SOFTWOOD GRAFTING AND IN VITRO PROPAGATION STUDIES IN JACKFRUIT (Artocarpus heterophyllus Lam.)

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I. INTRODUCTION

Jack fruit (Artocarpus heterophyllus Lam.) belonging to the family Moraceae, is a fairly large sized tree bearing largest fruit among edible known fruits. It is native to western ghats of India and also popular fruit in several tropical and sub-tropical countries. It is regarded as “poor man’s fruit” in eastern and southern parts of India.

Ripen fruits contribute significantly to the nutrition as a source of carbohydrates, minerals and β-carotene, a precursor of vitamin A. The juicy pulp is eaten fresh or preserved in syrup and has wide potential for preparing jam, jelly etc. In eastern India, tender fruit is popular as vegetable. Seeds of jackfruit can be used in many culinary preparations as boiled or roasted item. The pulp can also be used in preparation of pickles, chips, de-hydrated leather and thin papad. The rind is rich in protein and major protein has been extracted as “jacalin”. Jack timber is valuable for making furnitures as it is rarely attacked by white ants. The leaves and skin of fruits are a good source of cattle feed. The latex from the bark contains resins, and it is used to plug holes in earthen vats and buckets. The skin and core of the fruits, could be used commercially for pectin extraction.

Jack tree grows well not only in humid and warm climates of hill slopes but also in arid plains of south India, making it as one of the most suitable fruit crops for dryland horticulture. It is found largely in southern states viz., Kerala, Tamil Nadu, Karnataka and Andhra Pradesh, besides other states like Assam, Bihar, Orissa, Maharashtra and West Bengal. In Karnataka, area under jackfruit cultivation is about 7855 ha with a production of 2,36,524 tonnes annually (Anon., 2001).

Seed propagation is common method of propagation in jackfruit. Being highly heterozygous and cross pollinated, it has resulted in immense variation among populations for yield, size, shape, flesh colour, quality of fruit and maturity period. The significance of vegetative propagation in the maintenance of genetic uniformity and preservation of identity of an elite clone or cultivar is well recognized in horticultural crops. Therefore there is immense need to find out a suitable method of vegetative propagation, for quick multiplication of selected jack plants.

In grafting, attached method of grafting like approach grafting has shown greater success (Nazeem et al., 1984) but this method is cumbersome, laborious and costly. Detached scion methods of grafting like veneer, wedge, side and splice grafting have been tried in jack in different growing tracts as followed in mango and cashew with varying degree of success. The present day nursery practices will involve much cost and risk with respect to raising seedling root stocks and subsequent maintenance of seedling root stock till they attain graftable size. Healthy growth of rootstock and correct grafting technique probably will help to attain higher rate of success (Swamy, 1993).

In jackfruit conventional vegetative propagation methods like budding, grafting air layering etc., are generally cumbersome, time consuming and highly season bound with low multiplication rate. Further budded or grafted trees are not of much value for timber as the trunks do not retain their natural shape. Hence the alternative method is micropropagation having tremendous potential if exploited for mass multiplication of elite material.

While tremendous studies have been made in the application of in vitro clonal propagation technology in the propagation of herbaceous and temperate tree crops, whereas very limited success has been achieved in tropical and sub tropical fruit species. Most of researchers have been successful with seedling explants in case of micropropagation of jack. However, some workers carried out micropropagation with mature explants also in recent years, but the protocols developed for one clone do not satisfy for another clone.

Hence, the present investigation was undertaken to standardize the grafting technique and micropropagation protocol in jack fruit with the following objectives:

1. To standardize the method of grafting
2. To study the effect of age of rootstock and season of grafting on success of grafts.
3. To standardize the source of explant for micropropagation
4. To study the effect of source of growth regulators on shoot proliferation and rooting of explants.
II. REVIEW OF LITERATURE

In many parts of tropics numerous valuable fruit species are propagated primarily by seeds and are often of poor quality. Extensive use of vegetative propagation methods would be the greatest single step which could be taken for the improvement of tropical fruit cultivation. Several attempts were made to propagate fruit trees by grafting, budding, air layering, cutting and stooling. Inspite of development of many improved propagation techniques success has not been achieved yet in few fruit crops. This is also true for jackfruit in which several variations have been found and some of the types are worth multiplying.

Amin (1974) was the first Indian, who coined the term soft wood grafting in vegetative propagation of mango. Further, Gaur (1984) studied the comparative performance and economics of different grafting methods and opined that soft wood grafting is an economically viable technique in mango. Jackfruit is also one of the important tropical fruit crops and research work carried out in respect of standardization of softwood grafting technique is not adequate. The available information on various aspects of softwood grafting in jack is very meagre. Hence, the literature available on other fruit crops grown under similar situations have been reviewed in relation to present investigation and for better understanding of the subject. Following review is primarily on the grafting methods of propagation of the tropical fruit trees like jack, mango, cashew, sapota and other fruit crops.

2.1 ROLE OF AGE OF ROOTSTOCK ON GRAFT SUCCESS

2.1.1 Jack fruit

A comparative trial with different aged stocks was carried out at Taliparamba. The results showed that six months old seedlings when approach grafted with six months old jack scions a maximum of 76 per cent success was observed, but on two years old stocks the success rate dropped to 26 per cent. The use of younger stocks resulted in faster union (Anon., 1950).

Desai and Desai (1989) stated that softwood grafting is a simple and rapid method. They recorded success rate from 33.33 to 80.00 per cent by using six months old cuttings of 2.5 to 10.0 cm long as scions. Jose and Vasalakumari (1991) reported that epicotyl grafting on five days old stocks with pre-cured scions of three to four months age was found superior compared to soft wood grafting in jack. The maximum success rate recorded was 83.3 per cent.

Swamy and Melanta (1994) at University of Agricultural Sciences, Bangalore worked on softwood grafting in jack and obtained a success of 36.67 per cent 60 days after grafting on six months root stocks. Similar results were obtained with 4 months old root stocks in Dharwad conditions by Gaonkar (1998).

2.1.2 Mango

Jagirdar and Bhatti (1968) studied the effect of age of rootstock on success of veneer grafting in mango and reported that the age of the root stocks (three to nine months) did not affect the rate of success, but per cent graft take increased by the use of mature scion wood compared to immature scion wood. Amin (1974) tried wedge grafting in mango and reported 98.5 per cent success on one year old seedlings raised in nursery.

Singh and Srivastava (1979) obtained 80 per cent success when twelve months old root stocks were veneer grafted with six months old scions.

Reddy and Melanta (1988) working at University of Agricultural Sciences, Bangalore tried 3, 4, 5 and 6 months old rootstock for softwood grafting in mango. They recorded 68 per cent success with three month old rootstock raised in container.

Singh and Suryanarayan (1996) worked on softwood grafting in mango. The highest rate of success (87%) was obtained after 60 days of grafting on one month old rootstock.

2.1.3 Cashew

Phadnis (1971) suggested the use of rootstocks of less than five months age and not more than 50 cm height for veneer grafting. Nagabhushanam and Rao (1978) observed 35 to 96 per cent graft take with five to six months old rootstocks compared to 20 to 49 per cent
graft take with 15 to 20 months old rootstocks. Muniswami (1979) reported 90 per cent success on 4-5 months old seedlings. Harnekar (1980) reported 44 per cent success with the use of 4 and 8 weeks old seedlings in wedge grafting of cashewnut under Dapoli conditions.

Konhar and Das (1985) used 50 to 60 days old cashew seedlings for softwood grafting and recorded a success per cent of 43 to 100 per cent from June to May, except March. The highest success rate of 100 and 90 per cent was noticed during January and February respectively under Orissa conditions.

Jayarama Gowda and Melanta (1989) tried rootstocks of different age (2, 3, 4, 5, 6, 7 and 8 months) for wedge grafting in cashewnut and recorded highest success (60%) on 4 months old seedlings for softwood grafting under Bangalore conditions.

Kumar et al. (1989) suggested the use of 45 to 60 days old seedlings for softwood grafting in cashewnut under Ullal conditions.

Sarada et al. (1991) studied softwood grafting in cashew and obtained 65.43 per cent success rate on one month old rootstock in August month under Bapatla conditions.

Sawke (1992) at University of Agricultural Sciences, Dharwad obtained the best results (75%) with five months old seedlings as rootstock and 73 per cent success with four months old rootstocks in cashew under Dharwad conditions.

Shingre (2003) reported softwood grafting in cashew was more successful with rootstocks of age 45 days old seedlings under the conditions of Konkan.

2.1.4 Other fruit crops

Haldankar et al. (1987) reported that once the rootstocks attained a graftable size in Kokam (Garcinia indica L.), which takes about 22 weeks, further increase in age did not influence the percentage of success significantly. The percentage of success in softwood grafting ranged between 70 and 78 per cent and the highest success (78%) was observed with 26 week old rootstock.

Kulwal et al. (1988) recommended the use of about 12 to 15 months old root stock of rayan for softwood grafting in sapota. Rayan was found to be very slow growing in nature and attained graftable stage only after ten months. They reported 52 to 90 per cent success during July to October and the highest success (90%) was observed on 12-15 months old rootstocks in the month of August under Akola, Maharashtra conditions.

Waghmare (1990) studied the effect of age of rootstocks (6, 9, 12 and 15 months) of rayan on softwood grafting in sapota and recorded maximum success (59%) on 15 months old rootstock closely followed by 12 months old rootstock (56%).

Sadhu (1992) reported the highest rate of success (78.3%) in veneer grafting of sapota by using 2 years old rootstocks, under southern Bengal conditions.

Sathisha et al. (1997) in their studies at University of Agricultural Sciences, Bangalore on effect of age of rootstock on success of softwood grafting in tamarind recorded maximum success rate of 76 per cent on six months old rootstock followed by nine months old rootstock (75%). The minimum rate of success (13%) was recorded on 18 months old at 30 days after grafting.

Satish Kumar (2001) obtained highest graft success (51.96%) was observed with 8 month old rootstocks followed by 7 months (45%) old rootstocks in tamarind at University of Agricultural Sciences, Dharwad.

2.2 EFFECT OF SEASON ON GRAFTING

2.2.1 Jack fruit

Inarching in Artocarpus hirsuta and in jack seedlings during the month of July recorded 67 per cent success but on Rudrakshi jack seedlings, the success was higher to the tune of 80 per cent (Anon., 1950). Gunjate et al. (1980) reported 50 to 90 per cent success in epicotyl grafting. According to Vijay Kumar et al. (1991) about 45 to 50 per cent of successful grafts could be obtained within 40 to 50 days after grafting during April-May.
According to Desai (1987) the softwood grafting in jack recorded the highest success (69.33%) in April followed by May (56.0%) under Dapoli conditions. Swamy (1993) observed that the February and March were the most congenial months for softwood grafting in jack under Bangalore conditions.

