30
4	T_4	8.14	15.63	7.03	5.46
5	Control	8.61	15.06	8.50	5.00
CD		NS	NS	NS	NS

a) Length of the fruit

Treatments did not show any significant variation among themselves with regard to length of the fruit. All the treatments were on par with the control (8.61 cm) with respect to the length of the fruit.

b) Girth of the fruit

No significant variation was noticed among the treatments with respect to girth of the fruit. T_1 and T_2 had a fruit girth of 16.92 cm and 16.56 cm. T_3 and T_4

recorded a girth of 15.93 cm and 15.63 cm respectively. Minimum fruit girth was for control (15.06 cm).

c) Number of segments/fruit

Treatments did not differ significantly in number of segments of the fruit. T_2 and T_4 had less number of segments, viz., 7.66 and 7.03 respectively compared to control (8.5). The number of segments of T_1 and T_3 were 8.4 and 8.5 respectively and were very close to the control.

d) Number of seeds/fruit

Treatments did not show any significant variation in the number of seeds. Treatment T_4 had maximum number of seeds (5.46) compared to other treatments T_1 (4.26), T_2 (4.63), T_3 (4.3) and control (5.0).

4.3.3 Effect of cultar on fruit quality

Observations indicated that the treatments did not vary significantly among themselves and also with control with respect to fruit quality (Table 26).

Table 26. Effect of cultar on fruit quality

Sl.No.	Treatments	Total soluble solids TSS (°Brix)		Acidity (%)	HCA (%)	Recovery (%)
		Mucilage	Rind			
1	T_1	10.61	10.73	20.90	17.23	28.00
2	T_2	10.30	10.63	22.70	19.77	27.23
3	T_3	10.23	10.10	21.93	18.97	28.93
4	T_4	10.13	10.10	22.33	19.27	27.00
5	Control	10.10	10.00	20.60	17.90	28.00
CD		NS	NS	NS	NS	NS

a) Total soluble solids (TSS)

Total soluble solid content of mucilage and rind was recorded. It was observed that no significant variation was noticed between the treatments. Maximum TSS for mucilage and rind was recorded by T_1 with 10.61°Bx and 10.73°Bx respectively and minimum TSS was recorded by control with 10.1°Bx and 10.0°Bx respectively. TSS of the mucilage for other treatments T_2, T_3, T_4 was 10.30, 10.23 and 10.13°Bx respectively. For rind, the TSS was 10.63, 10.10 and 10.10°Bx for T_2, T_3 and T_4 respectively.

b) Acidity

With respect to acidity also, no significant variation was noticed. Maximum acidity was noticed for T_2 (22.70%) and minimum for control (20.6%). T_1 recorded a percentage acidity of 20.9, which was very close to control (20.6). T_3 and T_4 recorded a percentage acidity of 21.93 and 22.33 respectively.

c) Hydroxycitric acid (-) - HCA

Data on the mean value of hydroxycitric acid also indicated that the treatments did not vary significantly. The maximum value of hydroxycitric acid was for T_2 (19.77) and least for T_1 (17.23). T_3 and T_4 recorded a value of 18.97 and 19.27 per cent respectively and the control with 17.9 per cent.

d) Recovery percentage

No significant variation was noticed among the treatments in recovery percentage. The mean value ranged from 27 per cent to 28.93 per cent. The highest dried rind recovery was recorded for T_3 (28.93%) and minimum for T_4 (27%) followed by T_2 (27.23%). The dried rind recovery recorded for T_1 and control was 28 per cent

DISCUSSION

DISCUSSION

Kodampuli is an androdioecious tree, the cultivation of which is yet to be popularised. The commercial cultivation of the crop is handicapped due to various reasons. The seedling progenies segregate into male and female types. Difficulty in determining the sex in the early stage of growth is one of the limiting factors for commercial cultivation of kodampuli. Vegetative propagation by epicotyl grafting has been standardized in kodampuli but due to the lack of sufficient number of orthotrops, farmers invariably go for seeds or seedlings. Harvest season of the crop coincides with monsoon. Therefore drying and curing of rind is difficult which is another main problem faced by the farmers. In the present context, the thrust areas of research in this crop may be directed towards determining the sex in early stages of growth, standardization of a commercially viable method for conversion of unproductive males to productive females and efforts to shift flowering through bioregulator application. The results of the investigations on the possibility of regulating sex through controlled pollination and top working and to enhance early flowering and fruit set through bioregulator application in kodampuli (*Garcinia gummigutta)* are discussed in this chapter.

5.1 Regulation of sex through controlled pollination

Garcinia gummigutta was reported to be androdioecius in nature since male and bisexual flowers occur in separate trees (George *et al.*, 1992; Sherly, 1994). Fruit and seed set occurs through open pollination, cross pollination and apomixis in the related species of *Garcinia*, viz., G. *hombroniana* (Richards, 1990), *G. parvifolia*, *G. malacensis* and *G. scortechinii* (Sands *et al.*, 1988). The relationship between mode of reproduction and sex of the seedlings is discussed in this chapter.

