PROPAGATION STUDIES IN AONLA (*Phyllanthus emblica* L.)

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By

GOVINDA NAYAKA D.

DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD - 580 005

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I. INTRODUCTION

Aonla (*Phyllanthus emblica* L.) belongs to the family Euphorbiceae is one of the important minor fruit crops of our country. In India, it is called by various names such as Aonla, Nelli, Amla, Amlika, Dhotri, Emblica and Usuri. In the recent past growing of superior varieties of aonla is highly remunerative.

Aonla is indigenous to tropical South-east Asia, particularly central and southern India it is under cultivation since ancient times (Firminger, 1947). Under natural habitat, it is found in dry deciduous forests of India. In India, its cultivation is very common in the eastern districts of Uttar Pradesh particularly Pratapgarh and Varanasi. It is also grown in states like Haryana, Himachal Pradesh, Maharashtra and some parts of Karnataka.

The genus *Phyllanthus* comprises of about 350 (Horker, 1973) or even 500 species (Bailey, 1971). It is a deciduous tree of small to medium stature. It has the unique phyllanthoid branching habit, which bears determinate and indeterminate shoots. The trees are quite hardy and can be grown even in sodic and saline soils upto 35 ESP and EC 9 dSm^{-1} respectively (Pathak and Pandey, 1985).

The fruit is highly nutritious and is the richest source of vitamin C (400-1300 mg/100 g) among the fruits next only to Barbados cherry. It is also the richest source of pectin which is mostly useful in making jam and jellies. Medicinally, it acts as coolant, refrigerant, diuretic and laxative. It is the basic constituent of Chyavanaprash and Amrit Kalash, the Ayurvedic medicinal preparations. It is also used in tannin and dyeing industries.

According to Bajpai (1969), aonla is presently grown in forests from self sown seeds or by sowing seeds of unknown parentage. Hence, they exhibit wide range of heterozygosity with respect to growth, yielding capacity, quantity, quality, size and shape of fruits *etc.*

Freshly harvested seeds of aonla do not germinate even if exposed to favourable conditions of germination owing to seed dormancy (Srimathi *et al*., 2000). Dormancy may be because of internal (physiological) factors affecting embryo or morphological factor such as hard, thick testa, or due to incorrect storage or handling (secondary dormancy). Such seeds may require special treatments like stratification, scarification, soaking in water, growth regulators *etc.* for overcoming dormancy.

The adoption of systematic vegetative propagation technique for mass production of plants is prerequisite in crops like aonla, as the vegetatively propagated fruit trees are true-to-type and they come to bearing early (Hartmann *et al*., 1993). Shield/patch budding was tried successfully in aonla. Though it gives fairly higher percentage of success, it is cumbersome, time and labour consuming process and can only be done on seedling rootstocks of age, more than one year. Softwood grafting is reported to be easy, convenient to practice, involves simple skills and is a quick method of grafting.

The season and age of rootstock however play important role in the success of grafting in aonla. Rainy season and well matured rootstock favoured by high atmospheric humidity along with fairly high temperature, is found congenial for rapid callus production that ensures formation of an early and strong union between the stock and the scion. In view of this identifying a suitable age and season for grafting is most essential for standardizing softwood grafting protocol in aonla.

Tissue culture is also a better alternative to the conventional method of vegetative propagation in aonla for mass multiplication of true-to-type plants. Though many herbaceous species have been propagated extensively through this technique, the tree species have received very little attention, may be because of their slow growth coupled with long dormancy and problem of phenolic exudation (Kopp and Nataraj, 1990).

Hence, the present investigation was undertaken to standardize, use of chemicals to break the seed dormancy, the age and season of softwood grafting and micropropagation protocol in aonla with following objectives.

1. To study the effect of different dormancy breaking treatments
2. To find out the optimum season for softwood grafting

3. To find out best age of rootstock for softwood grafting
4. To standardize the protocol for micropropagation

II. REVIEW OF LITERATURE

The area under aonla is increasing day by day due to its popularization as a medicinal plant and also its potential for better adaptation to diversified soil and climatic conditions. There is great demand for genuine true-to-type planting materials in order to optimize production of quality fruits. But poor germination, slow growth of rootstock seedlings, lack of information on season and age of rootstocks for grafting and micropropagation protocol has rendered the clonal multiplication process more difficult to produce large scale planting material to meet the growing demand.

The available information on various aspects of seed dormancy, softwood grafting and micropropagation in aonla is very meagre, the relevant literature on other fruit crops, grown under similar situations have been reviewed. For better understanding of the subject the information is organized under the following headings.

2.1 Different seed treatments on breaking dormancy

2.2 Propagation of fruit crops by softwood grafting

2.3 Age of rootstock for wedge grafting

2.4 Influence of season of wedge grafting on graft success

2.5 Micropropagation

2.1 ROLE OF DIFFERENT SEED TREATMENTS ON BREAKING DORMANCY

2.1.1 Soaking in water

Seeds of different species of Pinus, Picea, Larix, Cunninghamia, Ptatychladus, Hippophas and Vitex germinated better when they were previously soaked in water for 15 to 24 hours. Soaking for more than 24 hours was detrimental to the germination of all the species (Ma and Liu, 1986). According to Mioma (1986) however, seeds of teak soaked in running water for 96 hours before sowing in the nursery showed better germination.

2.1.2 Gibberellic acid treatment

The stimulatory effect of the applied gibberellins on the germination of seeds has been widely reported. According to Diaz and Martin (1971), gibberellins are known to stimulate germination of seeds where dormancy is imposed by a wide variety of mechanisms such as incomplete embryo development, mechanically resistant seed coats, presence of inhibitors and factors relating to physiological competence of the embryo axis *etc.*

Shanmugavelu (1970), in his studies on the effect of GA_3 on the tree seeds, observed that seed germination was higher in some of the leguminous tree species with better shoot growth. But the treatment failed to induce root growth. He further confirmed that GA_3 treatment was superior to other growth regulators in jute seeds with respect to seed germination and shoot growth. But it had no effect on root growth.

Treatment with gibberellic acid produced excellent germination in samples of *Impatiens balsamina*, several origin of *Lavendula unguistifolia,* samples of *Brassica rapa* and one of *Viola odorata* (Renard and Clerk, 1978). Nagao and Sakai (1979) opined that pre-soaking of *Alexandra* palm seeds in water for 24 or 72 hours advanced germination. Further advancement was observed when pre-soaked in GA_3 100 or 1000 ppm for 72 hours.

Bhujbal (1979) reported highest germination per cent (92.50%) with minimum period when dried stones of aonla were treated with 500 ppm GA_3.

Mukhopadyaya *et al*. (1990) found that in *Peltoforum ferrugenium* in general 12 or 14 hours of seed soaking in GA_3 and KNO_3 solution or 6 hours of soaking in thiourea solution significantly increased the seed germination percentage and was superior to other treatments. Similar results with GA_3 were reported in several species like aonla (Dhankar and Singh, 1996 and Wagh *et al*., 1998), sapota (Pampanna, 1992) and Khirnee (Tendulkar, 1978 and Hegde, 1991).

2.1.3 Acid scarification

Rai (1976) observed good germination in *Acarocarpus fraxinifolium* seeds treated with concentrated sulphuric acid (H_2SO_4) for 10 minutes. He further observed that the seeds of *Albizia chinensis* treated with concentrated H_2SO_4 for 10 minutes gave 39 per cent germination in 7 days as against 8.3 per cent in the control. He further opined that H_2SO_4 acts on the seed coat and weakens it. In this case, impermeable seed coat seemed to be the cause for low germination. Similarly *Albizia falcata* seeds treated with concentrated H_2SO_4 for 10 minutes, gave only 29 per cent germination.

Anithakumari and Kohli (1984), noticed that seeds of *Cassia occidentalis* have dormancy partially due to hard seed coat which is impermeable to water and gas exchange. The presence of waxy substance covering the seed coat is attributed as the reason of impermeability. They further noticed that, of the many treatments tried, scarification of seeds with sulphuric acid was the best treatment for breaking dormancy. The content of total protein was found to increase in the embryonic axis and cotyledon and decrease in extraembryonal parts during initial phase of germination. The content of RNA in the extra embryonal parts after hydration increased with the advancement of germination. Preforced RNA instead of newly transcribed RNA was seen to play a role in germination. Under the influence of harmones, proteins get mobilized to embryonal zone from extraembryonal part.

Randhawa *et al*. (1986) reported that dormancy in *Cassia fistula* L. was due to hard seed coat and the dormancy could be easily broken by treating the seeds with concentrated H_2SO_4 for 5 to 11 minutes, which increased the germination from 4 to 67 per cent. They further claimed that, percentage of abnormal seedlings was very high when seeds were pre-soaked in water for 24 hours before treating with concentrated sulphuric acid.

Increase in germination percentage and production of vigorous seedlings was observed in *Cassia fistula* L. seeds when scarified or treated with concentrated H_2SO_4 for 45 to 90 minutes. They observed only one per cent germination in control (Babeley and Kandya, 1988). Savithakandya (1990) reported that acid scarification for different periods was effective in breaking the seed coat imposed physical as well as mechanical dormancy from the seeds of *Acacia auriculiformis*, *Albizia procera*, *Cassia glauca*, *Cassia siamea* and *Peltoforum ferrugenium*. Weight analysis of the seed coat after pre-treatment indicated that there was a reduction of 3.30 to 8.85 per cent in the weight of seed coat of different species due to the acid scarification for a period ranging from 5 to 45 minutes. They further claimed that, such a reduction in the weight of seed coat was due to the digestion of several compounds of seed coat by concentrated H_2SO_4. As a result, only the seed coat slightly porus and weaker. Increased porosity of the testa makes it fully or partially permeable to water and gases. Thus physical dormancy is removed. Similarly, weakening of the testa due to the removal of some cell layer or impregnations of the seed coat results in the removal of mechanical dormancy.

2.2 PROPAGATION OF FRUIT CROPS BY SOFTWOOD GRAFTING

Being a highly cross pollinated crop, there exists a high degree of variation in aonla, in the performance of precocity in bearing, bearing season, size of the fruits and fruit quality parameters. In order to overcome the disadvantage of sexual propagation, multiplication of superior types in aonla has been suggested by adopting vegetative methods (Gangawar *et al*., 1975). The work done on clonal propagation of aonla and other crops through softwood grafting is reviewed here under.

Amin (1974) was the first Indian who coined the term “softwood grafting” in vegetative propagation of mango. He tried this technique and succeeded in establishing *in situ* mango orchards in the arid zone of Gujarat state. Studies on green wood grafting in apple revealed that covering grafted part with polythene paper resulted in good survival and further, vigorous growth was noticed when the scions were collected from the basal part of the shoot (Park *et al*., 1974).