Jose and Vasalakumari (1991) noted higher percentage of sprouting and survival of epicotyl grafts prepared during the month of June compared to May, July and August months.

2.2.2 Mango

Amin (1974) tried softwood grafting and obtained 91.5 per cent success in nursery and 76 per cent in field at Anand by wedge grafting, during August. Nagawade et al. (1979) reported the success of 34.56 and 29.76 per cent in the month of July and August, respectively under extreme dry conditions of Rahuri.

Further, Amin (1979) studied the softwood grafting in mango and found that the period from March to September was very congenial in the drier parts of Gujarat.

Singh and Srivastava (1980) studied softwood grafting in mango and highest success (84%) during in July.

Hulamani and Nachegowda (1981) working at University of Agricultural Sciences, Bangalore reported better success (64.33%) in Greenwood wedge grafting, during the month of February and March irrespective of varieties in mango.

Gunjate et al. (1982) working at KKV, Dapoli achieved 55.0 to 64.9 per cent success during the period from June to October in mango. Shankara (1987) working at University of Agricultural Sciences, Dharwad tried green wood wedge grafting in mango, during February and March at fortnightly interval and observed the highest graft take (44.66%) during the second fortnight of March followed by first fortnight of March. He also tried epicotyl grafting during the months of June-July and obtained maximum success (42.26%) in first fortnight of July followed by second fortnight of June (34.33%).

Gupta et al. (1988) reported that stone grafting in mango was most successful from mid August to mid September with 50-55 per cent success on 10 days old rootstocks under subtropical conditions of Jammu.

Mango cultivars Alphanso, Pairi, Neelum, Totapuri and Mallika were wedge grafted on to one year old rootstock seedlings of local type at three fortnightly intervals during February-March. Highest percentage of sprouting (88.66%) and percentage of graft take (44.66%) was obtained with grafting done during the second fortnight of March. It was interesting to note that very less number of days for graft union formation were taken when the grafting was done during the first fortnight of March (32.6 days) (Shankara et al., 1991).

Kumar and Mitra (1994) in their studies on standardization of time and propagation techniques in mango cv. Himsagar, found better success rate for all techniques of grafting between June and August and then declined drastically.

In mango highest average graft success (50.33%) was achieved with grafts made in the first fortnight of August, followed by those made in second fortnight of July. Graft success which is lower in 1989-90 than in other two seasons, possibly due lower humidity and higher temperature (Basavaraja, 1996) at Dharwad.

Mango cv. Amrapali was propagated by softwood grafting from July 1991 to June 1992 at 15 days interval. Highest rate of success (86%) was obtained with July grafting, closely followed by August, September and April. Longest sprouts were also recorded with July grafting (27.34 cm) (Sanjay et al., 1996).

Krishnakumar (2000) recorded highest percentage of graft success during the month of August in case of softwood grafting in mango at ARS, Arasikere.

2.2.3 Cashew

Amin (1978a) tried softwood grafting during 1975-76 with 71.4 per cent success during August under Anand conditions. Nagabhushanam et al. (1979) observed high graft take (53%) with cleft grafting in September followed by August (50%). While the success in wedge grafting for the same months was 23 and 25 per cent respectively.
Nagabhushanam (1983) concluded from two years of experimentation, that epicotyl grafting in cashew can be successfully done on 15 days old seedlings during June to November by adopting cleft grafting. The grafting success was highest (60-68%) between June and August and declined in November (45-47%) under South Canara conditions in Karnataka.

Epicotyl grafting trials conducted during 1980-82 at Vengurla (Maharashtra) revealed that this method can be commercially exploited under Konkan (coastal) conditions. February to May was the congenial period with 62.4 to 67.7 per cent success on 10 days old seedlings (Sawke, 1983). Similar results were obtained by Aravindakshan et al. (1984) under Kerala conditions. Konhar and Das (1985) studied softwood grafting in cashew throughout the year under Bhubaneswar conditions (Orissa) and found that this technique was very much useful with a success ranging between 43 and 100 per cent was recorded during the months of January and February, respectively. Krishnamurthy et al. (1985) reported a success of 39-75 per cent in softwood grafting during the period from February to May in cashew at Ullal. Maximum success obtained was 57 per cent in the month of April.

Sawke et al. (1986) reported that softwood grafting was most successful in cashew, throughout the year except December and January, which coincides with the cold period. The mean graft success of consecutive three years ranged between 71.33 and 83.66 per cent during February to November under warm climate of Konkan region of Maharashtra. The lowest success of 22.33 per cent was observed in month of December.

Kumar and Khan (1988), while working on in situ softwood grafting in cashew recorded mean success of 42.7 to 60.0 per cent on 12 months old rootstock during March-May, when both day and night temperatures were higher.

Kumar et al. (1989) in their studies on softwood grafting of cashew in ARS, Ullal, reported success rate ranging from 39.5 to 86.0 per cent during the month of May (86%) followed by April (77.5%). They also reported that this method can be adopted from March to May under humid and warm conditions of coastal Karnataka for higher percentage of success.

Swami et al. (1990) in their correlation studies on success of softwood grafting with weather parameters, reported that monsoon season (June-October) was the ideal period for commercial production of grafts. The success rate ranged from 54 to 85 per cent. During other months the success rate was low (10 to 12%) due to unfavourable weather conditions and non-availability of suitable scion sticks.

Sarada et al. (1991) in their studies on softwood grafting in cashew reported significant differences among different months of grafting and with two types of scion shoots. A success rate of 65.43 per cent was recorded in the month of August, followed by 60.63 and 55.63 per cent in September and January. Height of the grafts and number of leaves produced was also highest in grafts prepared during the months of August, September and July, while it was lowest during December.

At University of Agricultural Sciences, Bangalore, vigorously growing scion sticks of cashew were wedge grafted to the selected rootstocks during September-October, December-January, March-April and June-July. In the June-July grafting, 23.3 per cent of scions sprouted, while only 3.3 per cent sprouted from the September-October grafting and complete failure was observed in the December-January and March-April graftings (Gowda and Melanta, 1991). Lenka et al. (1993) in their studies on softwood grafting in cashew under Orissa conditions for three years reported significant differences of graft success in different months of grafting. Average monthly (for all 3 years) success ranged from 21.7 per cent in December to 72.3 per cent in August. Graft success was positively correlated with minimum temperature and afternoon relative humidity.

Kadam et al. (1995) obtained the higher rate of success in cashew during July, August and September to an extent of 71.66 and 81.6 per cent respectively.

2.2.4 Other fruit crops

Amin (1978b) tried softwood grafting in fruits like aonla (Emblica officinalis L.), guava (Psidium guajava L.), phalsa (Grewia asiatica L.) and sapota (Manilkara achras (Mill.)
Forseberg) and obtained 73.3, 70.7, 100.0 and 91.6 per cent, respectively in the month of August at Anand.

Khalil et al. (1983) reported may to September period as the best time for veneer grafting of Guava, when the success rate was as high as 82-92 per cent. Haldankar (1985) reported October as the best month with a success of 80 per cent in Kokam under Dapoli conditions. Mishra (1985) studied softwood grafting in pecunno (Carya illinoensis (Wang) R. Koch), a new introduction in India and recorded a very high success of 90 per cent in August followed by 80.7 per cent in July.

Bankar (1989) in his studies on vegetative propagation in Annonas reported 84 and 86 per cent success rate with veneer grafting in cultivars like Balanagar and Pond-apple respectively. Bhuvn et al. (1990) in their studies on influence of environment on success of sapota propagation reported highest mean survival percentage (90%) for grafts made in February followed by January (86.55%).

Kulkarni (1990) tried softwood grafting in custard apple and noted highest success (63.55%) in March on six months old rootstock. The percentage of success was higher in sapota during the first half of the year till July, there after it was poor. Maximum success (82%) was recorded in July followed by May (67.3%). The least success (34.0%) was recorded in September (Madalageri et al., 1990). Again, Sulkeri et al. (1997) worked on softwood grafting technique in sapota and obtained maximum success of 61.83 per cent during May at Dharwad.

Softwood grafting of sapodilla cv. Kalipatti was performed on 15th day every month from January to December. The highest graft take (63.33%) was obtained for grafting done in May, which also resulted in the more scion shoot and leaf growth. In contrast, very low graft take (0-6.67%) was reported in September-February grafting (Pampanna et al., 1994).

Shinde et al. (1996) in their studies on softwood grafting in tamarind reported higher graft success (70.0%) in April (before flowering) followed by March (58.40%), when elite type tamarind cv. Sel-263 was grafted onto one year old rootstock seedlings of a local type of tamarind. They also reported the failure of grafts made during January-February or June-December under Aurangabad conditions.

Pampanna and Sulikeri (2000) reported that the initial and final graft take was highest with May grafting (both 60%) followed by April (both 43.3%) in their studies on use of invigorated roots for softwood grafting of sapota. Similarly, highest graft success (51.96%) with 8 months old rootstocks in tamarind. Grafting success was highest during February followed by January under Dharwad conditions (Satish Kumar, 2001).

2.3 MICROPROPAGATION

The in vitro propagation technique dates back to 1902 when Haberlandt predicted the totipotency of plant cells, i.e., the ability of a plant cell to develop into a complete plant. Efforts to demonstrate totipotency led to the development of tissue culture. Major breakthrough in plant tissue culture was seen after Skoog and Miller (1957) putforth the concept of hormonal control of organ formation and showed that differentiation of roots and shoots was a function of relative concentration of auxin and cytokinin in the medium. The early work of Morel (1960) on in vitro propagation of orchid provided the stimulus for propagating the ornamental species through tissue culture.

Development of nutrient media for tissue culture of tobacco by Murashige and Skoog (1962) was a great achievement, which is now being used for most of the species. Murashige (1974) was instrumental in giving the techniques of in vitro culture, the status of viable practical means for rapid and mass propagation of horticultural crops. He also described the concept of developmental stages of micropropagation.

Main commercial application of tissue culture has been in the production of clonal plants at very rapid rates compared to that of conventional methods. Numerous herbaceous plants have been propagated successfully in vitro. However, the number of woody trees, shrubs and coniferous trees, which commercially micropropagated is limited, because of problems like bacterial contamination, vitrification, browning of explants due to phenolic exudation, acclimatization of plantlets, the attempts of in vitro multiplication in woody perennial species is limited. However, in vitro clonal propagation studies have been reported
in economically important fruit crops like apple, plum, citrus, grape etc., but not much work has been done on in vitro propagation of tropical fruits in general, jack fruit in particular. Hence the present review deals with the other woody perennial trees to cover various aspects influencing in vitro shoot proliferation and in vitro rooting of plantlets.