5.1.1 Pollination and fruit development

Bisexual flowers, which were covered for eliminating the chances of cross pollination, failed to set any fruit. It shows that chances of self pollination do not exist

in kodampuli. This may be due to poor fertility of pollen of bisexual flowers. In this experiment, acetocarmine stain test showed 21.8 per cent fertility for pollen from bisexual flowers. Sherly (1994) reported 27.03 per cent fertility for pollen from bisexual flowers whereas Muthulakshmi (1998) recorded 22.94 per cent pollen fertility for normal bisexual flower. In this experiment also it was noticed that most of the anthers of bisexual flowers were without viable pollen grains, which might have resulted in the absence of fruit and seed set through self pollination.

The percentage fruit set was only 35 per cent under open pollinated conditions. This may be due poor pollen source even in the open conditions. Since pollination in kodampuli is entomophilous, fruit set can be enhanced by insect activity. The decreased fruit set by open pollination may be due to the absence of sufficient male trees in the near vicinity.

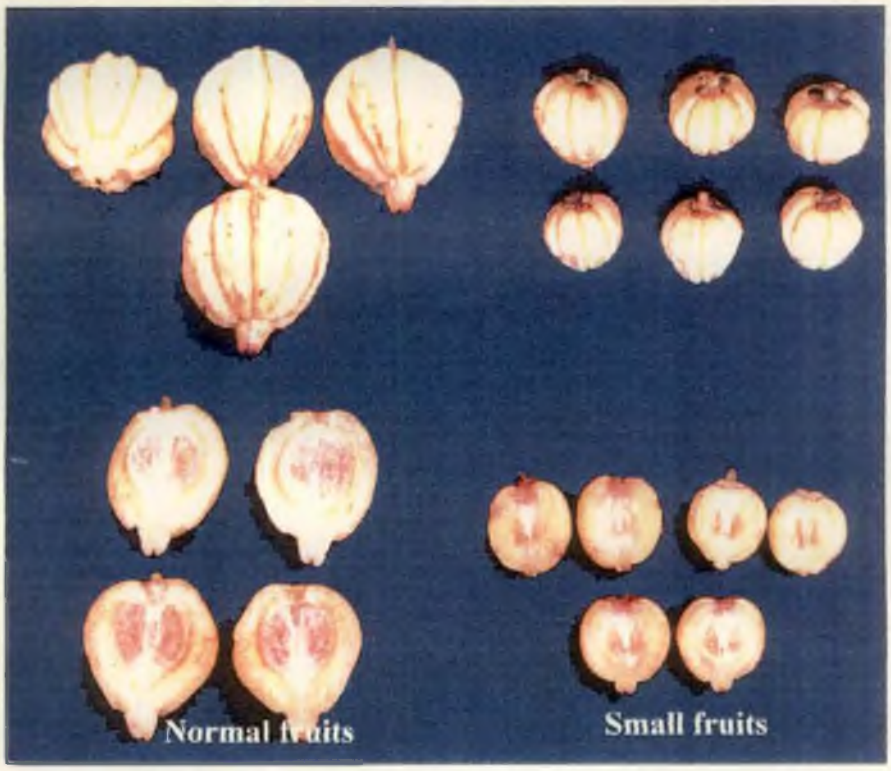
In the case of emasculated flower no fruit set was observed. This indicates that there may not be any chance for apomictic fruit development in kodampuli. At the same time, apomixis is a common phenomenon in *G. mangostana* and certain other related species of *Garcinia* viz.,*G. hombroniana* (Richards, 1990), *G. parvifolia*, *G. malacensis* and *G. scortechinii* (Sands *et al.*, 1988).

An increased fruit set (60 per cent) was obtained when the bisexual flowers were artificially pollinated with pollen from male flowers. This clearly indicates that hand pollination with pollen from male flowers could increase the fruit set compared to the natural conditions. The heterostylous nature of flower can be considered as an adaptation to cross pollination over self pollination. Karnik and Gunjate (1984) reported that in *Garcinia indica* fruit set was 78 per cent and 68 per cent after hand and open pollination respectively. Thus it may be concluded that a pollen source from a male tree is a must for good fruit set in *Garcinia gummigutta*.

Fruits obtained through hand pollination were generally larger in size compared to open pollinated fruits at the stage of harvest. Large sized fruits on an average contained five viable seeds and small sized fruits contained no or very few viable seeds (Plate 21 and Plate 22). Fruit size is correlated with number of full seeds/fruit. This is in accordance with the reports of Callan and Lombard (1978) that

Plate 21. Developed and undeveloped seeds of *Garcinia*

Plate 22. Size difference in fruits of *Garcinia*



cross pollination favours longer duration of seed growth although abortion may occur before seed maturity is achieved. Hand crossing or proximity to a polliniser increased fruit size. Fruit weight and/or diameter were highly correlated with seed content. Callan and Lombard (1978) also reported that in orchard where pollen transfer by bees was adequate, 94 to 97 per cent of the open pollinated fruits was seeded. Fruit size was not correlated with number of full seeds per fruit. But fruit size was positively correlated with total number of seeds over 7 mm long indicating that early seed development is important in fruit growth as well as fruit set. According to Gilbert and Breen (1986) the variation in fruit malformation among the strawberry cultivars may be related to their differences in anther quality and pollen production.

Time taken for fruit set in open pollination and artificial cross pollination was almost the same. Hand pollinated flower took 2.4 days and open pollinated flower took 2.6 days for fruit set. This indicates that mode of pollination makes no difference in the time taken for fruit set.