Grafting procedure followed for softwood grafting in mango consists of grafting on *in situ* rootstocks or on rootstocks raised in polythene bags for a year or more and grafting on them with scions selected from developing terminal shoots. The scion should be of same

thickness as that of terminal portion of the decapitated stock. Excellent results were obtained when terminal growth of rootstock was bronze in colour (Amin, 1978a).

Amin (1978b) tried softwood grafting in other fruits like aonla (*Phyllanthus emblica* L.), guava (*Psidium guajava* L.), jackfruit (*Artocarpus heterophyllus* L.), phalsa (*Grewia asiatica*) and sapota (*Manilkara achras* Mill.) and obtained success of 73.3, 70.3, 33.3, 100.0 and 91.6 per cent respectively in the month of August at Anand.

Sixty six to hundred per cent graft-take was obtained when softwood scion of the pear were grafted on to softwood shoots of pear rootstocks raised from seedlings (Turovskaya, 1980). Gaur (1984) evaluated different methods of grafting and found that softwood grafting was the best method with highest success (75-80%) compared to inarching, veneer grafting and stone grafting in mango.

Vegetative propagation trials by softwood grafting carried out at Bubaneshwar and Bapatla showed that softwood grafting was one of the most promising methods that could be adopted for large scale multiplication of cashew (Rao, 1985).

Purushotham and Narasimharao (1990) in their studies on propagation of tamarind by veneer and softwood grafting methods reported higher graft success in softwood grafting (68%) compared to veneer (49%) at 120 days after grafting. They also recorded higher sprout number in softwood grafting (3.22) compared to veneer grafting (3.00). However, the average sprout length was higher in veneer grafting (16.69 cm) compared to softwood grafting (15.95cm).

Minijose and Valsalakumari (1991) working on standardization of grafting techniques in jack, reported that grafts kept under mist conditions showed better survival percentage than those kept in the open field.

2.3 EFFECT OF AGE OF ROOTSTOCK ON SUCCESS OF GRAFTING

Softwood grafting is generally done on the newly emerged or emerging bronze coloured terminal shoots. Naturally, the height of grafting increases with an increase in the age of rootstock which is not considered to be a good graft.

Ahmad (1964) reported that a month old seedling was better than 12 months old seedling in veneer grafting of mango. Araque (1968) tried rootstocks of different age for veneer grafting of cashewnut and recorded highest success (80%) on six month old seedlings in containers.

Jagirdar and Bhatti (1968) studied the effect of age of rootstock on success of veneer grafting in mango and reported that the age of rootstocks (3 to 9 months) did not affect the rate of success, but the per cent graft-take increased by use of mature scion wood compared to immature scion wood.

Parente and Maciel (1973) used three, six and nine months old seedlings of cashewnut and obtained best results with six months old rootstock recording on an average 25 per cent success over other aged rootstocks. Ferraz *et al*. (1974) obtained the best results with four months old seedlings and the worst with two month old seedlings in a comparative trial using two, four, six and eight months old seedlings in cashewnut.

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 (1979a) studied effect of age of rootstocks (6, 12, 18 and 24 months) on the success of veneer grafting in mango. They noted higher success (70%) on 12 months old rootstocks in nursery.

Nagabushanam and Rao (1978) obtained 35-96 per cent graft-take in cashew with six months old rootstocks compared to 20-49 per cent graft-take with 15-20 months old seedlings. Muniswami (1979) tried veneer grafting in cashewnut under mist chamber and recorded 90 per cent success on 4-5 month old seedlings. Harnekar (1980) reported 44 per cent success with the use of 4 and 8 week old seedlings for wedge grafting in cashewnut under Dapoli conditions.

Singh and Srivastava (1979b) in their studies on veneer grafting in mango obtained 80 per cent success with 12 months old rootstocks and 6 month old scions, when 6, 12, 18

and 24 months old rootstocks were used. Same workers during 1982 tried softwood grafting in mango on three different flushes from top *i.e.* top current flush, second flush from top and third flush from top with the object to minimize height of grafting. They reported the highest success (87%) in top flush in all the three grafting seasons followed by third flush (73.33%). Hence, it is clear from this, that grafting was possible on a healthy young seedling at a desired height with good success.

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

Reddy and Melanta (1988) tried 3, 4, 5 and 6 month old rootstocks for softwood grafting in mango. They recorded 68 per cent success with three months old rootstock raised in containers. Under *in situ* trial, the highest success (90%) was obtained with 7 months old rootstocks and the lowest (25%) with 3 months old rootstocks under Bangalore conditions.

Kulwal *et al*. (1988) recommended the use of about 12 to 15 months old rootstock 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 month old rootstocks in the month of August under Akola conditions.

Bajpai *et al*. (1989) in their studies on the effect of age of rootstock on veneer grafting of mango obtained higher per cent of sprouting, survival and more scion growth with lower leaves on two year old rootstocks than with one or three year old roostocks.

Jayaramagowda and Melanta (1989) tried rootstocks of different age (2, 3, 4, 5, 6, 7 and 8 months) for whip grafting in cashewnut and recorded the highest success (60%) on 4 month old seedlings under Bangalore conditions. Experiments conducted at various cashewnut research stations in India, suggested the use of younger seedlings for softwood grafting. At Vengurla, rootstocks of different age (1, 2, 3, 4, 5 and 6 months) were tried and the highest success (84.63%) was observed on three months old seedlings closely followed by two months old rootstocks (81.85%). Similarly at Jhargram, rootstocks of four different ages were tried and the highest percentage of success (59%) was noticed on two month old rootstock (Anon., 1989).

Waghmore (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 month old rootstocks closely followed by 12 month old rootstocks (56%).

Pujari and Magdum (1991) found that by use of 3 months old scion of 10 cm length was suitable for sapota softwood grafting on one year old khirni seedling they recorded the higher success of grafts (63.33%).

Singh and Suryanarayana (1996) worked on softwood grafting in mango. The highest rate of success (87%) was obtained 60 DAG when grafting was done on one month old rootstock.

Satisha *et al*. (1997) in their studies, 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 rootstocks followed by nine months old rootstock (75%). The minimum rate of success (13%) was recorded on 18 months old at 30 days after grafting under Bangalore condition.

Sathishkumar (2001) obtained highest graft success (51.96%) with 8 months old rootstocks followed by 7 months (45%) old rootstocks under Dharwad condition.

Shingre *et al* (2003) reported softwood grafting in cashew was more successful with rootstocks of age 45 days under Konkan conditions.

2.4 INFLUENCE OF GRAFTING SEASON ON GRAFT SUCCESS

The graft success largely depends upon the environmental conditions under which it is performed. However, the developmental anatomy of graft healing process reveals that the method of grafting followed is equally important in the entire process of grafting (Hartmnan *et*

al., 1997). One of the important conditions for success outdoor is selection of the correct season of grafting which is conducive for rapid graft healing process.

Amin (1978a) was pioneer worker who tried softwood grafting in cashew during 1975-76 with 71.4 per cent success during August under Anand conditions. He also tried softwood grafting in fruits like aonla (*Emblica officinalis* L.), guava (*Psidium guajava* L.), jack fruit (*Artocarpus heterophyllus* L.), phalsa (*Grewia asiatica* L.) and sapota (*Manilkara achras* Mill.) and obtained success of 73.3, 70.7, 33.3, 100.0 and 91.6 per cent, respectively in the month of August at Anand. Further he studied the softwood grafting in mango and found that the period from March to September was very much congenial in drier parts of Gujarat.

Kolekar (1979), while working on vegetative propagation of jackfruit recorded maximum success of 80 per cent during February through softwood grafting followed by October (60%). Harnekar (1980) studied softwood grafting in cashew and obtained 56 and 44 per cent success in May and June, respectively under Dapoli conditions. Further, he tried softwood grafting in jackfruit and obtained the highest success (41.66%) in May. According to him rainy season was not congenial.

Patel and Amin (1981) reported that *in situ* softwood grafting in mango could be done from February to September, recording more than 70 per cent success. Further, they could obtain more than 85 per cent success between April and August. The spring and summer grafts produced more vegetative growth. The period from October to January was not found favourable in the Western parts of India. Same authors in their further investigations to know the best period for softwood grafting in mango reported that period between May and August is best for softwood grafting with 95-100 per cent success from February to May, the success rate ranged from 77-97 per cent. After third week of September there was considerable reduction in success rate of graft union. They also reported that the growth of grafts were not influenced by age of rootstocks but influenced by season of grafting.

Singh and Srivastava (1982) studied the factors contributing to the success of *in situ* softwood grafting. Grafting success was the highest in the month of August (90%) compared to July (67%) and September (70%). Height at which the grafting was done on the young seedling had no effect on the final success. Softwood grafting gave better success compared to veneer grafting. Softwood grafting was the most successful method of grafting during June month where the success rate was as high as 100 per cent (Singh *et al*., 1984). Konhar and Das (1985) studied the success of 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 with maximum recorded during the month of January. The highest success of 90 and 100 per cent was recorded during the month of January and February, respectively. They also tried epicotyl grafting and found this method successful almost through out the year on 5 to 7 day old seedlings with 56 to 100 per cent graft-take, except March during which, scion sticks were not available.

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 75 per cent in the month of April. They also reported a success ranging between 72 and 80 per cent during the same period in epicotyl grafting. Softwood grafting in pecan nut (*Carya illinoensis*), a new introduction to India recorded a very high success of 90 per cent during August followed by 86.7 per cent during July (Mishra, 1985).

Panicker (1986) observed 54 to 82 per cent final survival in a trial carried out on mango softwood grafting for 12 months at Dapoli. The maximum success of 82 per cent was during July followed by May (72%) and November (70%). Sawke *et al*. (1986) reported that softwood grafting was most successful in cashew, throughout the year except December and January, which coincided with the cold period. The mean graft success of consecutive three years was between 73.11 and 83.66 per cent during February to November under warm climate of Konkan region of Maharastra. The lowest success 22.33 per cent was observed in the month of December.

According to Desai (1987), the softwood grafting in jackfruit recorded the highest success (69.33%) in April followed by May (56.0%) under Dapoli conditions. *In situ* softwood grafting in cashew recorded on an average 42.5 to 60.0 per cent success on 12 month old rootstock during March-May when both day and night temperatures were higher. The success rate of 60.00 per cent during April and 42.5 per cent during March (Kumar and Khan, 1988).

Kulwal and Tayde (1989) studied mango propagation by softwood grafting on one year old seedling. The studies revealed that the graft ranged from 70 to 90 per cent during August and varieties like pairy, kesar, pundur and panchadharkalasa showed cent per cent survival after 120 days. August and September months was more suitable for softwood grafting under Akola conditions.