2.3.1 Establishment of culture

2.3.1.1 Explant

Plants grown under controlled conditions in a greenhouse and field grown plants can be used for in vitro isolation (Pierik, 1987). Factors like type of explant, its size, position and physiological age have great influence on their morphological activity and degree of differentiation. These points have to be kept in mind while selecting a suitable explant for micropropagation. Mascarenhas et al. (1987) reported that hypocotyl explants of tamarind developed 4-5 shoots per explant as compared to nodal segments and shoot tip explant which produced only one and two shoots respectively.

In pomegranate, different morphogenetic response of explants have been reported. These include callus formation from anther walls (Moriguchi et al., 1987). Petals (Nataraja and Neelambika, 1996), plantlets from leaf segments (Omura et al., 1987).

Goyal and Arya (1985) reported maximum number of shoots in case of Ber when shoot tips was used as explant, closely followed by cotyledonary node, nodal sections and hypocotyls of ber.

Jaidka and Mehra (1986) found in pomegranate Cv. Kandhari that callusing was affected by variation in size of the explants i.e. larger explants (5-8 mm) callused more frequently than smaller ones (2 to 3 mm). In view of this, Muralikrishna (1988) found the highest percentage of establishment with explants of 10 mm size followed by 7.5 mm. He opined that, as the size of explant increased, the establishment percentage of explant also increased only upto 10 mm but decreased with increase in size.

Shoot organogenesis has been successfully induced in sugar apple endosperm, anther and leaf explants as well and in Cherimoya hypocotyls, petiole internode and nodal cuttings. Hypocotyl and nodal cuttings of Alemoya sps. have proved suitable for in vitro culture (Rasai et al., 1995 and Nair et al., 1984).

Maximum number of shoot buds produced per explant by using vegetative buds from nodal explants in case of in vitro propagation of Annona hybrid (Nair et al., 1984) and also in grapes (Singh et al., 2002).

Studies on in vitro propagation of papaya cv. CO-5 revealed that highest callusability per cent within 25 days on MS medium supplemented with 1.0 mg/l IAA and 4.5 mg/l BA in case of shoot tip culture than explants like leaf bits and nodal segments (Suthamathi et al., 2002).

2.3.1.2 Surface sterilization

Contamination of explants is a major problem in tissue culture studies. These contaminants are generally confined to the outer surface of the plant. Plant tissue cultures must usually be established and maintained in aseptic conditions. Most of kinds of micro-organisms, particularly bacteria and fungi compete adversely for nutrients with plant material growing in vitro. Therefore, as far as possible, explants must be free from all microbial contaminants when they are placed on a nutrient medium. To achieve this objective explants are subjected to different sterilants either singly or in combination.

Khattak et al. (1990) assessed the efficiency of surface sterilants like HgCl₂, CaOCl₂ (calcium hypochloride), NaOCl or ethanol on guava (4-5 cm) taken from plastic wrapped or unwrapped branches of 10 years old guava trees. Treatment with 70 per cent ethanol for 1 minute + 5 per cent NaOCl for 5 minutes resulted in 70 per cent infection free and 70 per cent bud sprouting. However, Naidu et al. (1993) reported that one per cent NaOCl for 30 minutes was highly efficient in eliminating microbial contamination in coffee clones.

Surface sterilization of explants for 10-15 minutes with saturated chlorine water diluted with double glass distilled water in 1:1 proportion was found to be the best as compared to commercial powder (1%), mercuric chloride (0.1%) and silver nitrate (1%) in case of pomegranate, guava and grape (Muralikrishna, 1988).
In case of neem, treatment of explants with 70 per cent alcohol for 10 seconds and then with 0.5 per cent sodium hypochloride for 15 minutes was found to be the best for obtaining aspective cultures (Upadhyaya, 1995).

The surface sterilization of mature explants was effectively achieved in case of jack fruit when the explants were treated with a combination of Bavistin (1000 mg/l) + citrimide (500 mg/l) + streptomycin sulphate (100 mg/l) for 90 minutes followed by treatment with mercuric chloride of 0.1 per cent for 10 minutes (Adiga, 1996).

2.3.1.3 Media

Nutrient medium is a mixture of substances in which cells, tissues or organs can grow with or without agar. The nutrient media consists of macrosalts, microsalts, vitamins, growth regulators and sucrose which are essential for growth and development of plant. The requirement varies with the explant and species (Hartmann et al., 1997).

The studies on establishment of nodal explants of *Pistachia* on various media, viz., Murshige and Skoog (MS), knop, woody plant medium (WPM) and Anderson’s medium were taken up by Aboulsalim (1991). He observed that better callusing and establishment on MS medium (100%) callus, although there were more chlorotic leaves on MS medium than on others.

Singh and Singh (1992) obtained the efficacy of various media viz., MS, Murashige and Tuker (MT), Gresshoy and Doy’s (GD), Gambarg’s media and white’s media on callus formation and somatic embryogenesis from shoot tips and immature ovules of *Citrus junos*. They opined that best media for callusing was MS media with various supplements.

Chavan *et al.* (1996) reported good establishment of axillary buds and shoot tips explants of jack on MS and WPM. Adiga (1996) obtained better establishment of explants on ½ MS media in jack.

2.3.1.3.1 Role of growth regulators on shoot proliferation

The production of shoots that may eventually become new plant is called shoot proliferation. The formation of adventitious shoots or roots was first determined by Skoog and miller (1957) through discovery of the regulation of organ formation (Shoots and roots) by changing the ratio of cytokinin/auxin, when ratio of cytokinin/auxins is high, it favours the formation of shoot but root formation is inhibited. The reverse favours the root formation. The most commonly used cytokinins include kinetin, 6-benzyl amino purine (BAP) and 2-isopentenyl aminopurine (2ip). Several attempts have been made to establish protocols for efficient plant regeneration and production of number of shoots in woody perennials.

Amin and Jaiswal (1987) developed rapid clonal propagation of guava through *in vitro* shoot proliferation from nodal explants from mature tree. The best response was reported on MS medium supplemented with 4.5 µM BA alone.

Yadav *et al.* (1990) using nodal explants of *Syzygium cumini* L. obtained maximum number of shoots in the presence of 4.5 µM of BA after four weeks, whereas after 8 weeks of culture, highest number of shoots i.e., 30-40 shoots were obtained on 1.12 µM BA + 0.25 µM NAA.

Mishra *et al.* (1999) cultured determinate aonla cv. NA-7 on MS medium with slight modifications. The most suitable *in vitro* establishment of aonla was observed when modified MS was supplemented with Kn 0.4 mg/l + GA3 1.0 mg/l.

Siddiqui and Farooq (1997) achieved multiple shoots in guava when nodal sections from young plants inoculated on MS medium supplemented with 1 mg/l BAP alone after two weeks of incubation. In case of nodal sections from mature tree, micropropagation was achieved with 4 mg/l BAP after three weeks of incubation.

Mehta (2000) conducted an experiment on micropropagation of *Tamarindus indica* L. They cultured mature embryo axis on MS medium supplemented with 2.69 µm NAA, 44.39 µM BAP and 4 per cent sucrose for induction of adventitious shoot buds. A medium containing 0.91 µM zeatin, 2.22 µM BAP, 0.41 µM calcium pantotheine and 0.40 µM biotin supported the differentiation of buds to form elongated shoots.
Suthamathi *et al.* (2002) carried out *in vitro* studies on papaya cv. CO-5. They obtained highest callusability in case of shoot tips cultured on MS medium supplemented with 1.0 mg/l IAA and 4.5 mg/l BA. Development of shoots was observed from shoot tip callus cultured on MS medium supplemented with BA 4-5 mg/litre compared to kinetin.

Pattepur (2003) investigated the tissue culture studies in tamarind. He obtained multiple shoots when cotyledonary explants were cultured on MS medium supplemented with BAP 0.5 mg/l + coconut water (CW) 10 per cent. Auxiliary buds collected from mature tree gave highest per cent bud break and response to multiple shoot induction on medium containing BAP 2.0 mg/l + CW 10 per cent.

2.3.1.3.1 Role of growth regulators on rooting of *in vitro* shoots

Proliferated shoots are transferred to a rooting medium supplemented with high concentration of auxin, which stimulates the formation of roots. Rooted plantlets are acclimatized in a suitable compost mixture or soil in net pots under controlled environmental conditions. The commonly used auxins for rooting are indole acetic acid, indole butyric acid and naphthalene acetic acid. The concentrations of these vary with species. Attempts made for rooting of *in vitro* shoots of the some woody perennials have been summarized here.

*In vitro* rooting of grape shootlets regenerated through direct and indirect organogenesis occurred with great ease. The highest number of roots per shoot was 8.15 with 1.0 µM of IBA, whereas NAA at 1.0 µM concentration was able to induce an average of only 3.56 per shoot (Muralikrishna, 1988).

*In vitro* rhizogenesis and root growth of *Carica papaya* L. was studied by Drew *et al.* (1993). Maximum rooting per cent was achieved by exposure of shoots to IBA than with NAA or 4-CPA. Maximum rooting percentage (96%) was achieved by exposure of shoots to a medium containing 10 µM IBA. Root initiation in teak was maximum when the medium containing IBA at the rate of 2.0 mg/l (Tiwari and Pandey, 1995) was used.

For regeneration of roots in neem IBA was found to be the best as compared to either IAA or NAA. IBA at 2.04 mg/l, produced the highest number of roots per shoot (7.0) with a length of about 4.0 cm after 40 days of transfer from the elongation media to rooting media (Upadhyaya, 1995).

A cent per cent rooting of carambola was achieved by Prashantha (1996) with IBA and NAA. IBA is a best source of auxin as compared to NAA at a concentration of 1 mg per litre induced maximum number of roots per shoot (3.5) and length of roots (3.0 cm).

2.3.2 *In vitro* propagation studies in jack fruit

*In vitro* regeneration of jack fruit was first reported by Rao *et al.* (1981). In their studies on shoot tip culture for the micro-propagation of jack tree, MS medium supplemented with BA (30 mg/l), IAA (0.5 to 5.0 mg/l) or 2-ip (30 mg/l) and NAA (1.0 mg/l) produced multiple shoots. Rooting of shoots occurred in a medium containing IBA and NAA at 0.1, 0.5, 1.0 and 1.5 mg/l concentrations.

In jack proliferation from nodal explants was greater than that from shoot tips, BAP was more effective than either 2-ip or kinetin and produced maximum proliferation when used at 5 x 10⁻⁶ M shoot proliferation was optimal at 30°C with a 12 h photoperiod. Optimal rooting of shoots *in vitro* was obtained with IBA at 10⁻⁶ M. The number and length of roots was significantly increased in 12 h light as compared with dark (Rahman and Jannet, 1988).