Fruit drop observed was 63 per cent in open pollinated fruits and 60 per cent in hand pollinated fruits during the early stages of development. Differences in fruit drop were not observed with respect to the mode of pollination. Chadha and Singh (1963) attributed the competition between young developing fruits, as the main cause of fruit drop, especially during the early stages in mango. In kodampuli fruit drop occurred during the early stages. Since fruit drop was confined to the early periods of development, the probable reason for the drop may be lack of fertilization or improper fertilization. The fruit drop may be the result of an abscission mechanism as reported by Addicot and Lynch (1955), Chadha and Singh (1963) and Randhawa (1971) in different crops. Baradwaj (1975) suggested the imbalance between various plant growth regulators as the possible reason for fruit drop. Further studies are to be carried out in this line to identify the exact reasons for fruit drop in *Garcinia.*

5.1.2 Seed dormancy

Seeds of *Garcinia* exhibit a long-term dormancy of about one year. To break the dormancy the seeds were subjected to different treatments. Of the different treatments tried, seeds sown after the removal of seed coat germinated in one month

and gave sixty per cent germination compared to seeds with seed coat, which took 13 months for germination. The effect of removal of seed coat on germination was studied in mango by Simao (1960). According to him germination was enhanced and germination percentage was increased by dehusking treatment. Subramanya and Reddy (1989) also obtained early, higher and faster germination of mango stones by the removal of seed coat before sowing.Ilyas (1978) showed that dehusked clove seeds started germination 16 days after sowing while normal seeds germinated only after 27 days. Thus it may be stated that probable reasons of dormancy can be hard seed coat or some inhibitory substances present in the seed coat.

Seven per cent polyembryony was observed in *Garcinia*. The polyembryonic seed produced twin seedlings. The twin seedlings were independent having separate taproots. The seedlings grew as healthy normal seedlings. Muthulakshmi (1998) reported 10 per cent polyembryony in *Garcinia*. Alex (1996) reported that 10 per cent of *Garcinia mangostana* seeds were polyembryonic in nature.

In the present investigations, pollination studies revealed that, on bagging the flowers after emasculation seed set was absent. This may be due to the absence of agamospermy in *Garcinia gummigutta* unlike in other related species like *G. mangostana* and *G. hombroniana*. In this experiment, multiple seedlings with separate tap root system were observed in *G. gummigutta*, where pollination and fertilization had occurred. Agamospermy may be an adaptive technique for reproduction in the absence of sufficient number of male trees. Stimulation from pollen may also lead to agamospermy. Therefore it is not clear whether the multiple seedlings have originated from a single sexual embryo or whether sexual embryos and pro embryos develop side by side. To answer these questions, a detailed elucidation of the reproductive biology of *Garcinia gummugutta* is to be carried out. Further embryological and cytological studies are required to determine the exact mode of origin of embryos.

5.1.3 Morphological and biochemical variations in seedlings

Noticeable changes were not observed in growth rate with respect to height, number, length and width of leaves in open pollinated and artificially pollinated seedlings. Muthulakshmi (1998) reported that no significant differences existed between mature male and female trees in terms of morphological characters like plant height, plant spread, canopy shape and branching habit. Mathew *et al.* (1996) reported significant difference in leaf area between male and female trees of kodampuli.

Janse (1898) stated that male nutmeg trees had smaller leaves and less horizontal branches. But this difference was not clear and prominent enough in young seedlings and hence it was not possible to determine the sex in the seedling stage.

Growth rate alone may not be an indicator to distinguish the sex of the plant at seedling stage. Seedlings having different growth rates were found in both open pollinated and hand pollinated seedlings. So definite conclusion could not be drawn with respect to sex of the plant at seedling stage. Some difference could be observed with respect to colour of the newly emerging leaves at the seedling stage. Different types of pigmentation viz.,green, brown and red were noticed in both sets of seedlings. In the present study also seedlings did not show any marked difference in growth pattern like height, number of leaves, length and width of the leaf in three pigmentation groups namely green, brown and red.

Muthulakshmi (1998) reported that in male trees, the emerging leaves were light green in colour while they showed a pinkish red colour in female trees. Shanmugasundaram *et al.* (1999) reported that identification of sex at the seedling stage of nutmeg on the basis of leaf form and venation, colour of young sprouts, vigour of seedlings, and chemical constituents are not reliable. Krishnamoorthy *et al.* (1992) conducted a detailed study of character in nutmeg like leaf shape, size of leaves, venation and colour of new sprouts and days for germination of seeds and concluded that none of these characters can be considered as a marker for sex in nutmeg seedlings. Therefore it may be concluded that looking at the pigmentation and

growth pattern alone we cannot arrive at a definite conclusion with respect to sex of the seedling in kodampuli.