Kumar *et al*. (1989) in their studies on softwood grafting of cashew at ARS, Ullal, reported success rate ranging from 39.5 to 86.0 per cent with maximum success during the month of May (86%) followed by April (77.5%). They also reported that this method can be done from March to May under humid and warm conditions of coastal Karnataka for higher percentage of success. Srivastava (1989) observed that humidity and temperature were the main limiting factors for the success of mango propagation by softwood grafting. Ninety five per cent success was recorded when grafting was done during the last week of June, during this month the mean temperature was 33.5°C and humidity was 82 per cent.

Kulkarni (1990) in his studies tried softwood grafting in custard apple and noted highest success (63.55%) in March on six month old rootstock. Swamy *et al*. (1990) in their correlation studies on success of softwood grafting of cashew 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 poor (10 to 12%), due to unfavourable weather conditions and non-availability of suitable scion sticks.

Softwood grafting of sapodilla cv. Kalipatti was performed on 15th day of every month from January to December, 1999. The highest graft-take (63.33%) was obtained for grafting done in May, which also resulted in the maximum growth of scion shoot (16.06 cm) and the highest number of leaves (15.05). In contrast, very low graft-take (0-6.67%) was reported in September-February grafting (Pampanna *et al*., 1994). *In situ* softwood grafting in cashew was most successful during July, August and September (71.66, 70.00 and 81.66%, respectively). These months coincided with the rainy season. Grafting was least successful (26.66%) during November (Kadam *et al*., 1995).

Mango cv. Amrapalli 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).

Shinde *et al*. (1996) in their studies on softwood grafting in tamarind reported higher graft success (70.00%) in April (before flowering) followed by March (58.40%), when elite type tamarind cv. Sel-263 was grafted on to one year old root stock seedlings of a local type of tamarind. They also reported the failure of grafts made during January-February or June-December under Aurangabad conditions.

Singh and Suryanarayana (1996) carried out softwood grafting during June-October using one and two month old local mango seedlings as rootstocks cv. Banganapalli as scion. They reported highest rate of graft success (87%), tallest grafted plants (17.1 cm) and highest number of leaves (7.0) after 60 days on one month old roostocks in August. Studies on the influence of polyembryonic rootstocks on the success and survival of softwood grafts in mango revealed that graft success depends on the speed of graft union formation. Grafting success was highest (96.67%) in Movandon and Chandrakaran grafted with cv. Neelum during June and survival was highest (76.67%) in Puliyan grafted with Banganapalli during August.

Bharad *et al*. (1999) in their studies on seasonal variation in success of softwood grafting of tamarind reported significant influence of season on days taken to sprout, percentage of bud break, percentage of bud survival and per cent age of graft success. They reported March and April were the better months for grafting under Akola 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 rootstock for softwood grafting of sapota. The maximum success was ascribed to the prevailing favourable conditions like higher maximum temperature (36.6°C), the higher minimum temperature (20.9°C) and optimum humidity (65%) resulting in increased cell activity and better union of the stock and scion.

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

2.5 MICROPROPAGATION

The major branch of biotechnology which has become commercially viable is micropropagation. Micropropagation has been defined as '*in vitro*' regeneration of plants from organs, tissues, cells or protoplasts (Beversdorf, 1990) and as 'the true to type propagation of a selected genotypes using *in vitro* culture techniques' (Debergh and Read, 1991). True-to-type propagation has advantages for heterozygous fruit crops such as aonla, pomegranate, papaya *etc.*

The concept of totipotency is the basis for micropropagation. When Haberlandt attempted the first cell culture studies, his intention was to develop a versatile tool to explore morphogenesis and to demonstrate totipotency of plant cells (Haberlandt, 1902). The first report of viable callus culture was reported by Gautheret (1939) and White (1939) in tobacco and carrot, respectively.

Micropropagation has been successfully employed for rapid production of uniform and superior quality planting material. Rapid and large scale clonal propagation of many fruit crops is now possible tissue culture. The regeneration can take place as extension or proliferation of shoot tip or axillary buds, direct production of organs from explant *i.e.* organogenesis or through the production of somatic embryos *i.e.* embryogenesis. An *in vitro* propagation involving an unorganized callus phase may produce variant plants *i.e.* somaclonal variants, clonal *in vitro* propagation is achieved by using meristems and axillary buds (Kumar and Kumar, 1998).

In tissue culture studies, one has to make use of plant growth regulators in addition to the supply of other salts, mineral, vitamins and sugars to obtain the desirable response to achieve the set of objectives.

Auxins are a class of plant growth regulators which cause cell elongation, apical dominance and root initiation. The most frequently used auxins are 2, 4-D, NAA, IAA and IBA of which IAA occurs naturally in plants. NAA and 2, 4-D are the most effective auxins to initiate callus. Cytokinins are phytohormones which promote cell division, proliferation of tissues. The most widely used cytokinins are kinetin, BAP and 2-IP, auxins or cytokinins alone cannot show organogenesis through cell division or callus *in vitro*. The relative concentration of auxin and cytokinin decides the plant morphogenesis *in vitro* (Skoog and Miller, 1957).

Since many fruit crops are sensitive to photoperiod requirement these are cultured *in vitro* under day night regime and a light cycle of 16 hrs followed 8 hrs of darkness is the most preferred one (Conger, 1987). The optimum growth of tissue cultures has been observed at a temperature regime 24-26°C (Pierik, 1987).

Establishment of culture

Explant

Pierik (1987) reported that genotype, plant age, physiological state (vegetative or regenerative), stage and age of explants, general health of plant, position of explant within plant, size of the explant, method of inoculation *etc.* affects the growth of explant *in vitro*. While selecting a suitable explant for micropropagation, these factors should be taken into consideration.

Muralikrishna (1988) reported highest percentage of shoot establishment in explants of 10 mm size followed by 7.5 mm. As the size of explant increased the shoot establishment percentage of also increased upto 10 mm (56.3%) and later decreases as size increased. He also stated that the rapid adventitious shoot initiation was found only in axillary bud (9 shoots/culture) than shoot tip explants. The later were able to produce fewer (2.17) shoots per culture in pomegranate.

In Jackfruit, Rajmohan and Mohankumaran (1988) studied the influence of explant source on the *in vitro* propagation. They reported maximum response from the seedling explants (17.4 shoots) followed by the fresh stem sprouts from five year old trees (4.50 shoots). A drastic reduction was observed from explants excised from ten to thirty year old trees (2.80 and 2.09 shoots, respectively). Maximum number (15-20) of shoots were induced

in shoot tip closely followed by cotyledonary node (10-15), nodal section (8-10) and hypocotyls (6-8) of ber (Mathur *et al*., 1993).

Nodal segment explants of 1-2 cm length when cultured from mid portion of the indeterminate shoots of mature tree of aonla cv. NA-7 in the month of April-July to August-November gave better bud induction (Mishra *et al*., 1999).

Singh *et al*. (2002) studied the *in vitro* propagation of grapes. They obtained better culture establishment with nodal explants than the shoot tips.

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).

Micropropagation studies of explants from mature trees

Aonla

Verma and Kant (1996) cultured nodal segment explants of aonla on modified MS medium supplemented with BA (3-5 mg/l) and NAA (0.5 mg/l) during February-April and August-October. The bud break observed in only 8-10 per cent of explants. Leaching of phenolic compounds resulted in the loss of almost 90 per cent in cultures. The regenerated shoots were elongated on hormone free medium and subsequently rooted on half strength MS medium supplemented with IBA (3.0 mg/l) and sucrose (1.5%).

In aonla cv. NA-7, Mishra *et al*. (1998) reported heavy inborn contamination and phenol exudation were the major problem in establishing the *in vitro* cultures of aonla. Dipping of explants in bavistin (1%) and cholramphenicol (0.1%) for 60-90 minutes followed by treatment with $HgCl_2$ (0.1%) for 8 minutes significantly reduced the explant contamination. The application of paraffin wax on the cut end of explants reduced browning by 20 per cent and increased the survival rate to 80 per cent as compared to non-waxed explants with or without antioxidant. Non-waxed explants treated with PVP showed the 50.30 per cent survival and browning to an extent of 47.73 per cent.

In aonla, Kant *et al*. (1999) reported 3-4 multiple shoots from nodal explants from mature tree on MS medium supplemented with BAP (5 mg/l) and IAA (0.5 mg/l) along with antioxidants. These shoots were elongated on hormone free MS medium. Rooting of the shoots was achieved on half strength MS medium fortified with IBA (2 mg/l) and sucrose (1.5%).

Micropropagation studies in aonla were carried out from nodal explants by Mishra *et al*. (1999) to develop the protocol for mass multiplication of true-to-type plants. The better shoot proliferation (33-37.5%) on modified MS medium supplemented with kin 0.4 mg/l + GA 1.0 mg/l than in WPM. Higher GA_3 concentration (3 mg/l) caused complete defoliation and dropping of determinate shoots. Regenerated shoots from 3 week old cultures in MS media supplemented with growth regulators and antioxidants failed to produce roots.

Mishra and Pathak (2001) studied the effect of nodal position and seasonal influence on *in vitro* shoot proliferation of aonla. The highest bud induction rate (76.40%) and longest indeterminate shoot (0.53 cm) was obtained with explants excised from the 10^{th} to the 15^{th} node during August-November in MS medium supplemented with kin (0.4 mg/l) and GA_3 (0.4 mg/l).

Other fruit crops

Amin and Jaiswal (1987) developed a rapid clonal propagation through *in vitro* shoot proliferation from nodal explant of guava. The best response was reported on MS medium supplemented with 4.5 μM BA alone. The response of *in vitro* shoot nodal segments was better in comparison to the field grown trees. Maximum response with minimum contamination and browning of explant and medium was obtained during April-June.

Muralikrishna (1988) reported the strategies of micropropagation of pomegranate cv. Jyoti. To control browning in culture medium he claimed that the subsequent transfer of explants on fresh medium as well as soaking of explants in 0.1 per cent ascorbic acid for 1-2 hours in the solution. In micropropagation studies, the axillary buds and shoot tip explants

were regenerated on basal MS medium supplemented with BAP 0.5 µM. Growth of axillary bud was slower than shoot tips, but axillary bud produce about 9.5 buds per explant as compared to 2.33 buds per explant by shoot tip. Rooting occurred when shootlets were soaked in IBA 100 mg/l solution for one hour before subculture.

In jackfruit, Rajmohan and Mohankumaran (1988) reported that problem of polyphenol interference was minimized by incorporation of activated charcoal (1%) and GA_3 1 mg/l in the establishment medium, insoluble PVP in the proliferation medium and by frequent subculturing. They recorded cent per cent survival and production of healthy, growing cultures of shoot apices in MS medium supplemented with GA_3 1 mg/l along with AC (1%).

In woody plants, no tissue lacks phenolic compounds including growing cell. Oxidation of phenolic compounds released from the cut ends of explants by phenol oxidase, peroxidase cause lethal browning of plant and culture medium. This problem can be overcome by sealing the cut ends of explants with liquid paraffin wax (Bhat and Chandel, 1991).