Rajmohan and Mohankumaran (1988) studied the influence of explant source and plant growth substances on *in vitro* propagation of jack fruit. Shoot apices from seedlings registered a multiplication of 17.4 x with 100 per cent rooting. Explants from fresh stem sprouts of five, ten and thirty years old jack trees recorded shoot multiplication rates of 4.5 x, 2.8 x and 2.29 x, respectively in five years. Explants from six months old jack grafts failed to produce multiple shoots. *In vitro* rooting of shoots occurred when cultured on half strength of MS medium with IBA at 2 mg/l, NAA 2 mg/l, 30 g/l sucrose and 6 g/l agar for 5 days followed by transfer to a growth regulator free medium.

*In vitro* propagation of jack fruit using mature nodal explants was reported by Roy *et al.* (1990). The nodal buds along with a portion of stem 2 cm long were excised from a 30 years old tree. These explants were surface sterilized with 0.5 per cent mercuric chloride for
2-3 minutes and cultured on MS medium with a range (0.5, 0.1, 1.5 and 2.0 mg/l) of auxins; indole 3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA) or 2,4-Dichlorophenoxy acetic acid (2,4-D) and cytokinins, 6-benzyl amino purine (BAP) or kinetin. For shoot proliferation BAP at 1.0 mg/l and kinetin at 0.5 mg/l were given best results. Cuttings from in vitro proliferated shoots rooted in ½ MS medium with 1.5 mg/l NAA or 1.0 mg/l IBA. But 1.0 mg/l each of IBA and NAA in combination produced roots with profuse lateral rootlets in 95 per cent of explants, 70 per cent of in vitro plants survived in pots and 75 per cent of these plants survived in the field.

Apical buds of jack containing primordial inflorescences collected from the foot stalks of adult trees produced tiny inflorescence (7 – 1.5 mm) within 6 weeks of culture on MS medium supplemented with 1 mg/l BA. Upon isolation, sectioning and subculturing on similar medium containing 0.5 to 1.0 mg/l BA alone or with 0.1 mg/l NAA, each section of tiny inflorescence produced uniformly growing shoot buds along their full length within 7 weeks of incubation. The buds developed into rootable shoots, 4 weeks after additional subculture and multiplied on a medium containing 1 mg/l BA only. Microcuttings were rooted with 90 per cent success using 2 mg/l IBA in ½ MS medium (Amin, 1992).

In vitro response for apical bud explants from mature trees of jack fruit was reported by Amin and Jaiswal (1993). Shoot tip cultures were established on MS medium with 5-10 mm long explants dissected from terminal buds of new growth from the trunk. Benzyl adenine and kinetin (4.5 – 9.0 µM), either separately or together, supported shoot proliferation. Bud explants taken from emerging trunk sprouts invariably produced clumps of multiple shoots, where buds obtained from actively growing top branches generally elongated to form a solitary shoots. November to January was the best season for initiation of cultures from field grown trees. Rooting of shoots with 60-80 per cent success was observed using half strength MS and 10 µM IBA or NAA.

Roy et al. (1993) employed in vitro culture for the clonal propagation of jack trees identified as flood tolerant using shoot buds as explants. MS medium containing 8.88 µM 6-benzyl adenine (BA) and 2.68 µM NAA induced highest number of shoot buds. For shoot multiplication the concentrations of BA and NAA were lowered to 44.4 and 0.54 µM respectively and coconut water was added at a concentration of 10 per cent. Within three weeks of transfer, 80 per cent rooting was observed on a ½ MS medium with 5.37 µM NAA and 4.92 µM IBA. Young plantlets were transplanted directly from culture tubes to earthen pots containing sterile sand, soil and humus (1:2:1) and covered by transparent plastic bags.

Adiga (1996) studied in vitro propagation of jack fruit cv.. Singapore jack using mature explants. Shoot proliferation was found maximum from nodal explants with BAP than kinetin at 2 mg/l, cultured on ½ MS medium. Shoots cultured on ½ MS medium supplemented with 6 mg/l IBA produced highest extent of rooting. The rooted plantlets were transferred to portrays containing sand/vermiculite. Plantlets were hardened under high humidity (90-100%) for 6-8 weeks.
III. MATERIAL AND METHODS

Studies on standardization of soft wood grafting technique in jackfruit were conducted at the Golden Jubilee Block, Department of Horticulture, University of Agricultural Sciences, Dharwad. The place is situated in transitional tract of Karnataka at 76°-07' east longitude and 15°-26' north latitude, at an altitude of 667 m above mean sea level. The average rainfall of the area is about 602.10 mm, which is fairly well distributed from April to October. The average maximum temperature goes upto 36°C in the month of April and the minimum temperature reaches 13°C in the month of December-January. The relative humidity fluctuates between 50 to 87 per cent. The weather data recorded at Main Agricultural Research Station, Dharwad for the year 2004-05 and average of 54 years are presented in Appendix –I. The experiment was conducted during the period from May 2004 to April 2005.

3.1 GENERAL CONDITIONS OF PROPAGATION STRUCTURES

3.1.1 Mist house

A mist house of dimension 18 x 6 m was used. The frequency of misting was 6-7 minutes for duration of 30 seconds. The relative humidity ranged between 85 and 95 per cent and the temperature between 35 and 40°C.

3.1.2 Green house

The green house with fan and pad cooling system dimension of 27 x 9 m was used. The relative humidity inside the greenhouse ranged between 80 and 90 per cent and temperature between 25 and 27°C.

3.1.3 Shade house

A shade house of dimension 18 x 24 m with a height of 2.4 m was used. It was of High Density Polyethylene (HDPE) green shade net and allowing only 25 per cent sunlight. This structure maintained relatively low temperatures with high humidity was maintained in comparison with the outside environment.

3.2 PREPARATORY OPERATIONS

3.2.1 Collection of seeds

Fully matured jackfruits of a local variety were harvested and allowed to ripe in the room for a week. Then the seeds were extracted manually. Medium sized good shaped and heavy seeds were dipped in water. Floating seeds were discarded and rest were taken as seed material. The seeds were spread over the ground under shade for surface drying. After drying, the seeds were treated with one per cent captan and used for raising rootstocks.

3.2.2 Raising of rootstocks

Polythene bags of 400 gauge thickness and of size 20 x 10 cm were used for raising rootstocks. Potting mixture containing red earth, farm yard manure (FYM) and coir dust in 1:1:1 (v/v) proportion was used. In the month of May selected healthy, medium sized seeds were sown flat on the medium with their hilum part facing down. The polythene bags holding the seeds were maintained in the nursery with all necessary care.

3.2.3 Maintenance of rootstocks

The seeds sown in the polythene bags were watered regularly. Germination of seeds was noticed 20 days after sowing. The polythene bags were kept under shade to protect the seedlings from direct solar radiation. General prophylactic plant protection measures were taken by spraying with fungicides and pesticides to control the pest and diseases. Weeding was done as and when required.
3.3 DETAILS OF EXPERIMENTS

The study consists of following experiments.

3.3.1 Experiment-I : Effect of age of rootstock on success of grafting.

3.3.1.1 Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age of rootstock</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>4 months</td>
</tr>
<tr>
<td>T₂</td>
<td>5 months</td>
</tr>
<tr>
<td>T₃</td>
<td>6 months</td>
</tr>
<tr>
<td>T₄</td>
<td>7 months</td>
</tr>
<tr>
<td>T₅</td>
<td>8 months</td>
</tr>
<tr>
<td>T₆</td>
<td>9 months</td>
</tr>
<tr>
<td>T₇</td>
<td>10 months</td>
</tr>
</tbody>
</table>

Design : Completely Randomized Block Design
Replication : Three
Number of grafts/treatment : 50

3.3.2 Collection of scion

The scions were collected from the healthy, elite trees of var. NAH-92, in the morning hours on the day of grafting and defoliated with sharp secateur. The scions so prepared were further used for grafting on the same day.

3.3.3 Method of grafting

Soft wood grafting method was followed in the study. For this the top growth of rootstock was decapitated with sharp knife or secateur. Then longitudinal cut of 5 cm length was given from the terminally pruned rootstock. A scion shoot of about same thickness was selected.

The length of scion was about 8-10 cm. The basal end of scion was given two gentle sloping cuts of about 5 cm on opposite sides by removing the bark and a little wood, giving a wedge shape. Care was taken to retain some bark on remaining two sides. The wedge shaped scion thus prepared was inserted into the ‘V’ shaped slit of the stock and secured firmly with 150 gauge thickness white transparent polythene strip of 1.5 cm width and 30 to 45 cm length, to keep the stock and scion in firm contact. The scions were covered with small transparent tubular bag to prevent entry of water into the grafted portion and also to avoid desiccation of the scion by creating humidity in the microclimate near and above the graft union region.

The grafted plants were transferred immediately to the mist chamber and maintained there for 30 days. Then they were shifted to shade house.

3.3.4 Observations recorded

Periodically observations were recorded on following parameters.

3.3.4.1 Per cent grafting success

Graft union success was recorded on 30, 45, 60 and 90 days after grafting (DAG). The scion which remained green was also taken as successful graft after 30 days of grafting.

3.3.4.2 Number of sprouts on graft

Number of sprouts were recorded by counting the sprouts on scions, 30, 45, 60 and 90 DAG. The mean was calculated.
3.3.4.3 Number of leaves
   Number of leaves on scion at 30, 45, 60 and 90 days after grafting were recorded on all successful grafts and mean was calculated.
3.3.4.4 Sprout length
   Sprout length on scion was recorded at 30, 45, 60 and 90 DAG on all the successful grafts and mean was calculated.

3.4 MICROPROPAGATION
   In vitro propagation was conducted in the plant tissue culture laboratory of the Division of Horticulture, University of Agricultural Sciences, Dharwad.

3.4.1 Source of plant
   The explant material for the experiment was collected from healthy, elite clone of Singapore jack tree located in the garden of Horticulture Department, Dharwad. Shoot tips
and nodal segments of young sprouts of the tree were used as explants for initial establishment of cultures.

### 3.4.2 Preparation of plant material

The following operations were carried out in the preparation and surface sterilization of plant material.

1. Explants were washed with sterile water containing few drops of teepol.
2. They were surface sterilized with Bavistin for 90 minutes followed by surface sterilization inside the laminar air flow cabinet with mercuric chloride (0.1%) for 10 minutes.
3. Explants were then thoroughly washed with sterile distilled water for 3 to 4 times.

For the experiment involving comparison of different explant source, in order to raise seedlings, the seeds were sterilized as follows:

The seeds were washed with tap water and later with sterile distilled water containing few drops of teepol before final surface sterilization with 0.1 per cent mercuric chloride for 20 minutes followed by sterile distilled water wash for 3-4 minutes.