No difference could be observed in biochemical characters in the seedlings derived from different modes of pollination. The mean values of the total phenol content in open pollinated and hand pollinated seedlings were 1097.20 mg 100 g^{-1} and 1207.00 mg 100 g^{-1}, respectively. But the picture was not the same when we grouped the seedlings on the basis of pigmentation. In the case of total phenol content, mean values for green seedlings were on the higher side (1240.85 mg 100 g^{-1}) as compared to red (1031.28 mg 100 g^{-1}) and brown (1029.42 mg 100 g^{-1}) flushed ones. Very similar results were noticed when leaves of mature trees of known sex were analysed. The mean value of the total phenol content of mature male tree recorded was 1685.75 mg 100 g^{-1} and that of female tree was 1138.00 mg 100 g^{-1}. Therefore some definite relationship exist between the phenol content, pigmentation and sex of the seedlings. Muthulakshmi (1998) reported that, regarding the colour of young flush, pink and shades of pink were most widely prevalent in female trees of *Garcinia* than in male trees and the total phenol content of male was higher (1397.80 mg 100 g^{-1}) compared to that of female (749.53 mg 100 g^{-1}). Thomas (1997) reported that leaves of male nutmeg plants had higher phenol content than female ones. This could be supported by the findings of Bhattacharya and Rao (1982), that any treatment that decreases the phenolic content would enhance femaleness.

In the case of protein pattern, a single fast moving protein (Rm 0.38) was observed in mature female trees and in male trees two bands with Rm 0.38 and 0.63 was visible. But these differences could not be observed in the seedling progeny of open pollinated and artificially cross pollinated sets. All of them showed two bands each with Rm 0.38 and 0.63. The protein bands were fast moving and least prominent. This is in confirmation with the findings of Thomas (1997) where only a single fast moving band could be observed in both male and female plants of nutmeg. The absence of sufficient number of protein bands in male and female trees of *Garcinia*, unlike in many other crops, may be due to the presence of high content of protein degrading metabolites or protein inhibiting enzymes like protease present in the leaf.

Though three esterase bands with Rm 0.17, 0.24 and 0.37 were observed in both male and female the intensity of the bands varied with the sex. In general darker bands were observed in female trees, the darkest band at 0.37 Rm. Since in all the open pollinated and hand pollinated seedlings only one prominent band was seen, sex of the seedling could not be identified depending on mode of pollination. When esterase banding was analysed in different pigmentation groups, red flushed showed a darker band with Rm 0.37 which could be compared to that of female. The bands were not visible in other groups. This may be used as an indicator to distinguish the sex at the seedling stage. Detailed study is to be carried out to conclude the same.

Regarding the peroxidase banding pattern, the seedlings showed a great variation in number of bands when compared to mature male and female trees. Sex of the seedling could not be clearly distinguished based on the number of bands. But at the same time, intensity of the bands varied based on flush colour, throwing some light on the sex of the seedling. It was observed that seedlings with red flush colour had more intense bands compared to green flush colour. The intensity was prominent in the band with Rm 0.39 and 0.44. This could be compared to that of mature male and female trees, as the bands of female trees with red flush were more intense than that of male tree with green flush.

In this study the two polyembryonic seedlings from the same seed origin were not identical as expected. They showed differences in height, leaf number and leaf measurements. Bhanja (2000) reported polyembryony in *Madhuca indica* and observed that among the twin seedlings one was smaller but not so weak as compared to the other. Twin seedlings of kodampuli showed varied pigmentation (one red and one green) as well as same pigmentation either both red or both green.

Singh (1960) reported that in mango, of all the seedlings produced from polymebryonic stone, one was developed from the fertilised egg cell and was usually weak, stunted and quite different from the rest. The others were vigorous in nature and were developed from nucellus cells. Similar type of observations was also noticed in *Garcinia*.

Considerable variation was observed in the total phenol content of both the seedlings. The total phenol content of the vigorous seedling was low compared to the less vigorous seedling. Seedlings, which showed the same flush colour, both red or both green, had more or less the same phenol content. Red flush coloured seedlings showed values towards lower side and green towards the higher side.

Protein pattern of the twin seedlings did not show any difference. Peroxidase banding pattern varied between the seedlings. The intensity of the band varied. Vigorous seedling showed more intense bands. Variation in the intensity of bands could also be related with the pigmentation of the seedlings as well. Seedlings with red pigmentation had more intense bands compared to green flushed ones.

Certain other interesting observations noticed during the investigations are given below. In the College Orchard where male trees were cut down for top working very severe flower drop was observed. Fruit drop was also high. Many of the fruits that set did not attain full size resulting in small sized fruits without viable seeds inside (Table 27).

Table 27. Fruit characters of bearing tree in the absence of male trees

Treatments	Percentage	Length (cm)	Breadth (cm)	Number of segments	Number of seeds
Big fruits	10	10.5	17.99	7.9	4.60
Small fruits	90	5.49	12.46	7.9	0.00
CD		(0.86)	(1.66)	NS	(1.03)

Singh (1990) reported that size of the seed might contribute to the size of the fruit through hormonal activity. In orchards where seedless fruit was more common, extent of seed development was related more closely to fruit size. According to Callan and Lombard (1978) as bagged limbs of emasculated and unpollinated flowers do not set fruits, observations made in 'Comice pear' reported that fruit set can be due to stimulation of self pollination or stimulative parthenocarpy. The influence of cross-pollination on fruit set and size was apparently related to the presence of hormones, produced first by pollen tube and later by the developing endosperm and embryo. Seed abortion at each stage in fruit development could have led to the cessation of

hormone production by the seed and subsequent fruit drop or reduced growth. Hand crossing or proximity to a polliniser increased fruit size.