Shoot organogenesis in cv. Nana, a dwarf variety pomegranate was studied by Zhang and Stoltz (1991). Each culture produced a mean of 5.2 shoots with BA 1 µm+ NAA 2 µM while 6.6 shoots produced with BA 2 µM + NAA 1 µM. Study indicated that auxin (NAA) at 1 µM was optimum for *in vitro* shoot formation and that BA levels more than 2 µM inhibits shoot formation.

Amin and Jaiswal (1993) described a method of shoot tip culture for rapid multiplication of mature jackfruit trees. BA and kinetin (4.5 to 9 µM) either alone or in combination, supported shoot proliferation. The ideal season for collection of apical buds was during November to January. Shoots rooted with 60 per cent to 80 per cent success using half strength MS salts and 10 µM IBA or NAA.

In *Zizypus mauritiana*, Mathur *et al*. (1995) reported that stem explants from mature tree when grown on modified MS medium with 3800 mg/l potassium nitrate, 2475 mg/l ammonium nitrate, 11 µM BA produced 15-20 shoots per inoculation. Rooting was induced by pretreatment with 50 µM IBA or NAA for 24 hours followed by transfer to auxin free whites medium.

Roy and Roy (1996) reported that maximum shoots proliferation from shoot tips and nodal segments was induced in jackfruit on MS medium containing BA 2.5 mg/l and NAA 0.5 mg/l, with an average of 10.3 shoot/culture after 28 days. Rooting of excised shoots was achieved on half strength MS medium containing NAA and IBA each at 1.0 mg/l. Seventy five per cent of the plantlets survived after transplanting into the open field.

Roy *et al*. (1996) reported that multiple shoots were obtained from nodal explants of *S. cumini* on MS medium supplemented with kinetin 2.5 mg/l. Repeated subculture resulted in rapid shoot multiplication. Addition of 100 mg/l urea to media increased number of shoots rooted in half strength MS media containing 0.5 mg/l each of IBA, NAA and IAA. After transplanting in field, 75 per cent plantlets were survived with uniform growth.

In tamarind cv. Urigam, Balakrishnamurthy and Ganga (1997) reported the seasonal influence of explant collection on induction of multiple shoots from axillary buds. The monthwise collection and culture of axillary buds in MS medium fortified with six per cent sucrose, BA 0.5 mg/l and GA_3 0.5 mg/l indicated that best results with respect to per cent bud break (82.5%), number of days taken for bud break (9.33 days), per cent response to multiple shoot induction (22.50%), number of shoots per culture (2.17) and length of micro shoots (3.21 cm) were recorded by the axillary buds collected and cultured during the month of May. The axillary buds collected and cultured during the months December, January, February, March, September and October did not record any response.

Kantharajah *et al*. (1998) observed high frequency adventitious bud formation in pomegranate, when nodal cuttings of cv. Wonderful were cultured on half MS medium supplemented with BAP 0.5 mg/l + NAA 0.1 mg/l. NAA was most effective than BAP in root elongation and the highest shoot length occurred on media containing NAA 0.1 mg/l. Roots were induced after 12 days on WPM with NAA 2 mg/l.

Mehta *et al*. (2000) developed a protocol for *in vitro* regeneration of plants via adventitious bud formation from mature embryo axis of tamarind. Induction of adventitious shoot buds was achieved in the cut surface of the axis of tamarind. Induction of adventitious shoot bud was achieved in the cut surface of the axis when cultured on medium containing zeatin 0.91 µM, calcium panthothenats 0.41 µm and biotin 0.40 µM supported the differentiation of buds to form elongated shoots. Rooting occurred in half strength MS medium with 2 per cent sucrose following a 72 hrs treatment with auxin mixture in dark.

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/l compared to kinetin.

Pattepur (2003) did 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. Auxillary 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 was noticed.

III. MATERIAL AND METHODS

The germination, softwood grafting and micropropagation studies in aonla (*Phyllanthus emblica* L.) were conducted at the Department of Horticulture, College of Agriculture, Dharwad during the year 2005-06.

Dharwad is situated in the 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 total rainfall of the area is 1006.3 mm, which is fairly well distributed from April to October. The average maximum temperature goes upto 36.3°C in the month of April and the minimum temperature reached is 13°C in the month of December-January. The relative humidity fluctuates between 53 and 83 per cent. The meteorological data as recorded at the Meteorological Observatory, Main Agricultural Research Station, Dharwad for the years 2005-06 and average of last 54 years are presented in Appendix I. The details of experimental material and methodology followed are presented below.

3.1 EXPERIMENT I: EFFECT OF DIFFERENT TREATMENTS ON BREAKING SEED DORMANCY

3.1.1 Seed and its source

The required seed material was collected from the Forest Department, Government of Karnataka, Dharwad.

3.1.2 Treatments

Soaking of seeds in

T_1 – Tap water for 24 hours

T_2 – Cow urine for 24 hours

T_3 – Thiourea (2%) for 24 hours

T_4 – GA_3 500 ppm for 12 hours

T_5 – GA_3 500 ppm for 24 hours

T_6 – Stratification at 5°C for 10 days

T_7 – Acid scarification for 30 seconds

T_8 – Control

3.2 TREATMENT DETAILS

a. Water soaking: The aonla seeds were soaked in tap water for 24 hours under ambient temperature.

b. Soaking seeds in cow urine: The fresh urine from cow was collected from the dairy and the seeds were soaked in it for 24 hours.

c. Soaking of seeds in thiourea (2%): Thiourea of 2 g was initially dissolved in a little quantity of distilled water and then the volume was made upto 100 ml. Seeds of aonla were then soaked in the 2 per cent thiourea solution for 24 hours.

d. Soaking seeds in GA_3 solutions: 500 ppm of gibberellic acid was prepared by dissolving 50 mg of GA_3 in little quantity of ethyl alcohol. Then the volume was made upto 100 ml with distilled water to get 500 ppm solution. Seeds of aonla were soaked in such solution for 12 hours and 24 hours as per the treatments.

e. Startification: Seeds of aonla were soaked in distilled water and the seeds that settled at the bottom were taken and others were discarded. These seeds were kept in between two layers of germination paper and then it was rolled. These papers were kept on moist sand filled tray under refrigerated condition (5°C) for 10 days.

f. Acid scarification: The seeds were treated with concentrated sulphuric acid for 30 seconds. Then the seeds were taken out and thoroughly washed with running tap water followed by shade drying for two hours. Then they were used for germination.

g. Control: Seeds were sown without any pre-treatment.

Treatments – Eight

Replication – Three

Design – Completely Randomized Design

Method – Rolled towel test

3.3 OBSERVATIONS

The details of observations recorded in the laboratory on seed quality attributes are presented below.

3.3.1 Per cent germination

Germination test was conducted in three replications of 100 seeds each by adopting rolled towel method as described in ISTA Rules (Anon., 1999). The temperature of 25°C±1°C and relative humidity of 95 per cent was maintained during the germination test. The germination counts were made on the 20th days after sowing and germination was expressed in percentage.

3.3.2 Number of days taken for 50 per cent germination

The number of days taken from sowing to the time when 50 per cent of the seeds germinated was marked and noted.

3.3.3 Shoot length

From the germination test, 10 normal seedlings in each replication were sampled at random on the 20th day for measuring shoot length. The shoot length was measured from the collar region to tip of the shoot and mean shoot length was expressed in centimeter.

3.3.4 Root length (cm)

Ten normal seedlings that were adopted for recording shoot length were also used to measure root length. Root length was measured from the collar region to the tip of the longest root in centimeter.

3.3.5 Vigour index

The vigour index was calculated by adopting the method suggested by ISTA (Anon., 1985) as detailed below.

Vigour index = Germination percentage x Mean length of seedlings (cm)

3.4 EXPERIMENT II: EFFECT OF SEASON AND AGE OF GRAFTING ON SUCCESS OF SOFTWOOD GRAFTING

3.4.1 General conditions of propagation structures

Mist house

A mist house of dimension 18 x 16 cm was used for the purpose of study. The frequency of misting was 6-7 minutes for 30 seconds. The relative humidity ranged between 85 to 95 per cent and the temperature between 35 to 40°C.

Shade net house

A shade house of dimension 18 x 24 meter with a height of 2.4 meter was used for the hardening of grafted plants. It was of HDPE green shade net and allowing only 25 per cent sunlight. So, relatively low temperature with high humidity was maintained in comparison with the outside environment.

3.4.2 Preparatory operations

Collection of seeds

Seeds of aonla were collected from state Forest Department, Dharwad, Government of Karnataka. Medium sized, good shaped and heavy seeds were soaked in water. Floating seeds were discarded and only sunken seeds were taken as seed material. The seeds were spread over the ground under shade for surface drying. After drying, the seeds were treated with gibberellic acid (500 ppm) for 24 hours and used for raising rootstocks.

3.4.3 Raising of rootstocks in polythene bags

Polythene bags of 400 gauge thickness and of size 20 x 10 cm were used for raising rootstocks. Potting mixture containing red earth, farmyard manure (FYM) and coirdust in 1:1:1 (v/v) proportion was used. The sowing was carried out every month starting from June upto March and polythene bags holding the seeds were maintained in the nursery with all necessary care so as to obtain rootstocks of varying ages.

3.4.4 Maintenance of rootstocks

The polythene bags were initially kept under mist house for better germination and after germination, the bags were transformed under shade net house for subsequent growth of seedlings. General prophyllactic plant protection measures were taken by spraying with fungicides and pesticides to control the pest and disease. Weeding was done as and when required.

3.5 EXPERIMENT IIa: EFFECT OF SEASON ON SUCCEES SOFTWOOD GRAFTING

The experiment was conducted on the rootstocks of same age maintained in the polythene bags. Softwood grafting was taken up during 2nd fortnight of each month starting from November to June of following year.

3.5.1 Treatments

Treatment	Season
T_1	November
T_2	December
T_3	January
T_4	February
T_5	March
T_6	April
T_7	May
T_8	June

Design : Completely randomized design

Replication : Three

Treatments : Eight

Method of grafting: Softwood grafting

3.5.2 Collection of scion

The scions were collected from the healthy, elite trees in the morning hours on the day of grafting and defoliated with secateur. The scions so prepared were used for softwood grafting on the same day.

3.5.3 Method of grafting

Softwood grafting method (wedge method) was followed in the study.

For this, the top growth of root stock was decapitated with sharp knife or secateur. Then longitudinal cut of 5 cm length was given from the tip downwards. A scion shoot of about 8 to 10 cm length was matching thickness was selected.