### 3.4.3 Preparation of stocks

Murashige and Skoog medium was commonly used for all the experiments. Five stock solutions (8x) were prepared with double distilled water, poured into well stoppered bottle and were stored in refrigerator. Details of stock solutions are given below.

#### 3.4.3.1 Mineral and vitamin stock

<table>
<thead>
<tr>
<th>Stock</th>
<th>Stock A: Macro elements</th>
<th>Stock C: Calcium chloride stock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 ml (8x)</td>
<td>1000 ml (8x)</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>13.2 g</td>
<td></td>
</tr>
<tr>
<td>KNO₃</td>
<td>15.2 g</td>
<td></td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>2.96 g</td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.36 g</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock</th>
<th>Stock B: Microelements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 ml (8x)</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.2 g</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.2 g</td>
</tr>
<tr>
<td>MnSO₄·4H₂O</td>
<td>178.4 mg</td>
</tr>
<tr>
<td>KI</td>
<td>6.6 mg</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>86.4 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>49.6 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock</th>
<th>Stock D: Iron stock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 ml (8x)</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>279 mg</td>
</tr>
<tr>
<td>Na₂EDTA</td>
<td>373 mg</td>
</tr>
</tbody>
</table>

Preparation of iron stock

Na₂EDTA (373 mg) in double distilled water was boiled to which FeSO₄·7H₂O at 279 mg in double distilled was added gently. Volume was made upto 1000 ml.

<table>
<thead>
<tr>
<th>Stock E</th>
<th>Vitamin stock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 ml (50x)</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>2.5 mg</td>
</tr>
</tbody>
</table>
Glycine - 10 mg
Mesoinositol - 500 mg.

3.4.3.2 Preparation of growth regulator stock

Stock solutions of 6-benzyl amino purine (BAP), kinetin, naphthalene acetic acid (NAA) indole butyric acid (IBA), gibberellic acid (GA) and indole acetic acid (IAA) 100 ppm each was prepared by dissolving 10 mg of growth regulator in small quantity of ethanol. The volume was made up to 100 ml with distilled water to make 100 ppm stock.

3.4.4 Quality of chemicals

All chemicals used in experiments were of analytical grade.

3.4.5 Preparation and sterilization of media

The stock solutions were mixed in the required proportion along with growth regulators and sucrose (3%). Then volume was adjusted by adding double distilled water. The pH was adjusted to 5.8 by addition of 0.1N HCl or NaOH as required. After that agar at the rate of 0.6 per cent and charcoal at 0.7 per cent were added, and heated till the agar melts properly. The media is then poured into the test tubes and plugged with cotton plugs. Then these test tubers were autoclaved at 121°C at a pressure of 1.2 kg per cm$^2$ for 20 minutes and then allowed to cool to room temperature and stored in culture rooms until further use.

3.4.6 Culture establishment

3.4.6.1 Inoculation

Sterilized explants were inoculated into test tubes containing media. Cut ends of explants were kept in such a way that they have contact with medium.

3.4.6.2 Sub culture

Microshoots formed in the test tubes were taken out 4-5 weeks after inoculation. The nodes and shoot tips were separated by dissecting them in sterile environment of laminar air flow cabinet with sterile dissecting needle and forceps. Then they were placed in the test tubes containing fresh media.

3.4.6.3 Rooting

The microshoots which were more than 1 cm in height were taken out and then placed in the tubes containing half strength MS media with different concentrations of IBA and NAA for rooting.

3.4.6.4 Culture condition

The culture room was maintained at 25 ± 2°C temperature uniform light was provided by using fluorescent tubes (1500 lux) over a light/dark cycles of 16/8 hrs.

3.4.6.5 Transfer area and aseptic manipulations

All the aseptic manipulations such as surface disinfection of explants, preparation and inoculation of explants and subsequent subculturing were carried out under the laminar air flow cabinet. The working table of laminar air flow cabinet was sterilized by swabbing with absolute alcohol. All the required materials like media, spirit lamp, glasswares etc. were transferred onto the clean laminar air flow. The UV light was switched on for half an hour to achieve aseptic environment inside the cabinet where all the manipulations were conducted.

3.4.6.6 Hardening of in vitro plantlets

Young rooted plantlets were taken out of the test tubes washed with distilled water and planted in net pots, containing different hardening media. These plants were covered with a clean polythene sheet fixed over a frame to form a tunnel. The plantlets were watered twice a day initially then once a day after eight to ten days. Then they were transferred to greenhouse after 15 days (70-80% RH) for further acclimatization.

3.4.7 Experimental details

3.4.7.1 Experiment – II : Effect of source and type of explants on culture establishment
Treatment details

T1 - Shoot tips from axenic seedlings
T2 - Nodal segments from axenic seedlings
T3 - Shoot tips from mature plants
T4 - Nodal segments from mature plants

All explants were inoculated on ½ MS media containing 2 mg/l BAP, 3 per cent sucrose, 0.6 per cent agar and 0.7 per cent charcoal.

Design: Completely Randomised Block Design
Replications: Five
Number of culture tubes/treatment: Five

Observations recorded
1. Mean number of shoots produced/explant
   The number of shoots produced from single explant after inoculations were counted and mean was calculated.
2. Mean length of shoot
   Length of shoot was measured in cm during subculturing and their mean was calculated.
3. Mean number of leaves/shoot
   Number of leaves produced from single explant was counted and their mean was calculated.
4. Kind of response
   Type of response in terms of callus formation, bud break and shoot induction was recorded by visual observation.
5. Per cent survival of explants
   The number plants survived out of total inoculated was converted into percentage.
6. Per cent contamination
   The number of contaminated culture tubes were counted and converted into percentage.

3.4.7.2 Experiment – III: Effect of BAP and kinetin on shoot proliferation

Treatment details
T1 - ½ MS + BAP 1.0 mg/lit
T2 - ½ MS + BAP 1.5 mg/lit
T3 - ½ MS + BAP 2.0 mg/lit
T4 - ½ MS + BAP 2.5 mg/lit
T5 - ½ MS + Kn 0.4 mg/lit
T6 - ½ MS + Kn 0.6 mg/lit
T7 - ½ MS + Kn 0.8 mg/lit
T8 - ½ MS + Kn 1.0 mg/lit

Explants: Shoot tips
Design: Completely Randomized Block Design
Replication: Three
Number of culture tubes : 10

Observations recorded
1. Mean number of shoots produced/explant
   Number of shoots produced were counted and mean was calculated.
2. Mean length of the shoot :
   Length of shoots produced from explant was measured during subculturing and expressed in centimeters.
3. Mean number of leaves produced/shoot
   Number of leaves produced from single explant was counted and mean of them was calculated.

Experiment IV
3.4.7.3 Effect of source and concentration of auxins on \textit{in vitro} rooting of microcuttings

NAA and IBA were tried in different concentrations, as given below :

<table>
<thead>
<tr>
<th>Treatment details</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>½ MS + NAA 2 mg/lit</td>
</tr>
<tr>
<td>T_2</td>
<td>½ MS + NAA 4 mg/lit</td>
</tr>
<tr>
<td>T_3</td>
<td>½ MS + NAA 6 mg/lit</td>
</tr>
<tr>
<td>T_4</td>
<td>½ MS + NAA 8 mg/lit</td>
</tr>
<tr>
<td>T_5</td>
<td>½ MS + IBA 2 mg/lit</td>
</tr>
<tr>
<td>T_6</td>
<td>½ MS + IBA 4 mg/lit</td>
</tr>
<tr>
<td>T_7</td>
<td>½ MS + IBA 6 mg/lit</td>
</tr>
<tr>
<td>T_8</td>
<td>½ MS + IBA 8 mg/lit</td>
</tr>
</tbody>
</table>

Explants : Microcuttings of 3-4 cm length
Design : Completely Randomized Block Design
Replication : Three
Number of test tubes : 10 per treatment

Observations recorded
1. Mean number of roots per shoot
   Number of roots produced per microshoot were counted and mean was calculated.
2. Mean length of roots
   Length of roots was measured in cms, from base to tip and mean calculated.
3. Number of days taken for rooting
   Number of days taken for rooting from date of inoculation was recorded.

3.4.7.4 Statistical analysis

Completely randomized design was employed to analyse the standardization of both soft wood grafting and micropropagation. The data in percentage were transformed to arc sin values for statistical analysis. The data were subjected to ANOVA as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one per cent probability for laboratory experiment and at 5 per cent probability for field experiment where \textit{F} test was significant.
IV. EXPERIMENTAL RESULTS

The results of different experiments carried out are presented in this chapter. The studies were conducted to standardize the age of rootstocks for soft wood grafting and to develop protocol for micropropagation.

4.1 EXPERIMENT I: Effect of age of rootstock on success of grafting

This experiment was carried out to find out the optimum age of rootstock for carrying out soft wood grafting in jack. The grafting operation was carried out at monthly intervals on four to ten months old rootstocks.

4.1.1 Per cent graft union success

The data on the per cent graft success on 30th, 45th, 60th and 90th day after grafting are presented in Table 1 and Fig.1. The per cent graft success on 30th, 45th, 60th and 90th day after grafting showed significant differences among the treatments.

The per cent graft success gradually decreased from 30th to 90th day after grafting in all the root stocks of different ages. On 30th day after grafting the maximum graft union was noticed in seven months old rootstocks (58.67%), however it was on par with nine months old rootstock (56.95%). The lowest graft success was observed in case of ten months old rootstocks (43.80%) on 45th, 60th and 90th also the seven months old rootstocks showed highest graft union success. On 90th days after grafting the highest graft success (51.90%) was obtained with seven months old rootstocks and lowest graft success (39.99%) was observed with the 10 months old rootstocks.

4.1.2 Effect of age of rootstocks on average number of leaves per graft

The data on average number of leaves on 30th, 45th, 60th and 90th day after grafting are presented in Table 2.

The average number of leaves per graft on 30th, 45th, 60th and 90th day after grafting were significantly different among the treatments. There was gradual increase in number of leaves per graft at all the intervals.

On 30th day after grafting highest number of leaves (2.80) was noticed in four month old rootstocks. The minimum number of leaves (1.20) was observed in eight month old rootstocks. On 45th day after grafting maximum number of leaves (2.53) in four months old rootsocks and it was followed upto 60th day after grafting. On 90th day after grafting maximum number of leaves (5.20) was noticed in seven months old root stocks which was on par with four months old rootstocks (4.70). The lowest number of leaves (2.46) was observed with ten month old rootstocks, which was on par with eight month old root stocks and nine month old root stocks.