Recently, grafted bisexual plants alone are being used extensively by the farmers for commercial cultivation of kodampuli. The present study has revealed that bisexual tree alone is not sufficient for good fruit set. It has also shown that absence of sufficient pollen from male trees might lead to a reduced fruit set and considerable yield reduction. A natural seedling population generally consists more of male trees compared to females. It also indicates that in kodampuli, cross pollination is the nature's rule. All these observations lead to the fact that male trees are necessary for higher fruit set, fruit size, maximum fruit retention and thereby enhanced yield. Therefore steps should be taken to interplant male grafts as pollinisers in a commercial orchard of kodampuli.

In the present study there are indications regarding the correlation of the sex of the seedlings with mature male and female trees depending on the pigmentation of the flush and certain biochemical characters. These indications suggest that if more of red flushed seedlings are selected, chances to obtain female trees might be high. Brown flushed seedlings later turn to either red or green flushed but green pigmented ones do not change their flush colour. For conclusive results flush colour of the seedlings should be monitored till they reach bearing stage.

5.2 Alteration of sex through top working

Kodampuli is usually propagated through seeds. The trees are androdioecious in nature and the seedling progenies segregate into male and female types usually in 1:1 ratio. There is no foolproof technique for identifying the sex of the plant at the seedling stage. The sex of the tree can be identified only 7-8 years after planting when they begin to flower. The removal of male trees at this stage involves great economic loss to the growers. A single male tree may be sufficient for every 10 to 20 female trees for pollination and rest of the unyielding male trees can be made productive by converting them into female trees by top working. Top working is tried with advantage in nutmeg for sex conversion. Budding on three year old plants were found to be the best, which recorded cent per cent initial success (Beena, 1994).

In the present study the worth of top working to transform male kodampuli trees to productive females is being probed.

5.2.1 Top working

Male trees of kodampuli were beheaded about one metre above the ground level in April, just before monsoon and the cut end was smeared with Bordeaux paste to prevent fungal infection and decay. Singh (1953) reported that inferior trees of aonla could be headed back to a height of 1.2 m from the ground during March for top working. Khan *et al.* (1986) reported that beheading the trees 0.75 to 1.00 m above ground level was found to be ideal in cashew from the point of view of sprouting and aftercare of the successful graft and trees. The decapitated trees were irrigated regularly till the onset of monsoon. New shoots developed from the main trunk within 30 to 45 days. The shoots, which had a minimum length of 20 cm and maximum of 40 cm, were selected for grafting. In this experiment, beheaded trees produced large amount of root suckers. In *Garcinia*, production of root suckers has not been reported so far. Stress was induced in the crop by beheading and also by the removal of extra sprouts arising from the beheaded tree. These may be the reasons that facilitated the root sucker production in *Garcinia*.

The observations revealed that girth of the beheaded tree had a positive correlation with new sprout production. Trees with more girth produced more number of sprouts. But the girth of the tree had no influence on grafting success. Irrespective of the girth of the tree, top working through grafting was successful. Trees with 30 cm girth and 150 cm girth gave the same success percentage.

Newly emerged flushes were not good for flush grafting at all. But when they attained two months maturity, a graft success as high as 80 per cent was obtained. Three months old sprouts also showed a high success of 70 per cent. But with age there was reduction in graft success. Four months old sprout showed only 30 per cent success whereas grafting on mature sprout of five months was a complete failure. Rema *et al.* (2000) reported that the newly emerged shoots in nutmeg were ready for grafting when they attained about 20 cm length. Girth of the stock should be almost same as that of the scion for a successful graft.

Six to eight months old mature light brown scion recorded the highest graft take of 75 per cent. Twelve to sixteen months old scion shoot gave a success percentage of 50. Orthotropic shoots were selected for collection of scion as they gave an erect growth habit. Tender scion sticks of one to two months and three to four months age were not found good for grafting. The thickness of the scion should match that of the grafting flush. Krishnamoorthy (2000) also reported that in nutmeg the thickness of scion shoot must be exactly the same as that of the erect shoot, produced in the beheaded male tress.

Grafting was found to be influenced by season. The best season for grafting was found to be July and October with 75 per cent success followed by August and June with 71 per cent and 63 per cent success respectively. This observation is in confirmation with Srivastava (1962) and Palaniswamy and Hameed (1976) who reported that July was the best season for guava and cashew. Manithottam (1994) reported that in Kerala, July-August months were the best for budding in nutmeg. According to Shanmugasundaram *et al.* (1999) in the orthotropic shoots of nutmeg, cleft grafting was done during the end of rainy season. Thus it may be concluded that top working during rainy season ensures more success compared to other months.

Frequent watering at an interval of one to two hours was given to the grafts immediately after grafting and it continued till graft take. A temporary shelter was provided on two sides and on the top of the grafted tree to prevent harmful effects of rain, wind and scorching sun. A pot full of water was hung above the beheaded tree with provision for dripping of water through the whips fixed in the holes to maintain humidity around the grafts. Presence of moisture and humidity around the grafts considerably reduced desiccation of scion and helped faster graft take.