The basal end of the scion was given two gentle sloping cuts of about 5 cm on opposite sides by removing the bark to expose the cambium. The wedge shaped scion thus prepared was inserted into the 'V' shaped slit of the stock and secured firmly with white transparent polythene strip of 1.5 cm width and 30 to 45 cm length of 150 guage thickness, to facilitate cambiums of stions the stock and scion in firm contact. The stions were than covered with small transparent tubular polythene bag with perforation using pin to prevent entry of water into the grafted portion and also to avoid desiccation of the scion by creating humidity around the graft union.

The grafted plants were transferred immediately to the mist house and maintained these for 30 days after which they were shifted to shade net house.

3.6 OBSERVATIONS RECORDED

3.6.1 Per cent grafting success

Success of graft was recorded on 45th days after grafting (DAG). The number of successful grafts was counted and expressed as per cent.

3.6.2 Number of leaves

Mean number of leaves on scion at 30th, 45th day after grafting were recorded on all successful grafts.

3.6.3 Length of shoot

Mean length of shoot was recorded at 30th, 45th day after grafting on all the successful grafts and mean was calculated.

3.7 EXPERIMENT IIb: EFFECT OF AGE OF ROOTSTOCK ON SUCCESS OF SOFTWOOD GRAFTING

This experiment was conducted on the rootstocks of different ages maintained in the polythene bags. The softwood grafting was done at one time during third fortnight of April with precurred scion.

3.7.1 Treatments

The treatments included eight age group seedling as follows.

Treatment	Age of rootstock
T_1	3 months old
T_2	4 months old
T_3	5 months old
T_4	6 months old
T_5	7 months old
T_6	8 months old
T_7	9 months old
T_8	10 months old

Design : Completely randomized design
Replication : Three
Method of grafting : Softwood grafting
Treatments : Eight

3.7.2 Maintenance and selection of rootstocks

For this experiment the seeds were sown in polybags during the first week of every month from June to March, so that seedlings of different age groups were available at a given time for softwood grafting *i.e.* during April. Vigorously grown uniform rootstocks seedlings were selected for grafting.

3.7.3 Collection and preparation of scion

Collection was done as described in Experiment II.

3.7.4 Method of softwood grafting

Softwood grafting was done as described in Experiment II.

3.7.5 Observations recorded

1. Percentage of graft success at 30, 45 days after grafting. Number of successful grafts were counted and expressed as per cent.
2. Length of shoots per graft at 30, 45 days after grafting mean length of each shoot was measured in centimeter.
3. Number of leaves per graft at 30, 45 days after grafting. Mean number of leaves per graft were counted.

3.8 MICROPROPAGATION

The study on *in vitro* propagation was conducted in the plant tissue culture laboratory of the Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad.

3.8.1 Source of plant material

All required explants were collected from healthy trees maintained in garden of Horticulture Department, Dharwad. Nodal segments of young sprouts of the tree were used as explants for initial establishment of cultures.

3.8.2 Preparation of explant

After taking the explants of optimum size from plant source, they were washed with detergent (teepol) solution and with 0.1 per cent bavistin for 4-5 minutes followed by 3-4 washings with sterile distilled water. All the operations were carried out under aseptic conditions using laminar air flow cabinet. The explants were surface sterilized with 0.1 per cent $HgCl_2$ solution for 5 minutes and then rinsed with sterile double distilled water 3-4 times so as to remove all The traces of $HgCl_2$.

3.8.3 Preparation of stocks

Murashige and Skoog medium (1962) was commonly used for all the experiments. Stock solution (8x) were prepared with double distilled water, poured into well stoppered bottle and were stored in refrigerator. Details of stock solutions are given below.

3.8.3.1 Mineral and vitamin stock

Stock A:	Macro elements	– 1000 ml (10 x)
	NH_4NO_3	– 13.2 g
	KNO_3	– 15.2 g
	$MgSO_4.7H_2O$	– 2.96 g
	KH_2PO_4	– 1.36 g
Stock B:	Microelements	– 1000 ml (8 x)
	$CaCl_2 . 6H_2O$	– 0.2 g
	$CuSO_4 . 5H_2O$	– 0.2 g

$MnSO_4 . 4H_2O$ – 178.4 g

KI – 6.6 mg

$NaMoO_4 . 2H_2O$ – 2.0 mg

$ZnSO_4 . 7H_2O$ – 86.4 mg

H_3BO_3 – 49.6 mg

Stock C: Calcium chloride stock – 1000 ml (10x)

$CaCl_2 . 2 H_2O$ – 3.53 g

Stock D: Iron stock – 1000 ml (8x)

$FeSO_4. 7H_2O$ – 279 mg

Na_2 EDTA.$2H_2O$ – 373 mg

3.8.3.2 Preparation of iron stock

Na_2 EDTA (373 mg) in double distilled water was boiled to which $FeSO_4 . 7H_2O$ at 279 mg in double distilled was added gently. Volume was made upto 1000 ml.

Stock E : Vitamin stock – 100 ml (50x)

Thiamin HCl – 0.5 mg

Pyridoxine HCl – 2.5 mg

Nicotonic acid – 2.5 mg

Glycine – 10 mg

Mesoinositol – 500 mg

3.8.3.3 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 were prepared using analytical graded chemicals by dissolving 10 mg of respective growth regulators in small quantity of ethanol. The volume was made upto 100 ml with distilled water to obtain 100 ppm stock.

3.8.3.5 Preparation and sterilization of media

Specified quantities of stock solutions of macro, micronutrients, organic constituents and growth regulators were pipetted out in a beaker. Sucrose and vitamin stock were added and dissolved. Then volume was made up by adding double distilled water. The pH was adjusted to 5.8 by addition of 0.1 N HCl or NaOH as required. After that agar at the rate of 0.6 per cent and activated charcoal at 0.7 per cent were added and heated till the agar melted properly. The media was then procured into the test tubes and plugged with cotton plugs. The test tubes with media were autoclaved at 121°C temperature at a pressure of 1.2 kg per cm^2 for 20 minutes, allowed to cool to room temperature and stored in culture rooms until further use.

3.8.4 Culture establishment

3.8.4.1 Inoculation

The aseptically excised and surface sterilized explants were finally placed on media under sterile laminar air flow hood. The culture test tubes containing nutrient medium prepared as per various treatments were decapitated by holding them over spirit lamp and inoculations were performed by placing explants with the help of sterilized forceps and the cotton plugs were replaced tightly. During inoculation, the explants were properly positioned on the media and were gently pressed with forceps to secure their firm contact with the media. The placement of nodal segment in the media was such that the axillary bud was placed above the media and not in contact with the media.

3.8.4.2 Sub culture

After about 4-5 weeks of shoot proliferation, the test tubes were taken out, the nodes and shoot 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.

3.8.4.3 Culture condition

The culture room was maintained at 25±2℃ with uniform illumination provided by using florescent tubes (1500 lux) over a light/dark cycles of 16/8 hours.

3.8.4.4 Transfer area and aseptic manipulations

All the aseptic manipulations such as surface disinfection of explants, preparation and inoculations 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 on to the clean laminar air flow chamber. The UV light was switched on for half an hour to achieve aseptic environment inside the cabinet where all the manipulations were conducted.

3.8.4.5 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.

3.8.4.6 Hardening of *in vitro* plantlets

Young rooted plantlets were takenout 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 plants were watered twice a day initially, then once a day after eight to ten days. They were transferred to shade net house after 15 days (70-80% RH) for further acclimatization.

3.8.5 Experimental details

EXPERIMENT IIIa: EFFECT OF GA_3 AND KINETIN ON SHOOT PROLIFERATION

Treatment details

Tr. No.	Treatments
T_1	MS with Kinetin 0.4 + GA_3 0.0 mg/l
T_2	MS with Kinetin 0.4 + GA_3 0.5 mg/l
T_3	MS with Kinetin 0.4 + GA_3 1.0 mg/l
T_4	MS with Kinetin 0.4 + GA_3 2.0 mg/l
T_5	MS with Kinetin 0.5 + GA_3 0.5 mg/l
T_6	MS with Kinetin 1.0 + GA_3 0.5 mg/l
T_7	MS with Kinetin 2.0 + GA_3 0.5 mg/l

Explants – Nodal explants

Design – Completely Randomized Design

Replication – Three

Number of culture tubes – 12 per treatment

Observations recorded

a. Time taken for initiation of sprouting: The mean number of days taken to show initial sprout from date of inoculation of various explants was recorded.

b. Mean number of shoots/explant: Mean number of shoots produced was recorded.

EXPERIMENT IIIb: EFFECT OF AUXIN CONCENTRATION ON ROOT INITIATION

Treatment details

Tr. No.	Treatments
T_1	MS + NAA 1.0 mg/l
T_2	MS + NAA 1.5 mg/l
T_3	MS + NAA 2.0 mg/l
T_4	MS + NAA 2.5 mg/l
T_5	MS + IBA 1.0 mg/l
T_6	MS + IBA 1.5 mg/l
T_7	MS + IBA 2.0 mg/l
T_8	MS + IBA 2.5 mg/l
T_9	MS + NAA 2.0 mg/l + IBA 2.0 mg/l

Explants – Microcuttings of 3-4 cm length

Design – Completely Randomized Design

Replication – Three

Number of test tubes – 12 per treatment

Observations recorded

a. Per cent root initiation: The number of shoots that responded to the media for root initiation were counted in each treatment and the proportion of this to that of the total number of shoots inoculated was calculated and presented as response of root differential in percentage.

b. Root length: The root length was measured in centimeters from base to the tip of the root.

c. Number of roots: The plantlets were removed from the culture medium and the mean number of roots produced per shoot was recorded.

3.9 STATISTICAL ANALYSIS

The experiment for all aspects of standardization of softwood grafting, micropropagation and breaking dormancy in aonla were laid out in Completely Randomized Design (CRD) by following the procedure outlined by Panse and Sukhatme (1967). The data in percentages were transformed to arcsine values for statistical analysis (Gomez and Gomez, 1984) and subjected to ANOVA with critical difference values tabulated at one per cent probability for laboratory experiments and at 5 per cent probability for softwood grafting studies, probability wherever ‘F’ test was significant.

IV. EXPERIMENTAL RESULTS

The results obtained from the studies on (i) effect of dormancy breaking treatments in aonla, (ii) effect of season of softwood grafting and age of rootstocks on success of softwood grafting and (ii) micropropagation in *Phyllanthus emblica* L. are presented in this chapter.

4.1 EFFECT OF DIFFERENT TREATMENTS ON BREAKING DORMANCY IN AONLA

In this experiment, freshly extracted seeds of aonla were subjected to different dormancy breaking treatments and their response was studied.

4.1.1 Days taken for 50 per cent germination

The number of days taken for 50 per cent germination as influenced by different seed treatments varied significantly among the treatments (Table 1). The seeds subjected to acid scarification for 30 seconds recorded minimum number of days (12.27) for 50 per cent germination. This was followed by seeds treated with 500 ppm of GA_3 for 24 hours recording 12.69 days and GA_3 500 ppm for 12 hours recording 13.36 days. However, these were statistically at par. The seeds with stratification treatment at 5°C for 10 days was the last to attain 50 per cent germination.