4.1.3 Effect of age of root stock on number of sprouts per graft

The data pertaining to the average number of sprouts per graft on 30th, 45th, 60th and 90th day after grafting are presented in Table 3. The differences for average number of sprouts on scion portion of the graft on 30th, 45th, 60th and 90th day after grafting were significant among the treatments.

On 30th day after grafting the maximum number of sprouts recorded was (2.33) in four months old root stocks, and minimum (1.26) with seven months old rootstocks. In all the intervals i.e. 30th, 45th, 60th and 90th day after grafting maximum number of sprouts was observed in four months old rootstocks.

4.1.4 Effect of age of rootstocks on sprout length

The data on the average sprout length on 30th, 45th, 60th and 90th day after grafting was presented in Table 4.

The perusal of the data reveals significant differences among different age rootstocks. There was a gradual increase in sprout length at different intervals after grafting.
Table 1. Effect of age of rootstock on per cent success of grafts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 DAG</th>
<th>45 DAG</th>
<th>60 DAG</th>
<th>90 DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$ (4 months)</td>
<td>50.43 (45.24)</td>
<td>49.63 (44.79)</td>
<td>44.58 (41.88)</td>
<td>43.30 (40.93)</td>
</tr>
<tr>
<td>$T_2$ (5 months)</td>
<td>52.13 (46.20)</td>
<td>51.28 (45.73)</td>
<td>46.00 (42.68)</td>
<td>42.71 (40.79)</td>
</tr>
<tr>
<td>$T_3$ (6 months)</td>
<td>52.93 (46.66)</td>
<td>51.12 (45.63)</td>
<td>45.58 (42.43)</td>
<td>43.80 (41.44)</td>
</tr>
<tr>
<td>$T_4$ (7 months)</td>
<td>58.67 (49.97)</td>
<td>56.34 (48.62)</td>
<td>53.62 (47.04)</td>
<td>51.90 (46.09)</td>
</tr>
<tr>
<td>$T_5$ (8 months)</td>
<td>47.49 (42.56)</td>
<td>45.38 (42.07)</td>
<td>42.38 (40.61)</td>
<td>41.71 (40.22)</td>
</tr>
<tr>
<td>$T_6$ (9 months)</td>
<td>56.95 (48.96)</td>
<td>53.29 (46.83)</td>
<td>45.11 (42.17)</td>
<td>43.29 (41.11)</td>
</tr>
<tr>
<td>$T_7$ (10 months)</td>
<td>43.80 (41.40)</td>
<td>42.50 (40.62)</td>
<td>45.88 (41.45)</td>
<td>39.99 (39.21)</td>
</tr>
<tr>
<td>Mean</td>
<td>51.77 (45.85)</td>
<td>49.93 (44.90)</td>
<td>45.88 (42.61)</td>
<td>43.82 (41.40)</td>
</tr>
<tr>
<td>SEm±</td>
<td>0.815 (0.620)</td>
<td>0.582 (0.594)</td>
<td>2.47 (1.880)</td>
<td>1.765 (1.80)</td>
</tr>
</tbody>
</table>

*Figures in the parentheses indicate arc sin values
DAG – Days after grafting
Fig. 1: Effect of age of rootstock on percent success of grafts
Table 2. Effect of age of rootstock on number of leaves in grafts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 DAG</th>
<th>45 DAG</th>
<th>60 DAG</th>
<th>90 DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁ (4 months)</td>
<td>2.80</td>
<td>2.53</td>
<td>4.73</td>
<td>4.70</td>
</tr>
<tr>
<td>T₂ (5 months)</td>
<td>2.40</td>
<td>2.46</td>
<td>2.60</td>
<td>3.66</td>
</tr>
<tr>
<td>T₃ (6 months)</td>
<td>2.00</td>
<td>2.23</td>
<td>2.70</td>
<td>4.23</td>
</tr>
<tr>
<td>T₄ (7 months)</td>
<td>1.60</td>
<td>1.93</td>
<td>2.53</td>
<td>5.20</td>
</tr>
<tr>
<td>T₅ (8 months)</td>
<td>1.20</td>
<td>2.33</td>
<td>2.26</td>
<td>2.73</td>
</tr>
<tr>
<td>T₆ (9 months)</td>
<td>2.00</td>
<td>2.46</td>
<td>2.73</td>
<td>2.93</td>
</tr>
<tr>
<td>T₇ (10 months)</td>
<td>1.40</td>
<td>2.46</td>
<td>2.06</td>
<td>2.46</td>
</tr>
<tr>
<td>Mean</td>
<td>1.89</td>
<td>2.20</td>
<td>2.80</td>
<td>3.69</td>
</tr>
<tr>
<td>SEm±</td>
<td>0.094</td>
<td>0.077</td>
<td>0.068</td>
<td>0.154</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>0.285</td>
<td>0.233</td>
<td>0.206</td>
<td>0.467</td>
</tr>
</tbody>
</table>

DAG – Days after grafting

Table 3. Effect of age of rootstock on number of sprouts in grafts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 DAG</th>
<th>45 DAG</th>
<th>60 DAG</th>
<th>90 DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁ (4 months)</td>
<td>2.33</td>
<td>2.53</td>
<td>2.66</td>
<td>3.73</td>
</tr>
<tr>
<td>T₂ (5 months)</td>
<td>1.60</td>
<td>1.66</td>
<td>1.86</td>
<td>1.93</td>
</tr>
<tr>
<td>T₃ (6 months)</td>
<td>1.36</td>
<td>1.86</td>
<td>1.93</td>
<td>1.86</td>
</tr>
<tr>
<td>T₄ (7 months)</td>
<td>1.26</td>
<td>1.33</td>
<td>1.33</td>
<td>1.66</td>
</tr>
<tr>
<td>T₅ (8 months)</td>
<td>1.33</td>
<td>1.33</td>
<td>1.36</td>
<td>1.53</td>
</tr>
<tr>
<td>T₆ (9 months)</td>
<td>1.66</td>
<td>1.86</td>
<td>1.93</td>
<td>2.33</td>
</tr>
<tr>
<td>T₇ (10 months)</td>
<td>1.33</td>
<td>1.26</td>
<td>1.26</td>
<td>1.33</td>
</tr>
<tr>
<td>Mean</td>
<td>1.55</td>
<td>1.62</td>
<td>1.66</td>
<td>2.05</td>
</tr>
<tr>
<td>SEm±</td>
<td>0.058</td>
<td>0.076</td>
<td>0.080</td>
<td>0.113</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>0.175</td>
<td>0.230</td>
<td>0.242</td>
<td>0.403</td>
</tr>
</tbody>
</table>

DAG – Days after grafting
On 30\textsuperscript{th} day after grafting maximum length of sprout (3.52 cm) was observed in case of four month old rootstocks. This trend was carried upto 90\textsuperscript{th} day after grafting. On 90\textsuperscript{th} day after grafting highest length of sprout (4.32) was observed in case of four months old rootstocks. The lowest length of sprout (1.63) was observed in ten months old rootstocks, on 90\textsuperscript{th} day after grafting. It was true with all the intervals after grafting i.e., 30\textsuperscript{th}, 45\textsuperscript{th}, 60\textsuperscript{th} and 90\textsuperscript{th} day after grafting.

4.2 EXPERIMENT II: Effect of source of explants on culture establishment

In an attempt to standardize explants for tissue culture of jack fruit, different types of explants were tried for \textit{in vitro} shoot regeneration and multiplication. It was observed that explants exhibited differential response to the \textit{in vitro} conditions. The plants from mature tree and axenic seedlings were tried in the study. The survival and contamination percentages of explants are presented in Table 5. The highest percentage of contamination (20\%) was observed with modal explants from both mature tree and axenic seedling. The minimum percentage of contamination (10\%) was observed when shoot tips from mature tree and axenic seedling were used. Highest percentage of survival (50.40\%) was recorded in shoot tip explants from mature tree and minimum percentage (38.00\%) of survival was noticed in modal explants of axenic seedling which was on par with shoot tip explants of axenic seedling.

<table>
<thead>
<tr>
<th>Table 4. Effect of age of rootstock on sprout length of grafts (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>T\textsubscript{1} (4 months)</td>
</tr>
<tr>
<td>T\textsubscript{2} (5 months)</td>
</tr>
<tr>
<td>T\textsubscript{3} (6 months)</td>
</tr>
<tr>
<td>T\textsubscript{4} (7 months)</td>
</tr>
<tr>
<td>T\textsubscript{5} (8 months)</td>
</tr>
<tr>
<td>T\textsubscript{6} (9 months)</td>
</tr>
<tr>
<td>T\textsubscript{7} (10 months)</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SEm\pm</td>
</tr>
<tr>
<td>CD at 5%</td>
</tr>
</tbody>
</table>

DAG – Days after grafting
Table 5. Effect of explants on per cent survival and contamination of explants

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatments</th>
<th>Explant contamination percentage</th>
<th>Explant survival percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Shoot tips from axenic seedling</td>
<td>10.00 (18.43)*</td>
<td>50.40 (45.85)</td>
</tr>
<tr>
<td>T2</td>
<td>Nodal segments from axenic seedling</td>
<td>20.00 (26.57)</td>
<td>42.80 (40.85)</td>
</tr>
<tr>
<td>T3</td>
<td>Shoot tips from mature plants</td>
<td>10.00 (18.43)</td>
<td>39.00 (38.64)</td>
</tr>
<tr>
<td>T4</td>
<td>Nodal segments from mature plants</td>
<td>20.00 (26.57)</td>
<td>38.00 (38.03)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>15.00 (22.18)</td>
<td>42.55 (40.69)</td>
</tr>
<tr>
<td>SEM±</td>
<td></td>
<td>0.10</td>
<td>0.544</td>
</tr>
<tr>
<td>CD at 1%</td>
<td></td>
<td>0.432</td>
<td>2.350</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate arc sin transformed values

Explants from different sources produced differential responses in the form of callus formation bud break and shoot induction under in vitro conditions. Callus formation was observed in nodal explants from both mature tree and axenic seedling. Bud break and shoot induction responses were observed in shoot tip explants of both mature tree and axenic seedlings (Table 6).

Explants also affected the shoot proliferation which was presented in Table 7. There was no significant difference in mean number of shoots produced by different types explants used. Mean length of shoot was highest (1.20cm) when shoot tips from mature tree were used as explants. The minimum length of shoot (1.05 cm) was noticed in explants derived from axenic seedlings. Nodal explants derived from mature tree produced maximum number of leaves (2.10) and minimum number of leaves (1.98) were observed in nodal explants from axenic seedlings.

4.3 EFFECT OF GROWTH REGULATORS ON SHOOT PROLIFERATION

The results of the experiments conducted to study the effect of concentrations of cytokinins (BAP and kinetin) on shoot proliferation of jack fruit are presented in Table 8. Significant differences were observed among the treatments for mean number of shoots, length of shoot, mean number of adventitious buds and number of leaves.