Development of new leaves from the grafted scion was an indication of perfect union. It took 30 to 45 days for successful union. All the other shoots were removed from the main trunk periodically and Bordeaux paste was applied on the cut surface. The polythene ribbon tied around the grafted portion was removed on establishment of the grafts. Both the bark grafted as well as flush grafted or top

worked scions grew very fast. Growth pattern of bark grafts was slightly superior to that of flush grafts though the difference was not statistically significant.

5.2.2 Percentage success of grafting and budding

Initial success as well as establishment of grafts were almost equal with respect to bark grafting and flush grafting. But in both cases the values for initial success were considerably high whereas establishment percentage was very low. Husain *et al.* (1976) reported cleft grafting in *Bursera penicillata*. Mukherjee and Majumdar (1983) reported that large seedling trees of guava could be top worked either by cleft or bark grafting. Rao *et al.* (1957) described that side grafting could be successfully done in cashew with 70 per cent take by placing moist moss above and below the union.

Budding was a failure in kodampuli. Husain *et al.* (1976) reported that patch budding gave poor results in *Bursera penicillata*. Khan *et al.* (1986) from Ullal have reported that for top working, patch budding gave very low (18.3%) success and growth of sprouts was not rapid and so they considered this technique not promising for top working in cashew.

The percentage success in soft wood grafting of kodampuli seedlings was reported as 90 per cent by Nazeema (1992). Since it was carried out in mist chamber, the climatic conditions around the grafts could be controlled. Such high success percentage was not obtained while top working, as top working was carried out in field conditions. High percentage of initial success was obtained due to intense care but the establishment percentage was very low due to prevailing wind and hot weather. It was very difficult to control the weather parameters like wind, temperature, relative humidity etc. around the top worked tree. Therefore some methods are to be improvised for controlling the microclimate around the beheaded tree in the field conditions. Compared to flush grafting, bark grafting was more cumbersome and time consuming. So to adopt on a large-scale, flush grafting or cleft grafting may be recommended. The method adopted was very crude and refinement of the technique may offer a higher percentage establishment of the top worked trees.

The top worked male scion is yet to flower. Its flowering and fruiting characters should be studied to assess the performance of top worked trees.

5.3 Regulation of flowering using cultar

One of the major problems faced by *Garcinia* growers is the post harvest handling of the crop. As the harvest season coincides with monsoon showers curing and drying of rind is difficult. If flowering can be made early, harvest can be completed before rains, which would be a great boon to the growers. In this context an experiment was taken up to shift flowering to an early date through bioregulator application.

With regard to earliness in flowering, five ml cultar per m^2 canopy spread applied 90 and 75 days before flowering (ie. on 2^{nd} week of September and beginning of October) preponded flower emergence by twenty and ten days respectively. Similar findings were also reported by Burondkar and Gunjate (1992), that in mango paclobutrazol application induced early and profuse flowering for three consecutive years. Other treatments did not show any significant positive response. This gives an indication that quantity of cultar applied per m^2 canopy spread had a pronounced effect. None of the trees which received cultar @ three ml per m^2 canopy spread showed any response. Therefore it may be concluded that three ml cultar per m^2 canopy spread was not sufficient enough in *Garcinia* to induce flowering. Since we tried only two concentrations we are not in a position to conclude the optimum dose of cultar per tree. Higher doses may be tried in future, which might end up in better results.

Date of application of cultar was also an important factor, which influenced flowering. For a positive response cultar should be applied two and a half to three months before flowering after which the application yielded no response. In fact late application of cultar (60 days before flowering, ie. 2^{nd} week of October) resulted in negative effect evidenced from the absence of flowering in the treated trees. This finding is in confirmation with that of Ahmad *et al.* (2000). They reported that in litchi application of cultar 90 days before expected date of flowering was more effective compared to its application 60 days before expected date of flowering.

Coming to percentage fruit set, cultar @ five ml per m^2 canopy spread applied 90 days before flowering (2nd week of September) showed the maximum percentage fruit set (75%) as compared to the control (68.33%). Percentage fruit set was significantly low in other treatments compared to the control. Similar observations were recorded by Ahmad *et al.* (2000) in litchi. They recorded a maximum fruit set of 54.79 per cent with five ml cultar per metre plant spread when applied 90 days before flowering (2nd week of September). Results of the studies conducted by Burondkar and Gunjate (1992) in 'Alphonso' mango with paclobutrazol revealed that fruit set was significantly higher during first cropping year in soil application.

Regarding the time taken for maturity, the treatments did not vary significantly. All the treatments, including the control, completed maturity in 117-126 days. With respect to yield, cultar @ five ml per m^2 applied 90 days before flowering (2^{nd} week of September) recorded maximum yield of 22.6 kg which was very close to the control (22 kg).

With respect to the fruit size and fruit quality no significant response was observed with cultar application. This is in confirmation with the findings of Burondkar and Gunjate (1992) that in ripe mangoes none of the paclobutrazol treatments impaired or improved any of the fruit quality attributes. Paclobutrazol did not affect TSS and firmness in mango (Kulkarni, 1988).

Thus from the present investigations it may be inferred that paclobutrazol application in *Garcinia* as soil drench may not have a positive response with respect to yield and fruit quality but has a definite influence in shifting the flowering to an early date.