4.1.2 Per cent germination

There was significant difference in per cent germination due to different treatments used to break dormancy (Table 1). The maximum (80.39%) germination recorded in acid scarification for 30 seconds was significantly superior to other treatments and the least was recorded in (32.33%) control.

4.1.3 Seedling length

The seedling length differed significantly due to different dormancy breaking treatments (Table 2). The seedling length was higher in seeds treated with GA_3 500 ppm for 24 hours (15.23 cm) followed by acid scarification for 30 seconds (15.22 cm). However, these were statistically at par. The lower seedling length was observed in seeds treated with stratification at 5°C for 10 days (9.60 cm).

4.1.4 Root length

The data on root length as influenced by different dormancy breaking seed treatments are presented in Table 2. The highest root length (7.68 cm) was recorded in GA_3 500 ppm for 24 hours followed by GA_3 500 ppm for 12 hours (7.13 cm). However, they were at par with each other. The least (5.23 cm) root length was observed in both untreated and stratified seeds of aonla.

4.1.5 Vigour index

The data presented on vigour index in Table 3, revealed a similar pattern as that of per cent germination. The seedlings from the acid scarified seeds for 30 seconds registered maximum vigour index of 1223. This was followed by seeds treated with GA_3 500 ppm for 24 hours (1035). The lowest seedling vigour (326) was observed in seeds stratified at 5°C for 10 days.

4.2 EXPERIMENT IIa: EFFECT OF SEASON ON SUCCESS OF SOFTWOOD GRAFTING

This experiment was carried out to findout the best season for softwood grafting in aonla. The grafting operation was carried out from November 2005 of June 2006 using similar aged stocks.

4.2.1 Percentage of graft success

The data recorded at 45 DAG clearly revealed the significant influence of season of grafting on percentage success (Table 4).

Table 1. Effect of different seed treatments on days taken for 50 per cent germination and per cent germination in *Phyllanthus emblica* L.

Tr. No.	Treatments	Per cent germination	Days taken for 50 per cent germination
T_1	Water soaking for 24 hours	55.20 (48.01)	15.22
T_2	Soaking seeds in cow urine for 24 hours	60.34 (50.99)	14.76
T_3	Soaking seeds in thiourea (2%) for 24 hours	67.47 (55.26)	14.35
T_4	Gibberellic acid 500 ppm for 12 hours	68.00 (55.58)	13.36
T_5	Gibberellic acid 500 ppm for 24 hours	72.46 (58.38)	12.69
T_6	Stratification at 5°C for 10 days	34.01 (35.69)	16.70
T_7	Acid scarification for 30 seconds	80.39 (63.75)	12.27
T_8	Control	32.33 (34.67)	16.68
	S.Em±	0.19	0.37
	CD at 1%	1.54	0.78

Values in the parenthesis indicate arcsine transformed value

Table 2. Effect of different seed treatments on root length and seedling length in aonla

Tr. No.	Treatments	Root length (cm)	Seedling length (cm)
T_1	Water soaking for 24 hours	6.89	13.77
T_2	Soaking seeds in cow urine for 24 hours	6.83	11.27
T_3	Soaking seeds in thiourea (2%) for 24 hours	6.77	11.01
T_4	Gibberellic acid 500 ppm for 12 hours	7.13	13.56
T_5	Gibberellic acid 500 ppm for 24 hours	7.68	15.23
T_6	Stratification at 5°C for 10 days	5.23	9.60
T_7	Acid scarification for 30 seconds	7.07	15.22
T_8	Control	5.23	12.94
	S.Em±	0.20	0.32
	CD at 1%	0.81	1.33

Table 3. Influence of different seed treatments on vigour index in aonla

Tr. No.	Treatments	Vigour index
T_1	Water soaking for 24 hours	760
T_2	Soaking seeds in cow urine for 24 hours	679
T_3	Soaking seeds in thiourea (2%) for 24 hours	743
T_4	Gibberellic acid 500 ppm for 12 hours	982
T_5	Gibberellic acid 500 ppm for 24 hours	1035
T_6	Stratification at 5℃ for 10 days	326
T_7	Acid scarification for 30 seconds	1223
T_8	Control	418
	S.Em±	19.11
	CD at 1%	78.95

Table 4. Effect of season on success of grafting in aonla

Tr. No.	Month of grafting	Per cent graft-take
T_1	November 2005	87.50 (69.33)
T_2	December 2005	66.69 (54.78)
T_3	January 2006	50.04 (45.05)
T_4	February 2006	31.25 (34.00)
T_5	March 2006	43.43 (41.25)
T_6	April 2006	31.27 (34.01)
T_7	May 2006	25.10 (30.08)
T_8	June 2006	16.52 (23.99)
	S.Em±	0.18
	CD at 5%	0.55

Values in the parenthesis indicate arcsine transformed value

Table 5. Effect of season of grafting on average number of leaves and shoot length in aonla

Tr. No.	Month of grafting	Mean number of leaves		Average shoot length (cm)	
		30 DAG	45 DAG	30 DAG	45 DAG
T_1	November	8.7	13.00	2.43	2.62
T_2	December	7.7	11.53	1.93	2.17
T_3	January	7.2	10.87	1.83	2.07
T_4	February	6.3	9.47	1.93	2.13
T_5	March	5.4	6.20	2.78	2.83
T_6	April	5.2	5.97	2.99	3.13
T_7	May	3.8	5.73	2.71	2.84
T_8	June	3.2	5.13	1.68	1.83
	S.Em±	0.09	0.14	0.09	0.21
	CD at 5%	0.27	0.41	0.26	0.62

DAG – Days after grafting

The percentage graft success recorded after 45 DAG varied significantly from 16.52 to 87.50 under different months. The highest rate of success (87.50%) was recorded in the grafts prepared in the month of November followed by December (66.69%). The lowest rate of success (16.52%) was recorded in the grafts made during June.

4.2.2 Mean number of leaves

It is evident from the data presented in Table 5 that the mean number of leaves was significantly influenced by the season of grafting at all the stages of observation. The mean number of leaves observed at 30 DAG varied from 3.2 to 8.7. The highest number of leaves (8.7) were recorded in the grafts prepared during November, followed by December (7.77) and January (7.2). However, the November grafting was significantly superior to other months of grafting.

After 45 days, maximum number of leaves (13.00) was recorded in the grafts prepared during November while lower mean number of leaves was observed in the grafts prepared in all other months of grafting at 45 DAG. November grafted plants continued to produce the highest number of leaves after 45 days, while the lowest number of leaves (5.13) was recorded in June month of grafting.

4.2.3 Average shoot length

Perusal of the data in Table 5 revealed that there was a significant influence of time of grafting on shoot length. It varied from 1.68 cm to 2.99 cm. Longest shoots were observed in the grafts prepared during April (2.99 cm) followed by March (2.78 cm). However, they were statistically at par with each other.

After 45 days of grafting, similar trend was observed with the highest length of shoot (3.13 cm) being recorded in the grafts prepared in April while lowest length of shoot (1.83 cm) was recorded in the grafts prepared in June.

4.3 EXPERIMENT IIb: EFFECT OF AGE OF ROOTSTOCK ON SUCCESS OF SOFTWOOD GRAFTING IN AONLA

This experiment was carried out to find out the suitable age of rootstocks for undertaking softwood grafting in aonla. The per cent graft success and subsequent growth observations are presented in Table 6 and 7.

4.3.1 Percentage of graft success

The percentage of graft success was considerably influenced due to age of rootstock. On perusal of Table 6, it is evident that the success ranged between 26.77 to 62.88 per cent. Highest percentage (62.85%) of graft success was recorded with 8 month old rootstock which was significantly superior to the rest of treatments. The lowest success (26.77%) was observed on 9 month old rootstocks.

4.3.2 Mean number of leaves

It is evident from the data presented in Table 7 that the mean number of leaves produced in grafts on different age rootstocks differed significantly. Maximum number of leaves (9.73) was recorded on 4 months old rootstocks followed by 10 months old (7.33) and 9 months old rootstocks (727) at 30 DAG, which were at par with each other. Minimum number of leaves (6.47) was recorded on 7 months old rootstocks. Maximum number of leaves (10.02) were continued to be maintained in 4 months old rootstock at 45 DAG also, while the minimum number of leaves (7.87) were recorded in 9 months old rootstock.

4.3.3 Average shoot length

Shoot length recorded at 30 and 45 days after grafting is presented in Table 7.

The average shoot length recorded at 30^{th} and 45^{th} day was found significantly differing with respect to different age of rootstock. After 30 days the highest shoot length (2.92 cm) was recorded in 4 months old rootstock which was significantly higher than all other treatments. The lowest shoot length (1.52 cm) was recorded in 10 month old rootstock. Four months old rootstock continued to maintain longer shoot at 45 DAG, while 10 months old rootstock continued to record the lowest shoot length of 1.72 cm.

Table 6. Effect of age of rootstock on success of grafting in aonla

Tr. No.	Age of root stocks	Per cent graft take
T_1	3 months	34.62 (36.08)
T_2	4 months	56.25 (48.62)
T_3	5 months	45.00 (42.15)
T_4	6 months	44.62 (41.93)
T_5	7 months	38.92 (38.62)
T_6	8 months	62.88 (52.49)
T_7	9 months	26.77 (31.18)
T_8	10 months	42.88 (40.93)
	S.Em±	0.19
	CD at 5%	0.56

Values in the parenthesis indicate arcsine transformed value

Table 7. Effect of age of rootstock on average number of leaves and shoot length in aonla

Tr. No.	Age	Mean number of leaves		Average shoot length (cm)	
		30 DAG	45 DAG	30 DAG	45 DAG
T_1	3 months	7.07	8.87	1.74	2.47
T_2	4 months	9.73	10.02	2.92	3.31
T_3	5 months	6.60	9.60	2.31	2.64
T_4	6 months	6.67	8.60	2.52	2.88
T_5	7 months	6.47	9.37	2.25	2.43
T_6	8 months	6.60	8.47	2.41	2.57
T_7	9 months	7.27	7.87	1.63	1.88
T_8	10 months	7.33	9.00	1.52	1.72
	S.Em±	0.12	0.30	0.07	0.06
	CD at 5%	0.35	0.89	0.20	0.18

DAG – Days after grafting

4.4 MICROPROPAGATION

4.4.1 Experiment IIIa: effect of kinetin and ga_3 on shoot proliferation in aonla

The studies on micropropagation in aonla was taken up by culturing nodal segments from mature tree at bud break stage. The results on various observations recorded are presented.

4.4.1 Days taken for bud initiation

The effect of MS media and growth regulators in combination on the bud initiation and number of shoots per explant from the nodal segment as explants are given in Table 8.