4.3.1 Mean number of shoots

There were significant differences among the treatments with respect to number of shoots per explant. Highest number of shoots (2.60) was observed on medium containing BAP 2 mg per litre followed by BAP 1.5 mg per l (2.23). The medium with BAP 0.5 mg per litre and kinetin 0.5 mg per litre produced lowest number of shoots which were on par with each other.

4.3.2 Length of shoots

Significant difference was observed among the treatments except in medium with BAP 1.0 mg/l and kinetin 1.5 mg/l, which were on par with each other. The maximum length of
Table 6. Nature of response of different type of explants of jack fruit to culture condition

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Explants</th>
<th>Callusing</th>
<th>Bud break</th>
<th>Shoot induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Shoot tips from axenic seedling</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>T₂</td>
<td>Nodal segments from axenic seedling</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T₃</td>
<td>Shoot tips from mature plants</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>T₄</td>
<td>Nodal segments from mature plants</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>

(-) No response  (✓) Response.

Table 7. Effect of type of explants on shoot proliferation

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatments</th>
<th>Mean no. of shoots</th>
<th>Mean length of shoots</th>
<th>Mean number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Shoot tips from axenic seedling</td>
<td>2.06</td>
<td>1.20</td>
<td>1.98</td>
</tr>
<tr>
<td>T₂</td>
<td>Nodal segments from axenic seedling</td>
<td>2.00</td>
<td>1.10</td>
<td>2.10</td>
</tr>
<tr>
<td>T₃</td>
<td>Shoot tips from mature plants</td>
<td>1.98</td>
<td>1.05</td>
<td>2.00</td>
</tr>
<tr>
<td>T₄</td>
<td>Nodal segments from mature plants</td>
<td>1.98</td>
<td>1.14</td>
<td>1.98</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.025</td>
<td>1.12</td>
<td>2.00</td>
</tr>
<tr>
<td>SEm±</td>
<td></td>
<td>0.038</td>
<td>0.019</td>
<td>0.031</td>
</tr>
<tr>
<td>CD at 1%</td>
<td></td>
<td>NS</td>
<td>0.078</td>
<td>0.128</td>
</tr>
</tbody>
</table>

NS – Non significant
shoot (1.43 cm) was recorded with the medium containing BAP 2 mg/l. The shortest shoot (0.70 cm) was recorded with the medium having kinetin 2 mg/l.

4.3.3 Number of leaves

The number of leaves per explant was recorded to be highest (2.18) with basal medium containing BAP 2.0 mg/l. The lowest number of leaves (0.94) was recorded with basal medium containing 1.5 mg/l which was on par with kinetin 1.0 mg/l, kinetin 1.5 mg/l and kinetin 2.0 mg/l.

4.4 EXPERIMENT IV: Effect of growth regulators on *in vitro* root formation

The results of the experiment to study the effect of auxins on *in vitro* root formation are presented in Table 9.

The results of the experiment revealed that the medium containing IBA 8.0 mg/l has responded positively for root initiation, by producing four root initials. However all other treatments of auxins failed to show *in vitro* root formation in jackfruit. It had taken 31.00 days for root initiation.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatments (mg/l)</th>
<th>Mean no. of shoots</th>
<th>Length of shoots (cm)</th>
<th>Mean number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>½ MS + BAP – 0.5</td>
<td>0.56</td>
<td>1.03</td>
<td>1.04</td>
</tr>
<tr>
<td>2.</td>
<td>½ MS + BAP – 1.0</td>
<td>1.20</td>
<td>0.99</td>
<td>1.03</td>
</tr>
<tr>
<td>3.</td>
<td>½ MS + BAP – 1.5</td>
<td>2.23</td>
<td>1.06</td>
<td>0.94</td>
</tr>
<tr>
<td>4.</td>
<td>½ MS + BAP – 2.0</td>
<td>2.60</td>
<td>1.43</td>
<td>2.18</td>
</tr>
<tr>
<td>5.</td>
<td>½ MS + Kinetin – 0.5</td>
<td>0.55</td>
<td>1.01</td>
<td>1.08</td>
</tr>
<tr>
<td>6.</td>
<td>½ MS + Kinetin– 1.0</td>
<td>1.21</td>
<td>1.02</td>
<td>1.01</td>
</tr>
<tr>
<td>7.</td>
<td>½ MS + Kinetin– 1.5</td>
<td>1.00</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>8.</td>
<td>½ MS + Kinetin– 2.0</td>
<td>0.95</td>
<td>0.70</td>
<td>0.99</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.295</td>
<td>1.032</td>
<td>1.16</td>
</tr>
<tr>
<td>SEm ±</td>
<td></td>
<td>0.026</td>
<td>0.015</td>
<td>0.21</td>
</tr>
<tr>
<td>CD at 1%</td>
<td></td>
<td>0.107</td>
<td>0.061</td>
<td>0.867</td>
</tr>
</tbody>
</table>
Table 9. Effect of growth regulators on shoot formation

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatments (mg/l)</th>
<th>Mean number of days taken for root initiation</th>
<th>Mean number of roots</th>
<th>Mean length of roots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>½ MS + IBA-2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>½ MS + IBA-4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>½ MS + IBA-6.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>½ MS + IBA-8.0</td>
<td>31.00</td>
<td>4.00</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>½ MS + NAA-2.0</td>
<td>-</td>
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<tr>
<td>6.</td>
<td>½ MS + NAA-4.0</td>
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<td>7.</td>
<td>½ MS + NAA-6.0</td>
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<tr>
<td>8.</td>
<td>½ MS + NAA-8.0</td>
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</table>
V. DISCUSSION

Jackfruit is an important tropical fruit crop. The fruit is rich in nutritive value and very popular all over tropical regions. It is generally grown in the back yards and in the coffee and cardamom plantations, as shade tree. It has not received much attention towards its crop improvement and standardization of cultural practices. Standardization of vegetative propagation methods will serve as one of the important tools for boosting up the production and quality of crop, particularly in cross-pollinated crops like jack.

Although different vegetative propagation methods have been developed, the seed propagation has been particularly followed. As such, there are no standard varieties in jack. However, there are good number of trees which are superior in yield and fruit characters. Vegetative propagation of such selected trees assumes greater importance to ensure uniformity of planting material and for enhancing production.

Any vegetative propagation method developed should be easy to adopt and achieve high multiplication rate. In soft wood grafting approach, the optimum age of rootstock is important. In general, those plants that are easy to propagate conventionally are also easy to micropropagate (Murashige, 1974). But the success in micropropagation of woody perennials has been very limited due to the problems such as contamination, phenolic exudation, vitrification, induction of rooting, acclimatization etc. Jackfruit being woody perennial, shows many of the above undesirable characteristics. Hence, keeping these points in view, the present investigation was taken up during 2004-05 at Department of Horticulture, University of Agricultural Sciences, Dharwad and the results are discussed here under.

5.1 EFFECT OF AGE OF ROOTSTOCK ON THE SUCCESS OF SOFTWOOD GRAFTING

This experiment was conducted to find out the optimum age of the root stocks to get the highest success rate of softwood grafting.

5.1.1 Graft union success

The rate of graft union success recorded for 30th, 45th, 60th and 90th DAG has revealed significant differences among the rootstocks of different age groups (Table 1 and Fig. 1). Maximum graft union success was reported in case of seven months old and minimum was noticed with ten months old root stocks. Swamy and Melanta (1994) opined that graft union success varies with age of the rootstocks in jack. Similar results were also obtained by Reddy and Melanta (1988) and Prasad et al. (1990) in mango. Further, Satish Kumar (2001) in tamarind had reported highest graft union success in eight and seven months old rootstocks under Dharwad conditions. He attributed that the effect may be due to the physiological maturity of rootstock which plays important role in the success and growth of grafts. On the contrary Muniswami (1979) and Jayaramagowda and Melanta (1989) in cashew, opined that younger rootstocks are better amenable than older for soft wood grafting.

Dambal (1999) in sapota reported the highest graft union success with eight months old rootstocks followed by nine months old rootstocks. Similar results were obtained by Sulikeri and Rao (1999) when grafting was done with eight to ten months old aged rootstock in mango.

The age of rootstock has relationship with regenerating ability of a plant part which is found to be higher in younger root stocks and this is because of higher activity of meristematic cells resulting in faster formation of callus and quick healing of graft union. In general the lower graft union success could be attributed to the lack of intimate contact of cambial region of both stock and scion and to interferance of exudation of latex (Hartmann and Kester, 1997).

The graft union success also depends on temperature and humidity to the greater extent. The temperature affects the graft union by influencing callus formation. In general callus formation is optimum at about 26-29°C (Bose et al., 1986). Gaonkar (1998) obtained highest graft union success with four months old rootstocks under mist conditions. The reason for lower success in four months and ten months old rootstocks could be attributed to physiologically over and under activity respectively. Hartmann et al. (1997) reported that when
rootstocks are physiologically more active or under active are likely to be unsuccessful in graft take.

5.1.2 Effect of age of rootstock on attributes of sprouts on grafts

The average number of leaves produced on grafts was significantly influenced by rootstocks of different ages at the 30th, 45th, 60th and 90th DAG. Similar results were obtained by Swamy (1993) in jack and its superiority for shoots at early stages of soft wood grafting in mango (Singh and Srivastava, 1980 and Reddy, 1986).

The vigour of grafts are expressed by their successful graft union and growth characters. In the present investigation, the highest number of leaves was noticed in case of seven months old rootstocks age group, but number of sprouts and sprout length was maximum on four months old rootstocks. The lower number of leaves, sprouts and least sprout length were observed in case of ten months old rootstocks. This may be due to temperature and humidity factors prevailing at the time of grafting.

Reddy (1986) reported maximum number of leaves on grafts prepared on nine months old rootstocks in mango. Yogananda (1989) reported maximum leaves on seven months old rootstocks following soft wood grafting in cashew. However, Patel and Amin (1981) stated that age of rootstocks did not influence vegetative growth of grafts and it may be the environmental conditions that influence the growth of grafts in mango.

5.2 EFFECT OF SOURCE OF EXPLANTS ON CULTURE ESTABLISHMENT

An explant constitute a living unit, independent of mother plant containing all its genetic information and presenting all the functions that the explant tissues or cells performed at their original location. The success concerning the preservation of cell totipotency and adoption of explants to aseptic conditions depends on the nature and origin of explants and on their physiological state following sterilization, explantation and inoculation. Murashige (1974) recognized several factors that could be considered in explant selection including organ that is to serve as tissue source, the physiological and ontogenic age of the organ and size of explant.