Since the upper limit of cultar applied in this experiment was five ml per tree, 90 days before flowering and the very same treatment happened to be the best to shift flowering to an early date by 20 days, we are not in a position to suggest optimum dose as a recommendation. As future line of work higher doses of cultar may be tried and date of application may be standardized for further shift of flowering to still early date, may be one to one and a half months.

SUMMARY

SUMMARY

The present study was undertaken in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1999-2001. Major objectives of the study were to explore the possibility of regulating sex through controlled pollination, to convert unproductive males to productive females through top working and to advance flowering and fruit set through bioregulator application. The experiment was carried out in the College Orchard and Vegetable Research Farm of College of Horticulture, Vellanikkara and the results are summarized.

Pollination studies revealed that chances of self pollination do not exist in kodampuli. The probable reason for this may be the poor pollen fertility of bisexual flowers. Acetocarmine stain test also revealed the low pollen fertility of 21.8 per cent in bisexual flowers. Bagged emasculated flowers also failed to set fruits. At the same time a percentage fruit set of 35 and 60 was observed under open pollinated and hand cross pollinated conditions. Hand pollination with pollen from male flowers could increase the fruit set compared to natural conditions. Insects are the pollinating agents in the natural condition. Artificially pollinated fruits were larger in size compared to open pollinated fruits. Hand crossing or proximity to a polliniser increased fruit size. Fruit drop was high in the absence of sufficient pollen source. Time taken for fruit set was almost same under different modes of pollination.

These lead to the conclusions that there is no chance for apomictic seed development in *Garcinia* and pollination is a must for obtaining good fruit set, enhanced fruit size and maximum fruit retention which emphasises the need for planting male trees in a commercial orchard.

Seeds of *Garcinia* exhibit a long term dormancy of about one year. Seeds sown after the removal of seed coat germinated in a month and gave sixty per cent germination compared to control, which took 13 months for germination.

The seedlings raised from seeds obtained through different modes of pollination were evaluated for morphological and physiochemical variations.

Noticeable changes were not observed in growth rate and biochemical characters based on mode of pollination. Three types of pigmentations, viz. red, brown and green were noticed in the seedlings. Some variations were noticed in biochemical characters of the seedlings based on pigmentation. The mean values of total phenol content for green seedlings were comparatively on the higher side (1240.85 mg/100 g) as compared to red (1031.28 mg/100 g), which was comparable to the high phenol content of mature male trees. Coming to the banding pattern, red flushed ones showed more intense bands for esterase and peroxidase when compared to seedlings with green and brown pigmentation.

Polyembryony was also observed in *Garcinia* and the percentage polyembryony was seven. The polyembryonic seed produced twin seedlings. Twin seedlings with varied pigmentation (red and green) and same pigmentation (either both red or both green) were observed. The twin seedlings varied in growth, total phenol content and banding pattern. Certain seedlings with same pigmentation did not vary much in the total phenol content. Since purely vegetative apomictic seed development was not observed during the studies, it may be inferred that pollination is a prerequisite for triggering the polyembryonic development in *Garcinia*.

From this experiment, identification of the sex at the seedling stage of *Garcinia* on the basis of seedling growth and leaf characters are not reliable but colour of new sprout and certain chemical constituents can be considered as markers of sex in *Garcinia* seedlings. For conclusive results the seedlings utilised in the study should reach flowering stage, which takes 6 to 7 years.

Attempts were made to manipulate the sex of the tree through the technique of top working. Thus unproductive males were converted to productive females by top working. For top working, beheading was done during April. A positive correlation was observed between girth and number of sprouts. Bark grafting was done one month after beheading and flush/cleft grafting done two months after beheading. Root sucker production was noticed in the beheaded trees. Stress induced in the crop during beheading and removal of extra sprouts from the beheaded tree might have resulted in root sucker production in *Garcinia*.

Irrespective of the girth of the tree, bark grafting was successful in male trees with girth ranging from 30cm to 150cm. Sprouts from the beheaded trees which were two months old were considered best for flush grafting with 80 per cent success. Three months and four months old sprouts showed 70 per cent and 30 per cent success respectively. Diameter of the sprout should match with that of the scion for a successful graft union. Matured orthotropic shoots light brown in colour and 6 to 8 months old were the best as scion and gave 75 per cent success. Plagiotropic shoots should not be selected as they showed a spreading or drooping growth habit.

Grafting done during July and October gave highest percentage of success (75%). Presence of moisture and humidity around the grafted shoot played an important role in grafting success. Retaining high amount of humidity around the grafted shoot could considerably reduce time required for the scions to sprout.

Both bark grafting as well as flush grafting was equally successful in top working the male trees. Budding was a failure in kodampuli. Significant difference was not observed in the growth rate of scion of flush and bark grafts. Percentage success of bark and cleft grafts also did not differ significantly. Initial success of the grafts were as high as 71 to 75 per cent compared to the establishment percentage of 33 to 38 per cent. Success of the technique can be analysed from the growth of the top worked tree. The top worked shoots attained on an average a height of 117 cm and spread of 75 cm with 27 branches and profuse foliage in one year. The shoots are yet to start flowering. Though both methods were successful, bark grafting was more cumbersome and time consuming compared to cleft grafting. Therefore flush/cleft grafting may be recommended for large scale adoption. To increase the percentage of establishment of the grafts, methods should be standardised to provide a congenial microclimate around the top worked tree.