The mean number of days taken for sprouting of buds ranged from 7.07 to 10.67 days. The earliest (7.07 days) bud initiation observed was on MS medium with kinetin 2.00 mg per litre + GA_3 0.5 mg per litre. The MS medium with kinetin 0.4 mg per litre + GA_3 1.0 mg per litre were significantly recorded maximum number of days taken for bud initiation (10.67 days).

4.4.2 Mean number of shoots per explant

There were significant differences among the treatments with respect to number of shoots per explant (Table 8). Highest number of shoots (3.28) was observed on MS medium containing kinetin 2.0 mg + GA_3 0.5 mg per litre followed by kinetin 1.0 mg + GA_3 0.5 mg per litre (3.28). However, these treatments were statistically at par with each other. The MS medium with kinetin 0.4 mg per litre + GA_3 1.0 mg per litre produced lowest number of shoots which were recorded 1.81 mean number of shoots per explants.

4.4.3 Experiment IIIb: effect of auxin concentration on root initiation

The data obtained with regard to the effect of regulators NAA, IBA and their combination on rooting are presented in Table 9.

There was no response of rooting at various concentration of NAA, IBA and their combination. But callus growth was observed on medium containing combination of NAA and IBA.

Table 8. Effect of different nutrient media on the bud initiation and number of shoots

Tr. No.	Treatments	Days taken for bud initiation	Average number of shoots per explant
T_1	MS with kinetin 0.4 + GA_3 0.0 mg/l	0.00 (1.00)	0.00 (1.00)
T_2	MS with kinetin 0.4 + GA_3 0.5 mg/l	0.00 (1.00)	0.00 (1.00)
T_3	MS with kinetin 0.4 + GA_3 1.0 mg/l	10.67 (3.42)	1.81 (1.67)
T_4	MS with kinetin 0.4 + GA_3 2.0 mg/l	10.27 (3.36)	2.56 (1.89)
T_5	MS with kinetin 0.5 + GA_3 0.5 mg/l	9.20 (3.19)	2.70 (1.92)
T_6	MS with kinetin 1.0 + GA_3 0.5 mg/l	8.87 (3.14)	3.21 (2.05)
T_7	MS with kinetin 2.0 + GA_3 0.5 mg/l	7.07 (2.84)	3.28 (2.07)
	S.Em±	0.02	0.03
	CD at 1%	0.08	0.13

Values in the parenthesis indicate square root transformed value

Table 9. Effect of auxin concentration on root initiation in aonla

Tr. No.	Treatments	Response
T_1	MS + NAA 1.0 mg/l	—
T_2	MS + NAA 1.5 mg/l	—
T_3	MS + NAA 2.0 mg/l	—
T_4	MS + NAA 2.5 mg/l	—
T_5	MS + IBA 1.0 mg/l	—
T_6	MS + IBA 1.5 mg/l	—
T_7	MS + IBA 2.0 mg/l	—
T_8	MS + IBA 2.5 mg/l	—
T_9	MS + NAA 2.0 mg/l + IBA 2.0 mg/l	Callus at cut end

V. DISCUSSION

Aonla (*Phyllanthus emblica* L.) is one of the most important arid zone and wasteland fruit crops of commercial importance. The pressure on increasing the economic outcome from marginal lands have further strengthened the claim of this species (*Phyllanthus emblica* L.) as the most suitable crop component for sustainable and economical utilization of marginal and wasteland ecosystems. It requires least inputs, management and is relatively free from pests and diseases. The expansion in acreage of this species through natural spread is mainly by seeds. Further popularization of establishment of clonal orchards and spread of aonla is limited as the seeds possess dormancy and pose problems in germination. Though limited efforts on vegetative propagation have been carried out, non-availability of genuine true-to-type planting materials is still a bottleneck in popularization of this arid zone fruit crop. In this regard, studies were carried out on some methods of propagation of aonla, the results of which are discussed in this chapter.

5.1 EFFECT OF DIFFERENT TREATMENTS ON BREAKING DORMANCY

The spread in area of *Phyllanthus emblica* L. has been mainly through seeds, as sexual propagation is traditionally followed for large-scale multiplication of planting material. Grafting being an asexual method of large scale selected clonal multiplication. The essential component for this endeavour is the requirement of large scale healthy rootstocks. In this context, with conflicting reviews available on seed germination behaviour, the present experiment was implemented. The results highlighted that the seeds of aonla possesses a dormancy because of hard seed coat and presence of germination inhibitors. Hence, the present investigation to ascertain suitable dormancy breaking seed treatments in *Phyllanthus emblica* L. was undertaken. Seeds treated with concentrated sulphuric acid for 30 seconds gave highest germination (80.39%), took minimum number of days for 50 per cent germination (12.27 days) and had very high seedling vigour index (1223), as compared to rest of the treatments, indicating that treating aonla seeds in concentrated sulphuric acid for 30 seconds improves germination. Acid scarification brings about the softening of hard seed coat by dissolution of deposited lipids, pectic substances and high density waxes, which are responsible for hard seededness (Denny, 1917) and make it permeable to water. These observations are in line with the findings of Singhrot and Makhija (1979) in ber, Tadaria and Negi (1992) in *Cassia javanica*.

Similar results of increased germination per cent (72%) of *Cassia fistula* seeds soaked in concentrated sulphuric acid for 9 minutes as compared to control (4.00%) was observed by Randhawa *et al*. (1986).

The treatment with gibberellic acid at 500 ppm for 24 hours of soaking proved to be the second best alternative to acid scarification treatment. The exogenous application of GA antagonizes the ill effect of inhibitors (Brain and Hemming, 1958: Wareing *et al*., 1968) and increases endogenous gibberellin like substances (Mathur *et al*., 1971). GA helps in the synthesis of enzymes and one of them is α-amylase which converts the starch into simple sugars during the process of germination. These sugars provide energy that is required for various metabolic and physiological process associated with germination. Other enzymes activated by GA include those which weaken the seed coat and allow the axis to burst through. GA also enhances cell elongation, so the radicle can push through the endosperm and seed coat that restrict its growth (Hartman and Kester, 1979).

In similar way, Gholap *et al*. (2000) observed better germination and seedling growth with GA_3 200 ppm in aonla. Similar results were also obtained by Dhankhar and Singh (1996) in aonla and Shanmugavelu (1970) in some of leguminous plant species.

5.2 EFFECT OF SEASON ON THE SUCCESS SOFTWOOD GRAFTING

Season of grafting plays an important role in softwood grafting success. If the season is not conducive, the favourable effects of other factors are likely to be nullified, resulting in lower rate of success. The success of grafting is dependent upon the weather conditions and thus vary from region to region within a season. The seasonal influence could be ascribed to

the influence of prevailing temperature and humidity. The present studies revealed that the grafting success ranged from 16.52 to 87.50 per cent in June to November (Plate 2), respectively. The maximum success of grafting was obtained in grafts prepared during November followed by December. The lowest graft success was recorded in the grafts prepared during June. It may be due to the weather condition that remained moist causing lower cambial activity. However, Keskar *et al*. (1991) obtained maximum success in budding of aonla through patch budding (91.6%) in mid July followed by shield budding (81.6%). But, total failure of softwood grafting performed during June to August months was also observed.

Sandhu (1992) in their studies on standardization of grafting technique in sapota reported better graft success during September-November. Further he opined that the period from September to November seems to be ideal for grafting of sapota, when the temperature (22-25°C) and humidity (65%) were found to be optimum for maximum graft success. Similarly, Hegde (1988) tried modified epicotyl grafting in cashew and noted 50 to 75 per cent success during June to November.

Hartmann *et al*. (1997) opined that the environmental conditions during and following grafting under mist, can be readily controlled thereby, permitting greater reliability of grafting over a long period of time, when compared to out door grafting operation.

Significant differences among the treatments were observed for the number of leaves and shoot length of grafts. The differences may be due to the active growth period of mother trees with higher level of nutrients in scion shoots. Better growth of grafts during November can also be correlated to higher cell activity and early sprouting which are responsible for higher number of leaves and shoot length, this might be because of synthesize more food material and photo synthates might have increased the height of scion shoot. Poor growth and success of grafts during June may be attributed to the reduced rate of division of cambial cells, their differentiation and consequent development in healing of stock scion union. This inturn may be due to decreased synthesis of endogenous auxin and mobilization of reserved food material caused by reduced activity of hydrolyzing enzymes.

For commercial graft production, November to December was found to be ideal as the success was above 60 per cent during this season. During the other months, success rate was poor due to unfavourable weather conditions and non-availability of suitable scion sticks.

LEGEND

Plate 1.

A - 3 months old

B - 4 months old

C - 5 months old

D - 6 months old

E - 7 months old

F - 8 months old

G - 9 months old

H - 10 months old

Plate 2.

A - November

B - December

C - January

D - February

E - March

F - April

G - May

H - June

Plate 1. Influence of age of rootstock on the growth of softwood grafts in aonla

Plate 1. Influence of age of rootstock on the growth of softwood grafts in aonla

Plate 2. Influence of season of grafting on the growth of softwood grafts in aonla

Plate 2. Influence of season of grafting on the growth of softwood grafts in aonla

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

This experiment was conducted to findout the optimum age of the rootstock to get maximum success in softwood grafting.

The study revealed that success of grafting significantly differed among rootstocks of different age. Maximum graft success was reported in case of eight months old rootstocks and minimum was noticed with ten month old rootstocks (Plate 1). Jayaramagowda and Melanta (1989) opined that the young rootstocks give more success in softwood grafting than older ones. Similar results were also obtained by several workers in different crops (Parente and Maciel, 1973; Muniswami, 1979; Reddy and Melanta, 1988). Further, Satishkumar (2001) in tamarind reported highest graft 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 an important role in the success and growth of grafts as reported by many other workers in different crops.

Hartmann and Kester (1979) opined that, the age of rootstock has relationship with regenerating ability of a plant part which is found to be higher in younger rootstocks and this is because of higher activity of meristematic cells resulting in faster formation of callus and quick healing of graft union.

It was evident from the present investigation that the rootstocks with appropriate vigour and physiological maturity recorded maximum success in grafting, which was noticed in comparatively younger rootstocks like 8, 5 and 4 months old than the 10 months old rootstocks.

Dambal (1999) working on sapota at UAS, Dharwad reported the highest graft success with eight months old rootstocks followed by nine months old rootstocks. Further in Tamarind, Satish *et al*. (1997) reported lowest graft success in root stocks aged 9 month and more. Similar results were obtained by Reddy and Melanta (1988) in mango, Gowda and Gowda (1989) in Champaka.

The present studies also revealed that the average number of leaves and shoot length were significantly influenced by rootstocks of different age. The average number of leaves produced on grafts were higher in 4 month old rootstock which is significantly superior over other ages of rootstocks. Similar results were obtained by Singh and Srivastava (1980), in softwood grafting recording better results in success as well as extension of growth of shoots at early stage in mango.