In the present study, various explants viz., shoot tips and nodal explants from both mature tree and axenic seedling were used and their effects to in vitro response was tested (Tables 5, 6 and 7 and Plates 2, 3, 4, 5 and 6). It was revealed that shoot tips from mature tree exhibited maximum survival percentage than others and also the regeneration capacity was better in case of shoot tips derived from mature trees as explants. This indicates that the aptitude of cells belonging to in vitro cultured explants to differentiate is very unevenly distributed within various species, organs and tissues. The regenerative aptitude differs from cell to cell, tissue to tissue, organ to organ, species to species and even within same species, cultivar to cultivar (Cachita-Cosma, 1991). Similar results were obtained in Hortensia, citrus sps., Annona cherimola (Lazar and Cochita, 1984; Duranvila et al., 1989 and Jordan et al., 1991). In woody plants, greater the distance between meristems and the roots, less water, nutrients and phytohormones reach the meristems. Consequently meristematic cells present a decreased regenerative ability (Cachita-Cosma, 1991).

5.3 EFFECT OF GROWTH REGULATORS ON SHOOT PROLIFERATION

The main purpose of multiplication stage is to maintain the microculture in a stabilized state and multiply the microshoots to large extent. The basic medium of shoot multiplication stage is similar to stage-1, but often the growth regulators and mineral supplement levels are varied. The growth regulators are used to support basic level of growth. It is also equally important to direct the developmental response of the propagule (Hartmann et al., 1997). In vitro culture of higher plants with growth regulators – auxins and cytokinins are very significant to get the large propagules.

Discovery of cytokinins has helped in vitro propagation of plants by shoot multiplication through regulating shoot growth (Skoog and Miller, 1957). Cytokinins are often used to stimulate growth and development. Kinetin, BAP and 2-ip are commonly used. In the
Plate 2: Axenic seedling

Plate 3: Axenic seedling taken out for preparation of explants.
Plate 4: Type of explant – Nodal and shoot tip from axenic seedling

Plate 5: Mature tree branch collected from Singapore jack tree.
present study, BAP and kinetin were used at different concentrations. Among the BAP at 2 mg/l has given better response with respect to shoot multiplication, length of shoot, mean number of adventitious buds produced and number of leaves per explant, followed by BAP at 1.5 mg/l (Table 7 and Plate 10). Similar results were obtained by Adiga (1996) in jackfruit. However, Singh and Tiwari (1998) in jackfruit obtained maximum establishment and number of shoots per explant, when BA 1.8 mg/l + IBA 0.2 mg/l combination was tried. But Roy et al. (1990) obtained higher establishment of explants and more number of shoots per explant in jack fruit when medium was supplemented with BAP 1 mg/l + kinetin 0.5 mg/l. Similarly Siddiqui and Farooq (1997) obtained maximum shoot proliferation in guava when medium was supplemented with lower concentrations of BAP (1 mg/l).

These results clearly reveal that the effect of cytokinins on tissue organ cultures can vary according to type of cytokinins used, type of culture, the variety of plant and source of explant. It was confirmed from present investigation that BAP is the best source of cytokinin for induction of multiple shoots.

This result is in conformity with the findings of previous workers in various species. The optimal level of BAP for shoot bud development from seedling explants of mangosteen (Garcinia mangostana) was 5.0 mg/l. However, higher concentrations were effective but shoot buds were clustered and stunted (Goh et al., 1995). Among cytokinins tested, BAP was more effective than kinetin with maximum shoot proliferation from nodal explants in jack fruit when used at 0.5 mg/l (Rahman and Blake, 1988). Similarly, Upadhyaya (1995) opined BAP as a best source of cytokinin for shoot proliferation in neem.

5.4 INFLUENCE OF GROWTH REGULATORS ON IN VITRO ROOTING

The function of root formation stage is to root the microshoots and to prepare them for transplanting out of the aseptic protected environment of test tube to out door conditions of greenhouse or transplant area (Brainerd et al., 1981). Media having a low concentration of salts have proven satisfactory for rooting of shoots. But in vitro rooting of herbaceous plants is much easier than woody perennials. Although the progress is considerable in relation to primary establishment and multiplication rates, rooting remains a major problem in woody perennials (Nemeth, 1986). Many workers have reported failure of micropropagated shoots to
Plate 7: *In vitro* callus production from nodal explants.

Plate 8: Phenolic exudation from explants.
Plate 9: Use of charcoal in the medium to overcome phenolic exudation.

Plate 10: Multiple shoot production on medium supplemented with BAP 2mg/l
root in various crops viz., *Persea americana* (Skene and Barlans, 1983), *Gravillea literana* (William et al., 1984), *Averrhoa carambola* (Litz and Griffis, 1989), Troyer citrange (Lukman et al., 1990) and curry leaf (Kalpana, 1999).

In the present investigation, best treatment for rooting was found to be half strength MS medium supplemented with IBA 8 mg/l (Plate 11). These results were in line with Adiga (1996) who obtained maximum per cent of rooting with 6 mg/l IBA, in jack fruit.

All other treatments failed to initiate rooting. The reason may be attributed to physiological status of explant taken for rooting which interacts with hormones and environmental factors (Nemeth, 1986). Lack of rooting morphogenesis may be due to lack of cell sensitivity to respond to morphogenesis even though auxin may or may not present in abundance (Hartmann et al., 1997).

**Protocol for in vitro multiplication of jack fruit**

Based on results, protocol for *in vitro* multiplication of jack fruit is given below.

1. Shoot tips from mature tree branches directly from field can be used as explants.
2. Explants from mature tree are to be treated with detergent teepol for 5 minutes followed by Bavistin for 90 minutes and again rinsed four to five times with water.
3. The explants are then surface sterilized with 0.1 per cent mercuric chloride for ten minutes inside the laminar air flow, followed by thorough washing of surface sterilized explants with sterile distilled water for 3 to 4 times.
4. Then explants are cultured on ½ MS medium containing charcoal (0.7%) + BAP (2 mg/l) for two weeks. The cultures are kept in culture room with 25 ± 2°C temperature and 16 hrs light and 8 hrs dark conditions.
5. Explants are to be sub cultured on media containing 2 mg/l BAP for better shoot proliferation.
6. After attaining 2-3 cm length, microshoots are transferred to ½ MS media containing 8 mg/l IBA for rooting.

**FUTURE LINE OF WORK**

1. Further investigations on the soft wood grafting under different agro-climatic conditions and varietal responses may be carried out.
2. Studies may be conducted on nutritional and hormonal influences on stock for increasing graft success and development.
3. Further investigations may be carried out to improve the *in vitro* rooting in jackfruit.
4. Comparative studies on performance of *in vitro* plants v/s. conventional grafted plants under field conditions may be tried.
VI. SUMMARY

The investigation entitled “Softwood grafting and in vitro propagation studies in jackfruit (Artocarpus heterophyllus Lam)” was conducted at the Golden Jubilee Block and Tissue Culture Laboratory of Department of Horticulture, University of Agricultural Sciences, Dharwad, during 2004-05. The experiments were carried out to find out appropriate age of root stock for soft wood grafting in order to achieve maximum graft success and to develop protocol for micropropagation of jack fruit.

A brief account of findings has been summarized below.

1. Root stock age was found to be significant with graft success at all the stages of observations. Among different aged rootstocks seven months old root stock has shown the highest graft success. At 90 days after grafting seven months old rootstocks showed 51.90 per cent of graft success. At 90 days after grafting, significantly more number of sprouts (2.33) and longer sprouts (4.32 cm) were observed in case of four months old root stocks.

2. Among different explant sources viz., shoot tip and nodal explants from both mature tree and axenic seedlings used, shoot tips from mature trees registered the quickest and better response with maximum survival percentage (50.40%).

3. In the study, on use of growth regulators (BAP and kinetin) at various concentration using shoot tips from mature trees as explants for shoot proliferation, BAP at 2 mg per litre has given the highest number of shoots, more number of leaves, highest number of adventitious buds and longer shoots. Lower concentrations of BAP and kinetin had given poor responses. BAP at 2 mg per litre has emerged as the best treatment for shoot proliferation than kinetin.

4. All concentrations of IBA and NAA used for in vitro rooting of microshoots failed to induce rooting except IBA at 8 mg per litre.

From the present study it is evident that seven months old rootstocks can be used for soft wood grafting to get maximum graft success. In case of micropropagation studies the shoot tips from mature tree was the best explant for in vitro propagation. Shoot tips responded better to BAP than kinetin but in vitro rooting of microshoots was problematic. Therefore, efforts are needed to overcome this problem.
VII. REFERENCES


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Appendix I: Monthly meteorological data for the experimental year (*Kharif*, 2004) and the mean of past 54 years (1950 – 2004) as recorded at the Meteorological Observatory, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (Karnataka)

<table>
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<th>Month</th>
<th>Rainfall (mm)</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
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<tbody>
<tr>
<td></td>
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<td>Mean* 2004</td>
<td>Mean* 2004</td>
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<tr>
<td></td>
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<td>Mean maximum</td>
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<tr>
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<tr>
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<td>September</td>
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<td>October</td>
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<tr>
<td>November</td>
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<td>30.2</td>
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<tr>
<td>December</td>
<td>0.0</td>
<td>54.5</td>
<td>29.4</td>
</tr>
<tr>
<td>Total</td>
<td>602</td>
<td>798.75</td>
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* Mean of 54 years (1950 – 2003)
A study on softwood grafting and *in vitro* propagation studies in jackfruit was conducted at the Golden Jubilee Block, Kumbapur Farm and Tissue Culture Laboratory of Department of Horticulture, University of Agricultural Sciences, Dharwad during 2004-05. The experiment on softwood grafting revealed that among different aged rootstocks, seven months old root stocks recorded the highest graft success (51.90%), and more number of leaves (52.), 90 days after grafting. Significantly more number of sprouts (2.33) and longer sprouts (4.32 cm) were recorded in case of four months old root stocks.

In case of *in vitro* propagation studies, shoot tips from mature tree registered the quickest and better response with maximum survival percentage (50.40%) among different explant sources used viz., shoot tip and nodal explants from mature tree and axenic seedlings. Mean number of shoots produced was non-significant among the treatments while mean length of shoot was highest (1.20 cm) in case of shoot tip explants from axenic seedlings and mean number of leaves (2.10) in shoot tips from mature tree.

Among different growth regulators (BAP and kinetin) at various concentrations for shoot proliferation BAP at 2 mg per litre has given the highest number of shoots (2.60), longer shoots (1.43 cm) and more number of leaves (2.18). Lower concentrations of BAP and kinetin gave poor response. All concentrations of IBA and NAA used for *in vitro* rooting of microshoots failed to induce rooting except IBA at 8 mg/litre.