Another experiment taken up was to advance flowering and fruit set through bioregulator application. Cultar @ 3ml and 5ml per m^2 canopy diameter was applied as soil drench 60, 75 and 90 days before the expected date of flowering. Among the various treatments tried, 5ml cultar per m^2 canopy diameter drenched 90 days before flowering (2^{nd} week of September) showed 20 days early flowering followed by 5ml cultar applied 75 days before flowering (beginning of October)

showing 10 days early flowering. Quantity of cultar applied per m^2 canopy diameter had a pronounced effect on flowering. Cultar @ 3ml per m^2 canopy spread was not sufficient enough in *Garcinia* to induce flowering.

Date of application of cultar was also important. Application of cultar 90 days before expected date of flowering was more effective compared to its application 60 days before flowering. Cultar applied @ 5ml per m^2 canopy spread, 90 days before expected date of flowering showed maximum percentage of fruit set (75%) compared to control (68.33%).

No significant variation was noticed among the treatments in time taken for maturity. Fruit size and fruit quality were also not affected by cultar application.

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REGULATION OF SEX IN *Garcinia gummigutta* (L.) Robs. THROUGH CONTROLLED POLLINATION AND TOP WORKING

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ABSTRACT OF THE THESIS

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ABSTRACT

The present investigation entitled "Regulation of sex in *Garcinia gummigutta* (L.) Robs. through controlled pollination and top working" was undertaken in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1999-2001. The trees maintained in the College Orchard and Vegetable Research Farm were utilized for the study. The objectives of the study included regulation of sex in *G. gummigutta* through controlled pollination, conversion of sex through top working and regulation of flowering through cultar application.

Cross pollination was found to be the main mode of reproduction in *Garcinia*. Fruit set did not occur by self pollination. Fertility of pollen in bisexual flowers was very low (21.85%). A percentage fruit set of 35 and 60 was observed under open pollinated and artificially cross pollinated conditions, respectively. Hand pollination with pollen from male flowers could increase the fruit set compared to open pollination. Artificially cross pollinated fruits were comparatively larger in size than open pollinated fruits. Hand crossing or proximity to a polliniser increased fruit size. Heavy fruit drop was observed in the absence of sufficient pollen source. Apomictic fruit development also did not occur in kodampuli. These facts helped to arrive at a conclusion that sufficient amount of pollen source should be present around the female trees for good fruit set, increased fruit size and maximum fruit retention. Therefore care should be taken to interplant male grafts, as pollinisers in an orchard of grafts of kodampuli.

The dormancy of the seeds of *Garcinia* could be overcome by sowing the seeds after the removal of seed coat. Seeds without seed coat germinated in one month whereas those with seed coat intact took one year for germination.

Seedlings raised by different modes of pollination did not show morphological and physiochemical variations. Based on pigmentation viz., red, brown and green, some differences were noticed in biochemical characters. Seedlings with red flush colour showed a lower total phenol content when compared to green flushed

ones. Esterase and peroxidase banding pattern of the red flushed seedling was more intense than green flushed ones. These characters of red flushed and green flushed seedlings were comparable to that of mature female and male trees, respectively. Morphological characters like leaf length, leaf width, number of leaves and height were not found to be contributing towards sex expression. The seedlings have to be established in the field and observed until flowering to verify any positive correlation of biochemical characters and pigmentation with sex expression.

Seven per cent polyembryony was observed in *Garcinia*. The polyembryonic seeds in general produced twin seedlings, which had same pigmentation or varied pigmentation. Variations were observed between the two seedlings in morphological and biochemical characters. The multiple seedlings might have originated from the cleavage of a single sexual embryo or from the sexual embryos and pro embryos, which might have developed side by side after pollination and fertilization.

Techniques tried for top working in *Garcinia* included bark grafting, flush/cleft grafting and patch budding. Among these, bark grafting and flush/cleft grafting were successful. Beheading was done during April and grafting was carried out from June to October.

Two months old sprouts from the beheaded trees were considered best for flush grafting. Matured orthotropic shoots light brown in colour and 6 to 8 months old were the best as scion, which showed highest percentage of success.

Grafting done during July and October gave highest percentage of success. Presence of moisture around the grafted shoot played an important role in graft success. Percentage of graft success was 71 in bark grafts and 75 in flush grafts. The growth rate of bark grafted and flush grafted scions were almost similar. The top worked shoots attained on an average, a height of 117 cm and spread of 75 cm with 27 branches and profuse foliage in one year. Through this study the possibility of transforming male kodampuli trees to productive females is revealed.

Cultar was drenched @ 3ml and 5ml per m^2 canopy diameter or spread, 60, 75 and 90 days before the expected date of flowering. The treatment with 5ml cultar per m^2 canopy diameter applied 90 days before flowering showed 20 days early blooming followed by 5ml cultar per m^2 applied 75 days before flowering which advanced flowering by 10 days. Three ml cultar per m^2 was not sufficient enough to induce flowering in *Garcinia*.

Maximum fruit set of 75 per cent was observed with cultar @ 5 ml per m^2 canopy spread, applied 90 days before expected date of flowering. Cultar treatment neither impaired nor improved any of the fruit quality attributes.