It was evident from the present investigation that the shoot length was also maximum in 4 months old rootstock followed by 5 and 8 months old stocks. The lower number of leaves and shoot length were observed in case of 10 months old rootstocks. Better growth of grafts in the present study with young rootstock may be attributed to the higher meristematic activity and juvenility of younger scion, which inturn helped for early sprout initiation. Perhaps early sprouting followed by optimum temperature and humidity might be responsible for production of more number of leaves and higher shoot length. This may be due to synthesis of more photosynthates. Patel and Amin (1981) opined that age of rootstocks did not influence vegetative growth of grafts and it may be the environmental condition that influence the growth of grafts.

Plate 3. Nodal segments used as explants for micropropagation in aonla

Plate 3. Nodal segments used as explants for micropropagation in aonla

Plate 4. Nodal segment showing growth of axillary bud on MS medium containing 2 mg kinetin + 0.5 mg/l GA_3 + citric acid +ascorbic acid

Plate 4. Nodal segment showing growth of axillary bud on MS medium containing 2 mg kinetin + 0.5 mg/l GA3+citric acid + ascorbic acid

5.4 EFFECT OF GA_3 AND KINETIN ON SHOOT PROLIFERATION

Although there is voluminous work on standardization of vegetative propagation in aonla, the fact that still remains is perpetual scarcity of quality planting material. Application of *in vitro* multiplication in aonla on the analogy of other fruit crops has been long desired and extensive attempts have been made to develop reproducibly workable protocols for micropropagation of this arid fruit crop as it is evident from the reports.

The available reports on *in vitro* clonal propagation of aonla are scanty. Regeneration of plantlets from cultured endosperm through callus cultures have been reported (Sehgal and Khurana, 1985). Similarly *in vitro* induction of multiple shoots from nodal explants have been reported by Verma and Kant (1996), Kant *et al*. (1999), Mishra *et al*. (1999) and Mishra and Pathak (2001).

In this study, it was observed that the combination of kinetin and GA_3 which has positive response in shoot proliferation from explants taken from mature trees of aonla. The highest mean shoot per explant (3.28) and less number of days taken for bud initiation (7.07 days) were recorded in medium containing kinetin 2.0 mg/l + GA_3 0.5 mg/l (Plate 3 and 4). In the present investigation, differential response of GA_3 to shoot growth from nodal explants have been observed. In order to increase the shoot length, kinetin was supplemented with GA_3, as GA_3 is known to be involved in the stem elongation. These results are in agreement with results obtained by Mishra *et al*. (1999) who obtained better shoot proliferation (33-37.5%) on modified MS medium supplemented with kinetin 0.4 mg/l + GA_3 1.0 mg/l from nodal explants of aonla. Further, they reported that higher GA_3 concentration (3 mg/l) caused complete defoliation and dropping of determinate shoots.

Kant *et al*. (1999) obtained 3-4 multiple shoots from nodal explants in aonla, when MS media was supplemented with BAP (5 mg/l) and IAA (0.5 mg/l) along with antioxidants. Similarly Pattepur (2003) obtained higher per cent bud break and response to multiple shoot induction in tamarind when medium was supplemented with BAP 2.0 mg/l + coconut water (CW) 10 per cent.

From the present investigations it was clear that among the different treatments used, kinetin 2.0 mg/l + GA_3 0.5 mg/l was the best combination for shoot proliferation (Plate 5) of nodal explants derived from mature trees of aonla.

5.5 EFFECT OF AUXIN CONCENTRATION ON ROOT INITIATION

The function of rooting stage is to make the microshoot to root and to prepare them for transplanting out of the aseptic protected environment of the test tube to the outdoor conditions of the green house or transplanted area (Brainerd *et al*., 1981). *In vitro* rooting of herbaceous cutting is much easier than woody perennials. Although the progress is considerable in relation to primary establishment, multiplication rates, rooting remains a major problem in woody perennials (Nemeth, 1986).

In the present investigation, the shoots inoculated on any of the rooting media tried could not produce roots and longer incubation of these shoots in the media caused complete leaf fall. Callusing near cut end was observed with IAA and NAA. Slight swelling at bottom of shoot followed by splitting of outer tissue was observed with combination of IAA and NAA (Plate 6). Mishra *et al*. (1999) observed that, regenerated shoots from three weeks old cultures in MS media supplemented with growth regulators and antioxidants failed to produce roots. Many workers have reported failure of micropropagated shoots in various crops *viz*., *Persea indica* (Nel and Kotze, 1982). *Troyer citrange* (Lukman *et al*., 1990), curry leaf (Kalpana *et al*., 1999).

The reason may be attributed to genetic makeup, physiological state of mother plant and the explant taken for rooting which interacts with harmones and environmental conditions (Nemeth, 1986). Also, may be lack of rooting morphogenesis or lack of cell sensitivity to respond to morphogenesis even though natural auxin may or may not be present in abundance. External application of auxin gives little or no rooting response (Hartmann *et al*., 1997).

Plate 5. Nodal segment showing growth of axillary bud on MS medium containing 2 mg kinetin + 0.5 mg/l GA_3 + 0.25 % activated charcoal

Plate 5. Nodal segment showing growth of axillary bud on MS medium containing 2 mg kinetin + 0.5 mg/l GA3 + 0.25 % activated charcoal

Plate 6. Micro shoot showing callus induction on MS medium with IAA 2.0+ IBA 2.0 mg/l

Plate 6. Micro shoot showing callus induction on MS medium with IAA 2.0 + IBA 2.0 mg/l

FUTURE LINE OF WORK

1. Investigations on physiological and biochemical aspects of seed dormancy may be carried out.
2. Further investigation should be carried out to evaluate the different methods of propagation under different agro-climatic conditions.
3. Investigation should be carried on interaction of age of rootstocks and season of softwood grafting.
4. Nutritional and hormonal influences can be studied for increasing graft success and development.
5. Studies may be conducted on *ex vitro* rooting and survival of hardened plants.
6. The performance of *in vitro* plants v/s conventional grafted plants may be tested.

VI. SUMMARY

The present investigation entitled “Propagation studies in aonla (*Phyllanthus emblica* L.) was conducted at Department of Horticulture and Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Dharwad during 2005-06. The experiments were carried out to findout suitable seed treatments to break the seed dormancy, identify ideal age of rootstock and best season for softwood grafting to obtain maximum graft success and development. The micropropagation technique was also attempted.

A brief account of findings have been summarized below :

1. Scarification of seeds with concentrated sulphuric acid for 30 seconds significantly increased germination per cent to 80.39, taking least number of days for 50 per cent germination (12.27) and highest vigour index (1223.20) as compared to the rest of treatments. GA_3 at 500 ppm for 24 hours resulted in vigorous seedling growth.
2. Season of grafting had profound influence on graft success. Maximum success per cent of (87.50%) was recorded in November grafted plants followed by December (66.69%). The least success of 16.52 per cent was recorded in June. The higher number of leaves and shoot length were also found in the grafts prepared during November and April.
3. Influence of rootstock age was found to be significant for success of grafts. The higher per cent of successful grafts (62.88) was observed in 8 month old root stocks followed by 4 month (56.25%) and 5 month (45.00%) old rootstocks. Significantly more number of leaves (10.02) and shoot length (3.31 cm) were observed in case of four month old root stocks.
4. In the studies on micropropagation, nodal segments from mature tree as explants were used with use of growth regulators (Kinetin and GA_3) at various concentrations for shoot proliferation, MS medium supplemented with kinetin at 2 mg/l with GA_3 0.5 mg/l resulted in more number of shoots (3.28) and less number of days taken for bud initiation (7.07). Lower concentration of kinetin and higher GA_3 concentration gave poor response.
5. All concentrations of IBA and NAA used individually for *in vitro* rooting of microshoots failed to induce rooting. However, combinations of IBA (2.0 mg/l) and NAA (2.0 mg/l) has resulted in callus induction at cut end of *in vitro* shoots.

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APPENDIX I

Monthly meteorological data for the experimental year (2005-06) and the mean of past 54 years (1950-2004) of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Month	Rainfall (mm)		Temperature (°C)				Relative humidity (%)	
			Mean maximum		Mean minimum			
	2005-06	1950-2004	2005-06	1950-2004	2005-06	1950-2004	2005-06	1950-2004
March	0	0.14	36.0	36.49	18.9	19.59	42	56
April	75	48.88	36.3	37.38	21.3	19.83	53	76
May	29.4	80.45	37.0	33.66	21.5	21.40	55	66
June	151	109.86	30.9	28.84	21.4	21.50	76	81
July	290.2	148.33	27.4	29.17	21.5	21.01	83	87
August	138.8	96.09	27.1	27.00	20.4	20.30	81	86
September	194.5	102.21	27.5	28.58	20.3	19.91	85	82
October	89.4 (9)	130.15	29.6	30.09	19.1	18.41	70	76
November	38.0 (1)	32.11	29.4	30.19	14.9	15.88	51	68
December	0	53.51	28.9	29.39	13.1	12.51	53	63
January	0	0.08	29.9	29.61	12.9	14.67	52	63
February	0	1.14	33.4	32.52	14.8	16.37	62	51
Total	1006.3	802.52						

Note: Figures shown in parenthesis indicates number of rainy days

PROPAGATION STUDIES IN AONLA (*Phyllanthus emblica* L.)

GOVINDA NAYAKA D. **2006** **Dr. A. N. MOKASHI**
MAJOR ADVISOR

ABSTRACT

Studies on seed germination, softwood grafting and micro propagation were carried at Department of Horticulture, University of Agricultural Sciences, Dharwad during 2005-2006.

In seed germination studies, the seeds scarified with concentrated sulphuric acid for 30 seconds were found to be the best one with very high per cent germination (80.39), taking least number of days for 50 per cent germination (12.27) and highest vigour index (1223).

Propagation studies revealed that, season of grafting had profound influence on graft success. Maximum success per cent of 87.50 was recorded in November grafting followed by 66.69 per cent in case of December. Least success was recorded during June grafting (16.52%). Influences of rootstock age on success of grafts were significant. The higher per cent successful grafts (62.88) were observed in eight months old rootstocks followed by four months (56.25%) old rootstocks.

In the studies on micropropagation, nodal segments from mature tree as explants were used with use of growth regulators (Kinetin and GA_3) at various concentrations for shoot proliferation. MS medium supplement with Kinetin at 2.0 mg/l with GA_3 0.5 mg/l resulted in more number of shoots (3.28) and less number of days taken for bud initiation (7.07). All the concentrations of IBA and NAA used individually for *in vitro* rooting of micro shoots failed to induce rooting. However combination of IBA (2.0 mg/l) and NAA (2.0 mg/l) has resulted in callus induction at cut end of *in vitro* shoots.