STANDARDISATION OF TOP WORKING IN NUTMEG (*Myristica fragrans* Houtt.)

By

BEENA S.

THESIS

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DECLARATION

I hereby declare that this thesis entitled **'Standardisation of top working in nutmeg (*Myristica fragrans* Houtt.)'** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award of any degree, diploma, associateship and other similar title, of any other University or Society.

Vellanikkara,
19 3 1994

BEENA, S.

Dr.ALICE KURIAN
Associate Professor
Department of Plantation Crops
and Spices

College of Horticulture
Vellanikkara
Date 19 3 1994

CERTIFICATE

Certified that this thesis entitled **'Standardisation of top working in nutmeg (*Myristica fragrans* Houtt.)'** is a record of research work done independently by **Miss.Beena,S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr.Alice Kurian
Chairperson
Advisory Committee

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Miss.BEENA, S.**, a candidate for the degree of Master of Science in Horticulture agree that the thesis, entitled **'Standardisation of top working in nutmeg (*Myristica fragrans* Houtt.)'** may be submitted by Miss.Beena, S. in partial fulfilment of the requirement for the degree.

Dr.Alice Kurian
(Chairperson)
Associate Professor
Department of Plantation Crops & Spices

Dr.G.Sreekandan Nair
Professor and Head
Department of Plantation Crops and Spices
College of Agriculture
Vellayani
(Member)

Dr.E.V. Nybe
Associate Professor
Department of Plantation Crops and Spices
(Member)

Dr.N.K.Vijayakumar
Associate Professor
College of Forestry
(Member)

External Examiner
Mr ABDUL KHADER
TNAU.

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Dedicated to
my loving parents

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Introduction

INTRODUCTION

Nutmeg (Myristica fragrans Houtt.) is an important tree spice belonging to the small primitive family Myristicaceae. It is unique, among spice plants as it produces two separate and distinct spices, the nutmeg and mace. The nutmeg plant, a native of the Eastern Islands of the Moluccas was introduced to India and other tropical countries during 18th century. The shade loving nature of the crop makes it ideal for intercropping in coconut plantations and is extensively cultivated in the homesteads of India.

The cultivation of this crop in India is mainly confined to the southern states of Kerala, Karnataka and Tamil Nadu. The total area under this crop in India during 1989-'90 was estimated to be 3120 ha with a production of 5322 tonnes, of which Kerala accounts for more than 90 per cent (Spices Board, 1991). The production of nutmeg is not sufficient to meet the internal demands and a part of the requirement is met through imports.

Dried nutmeg and mace find their major use as spices for flavouring. The aromatic oil is used for flavouring baked goods, cakes, cookies, puddings, pickles etc. Medicinally nutmeg acts as a stimulant and carminative. Nutmeg butter, the fat extracted from the seed is used in the manufacture of scented oils, perfumes and soaps and as flavouring agent in cookery and confectionary.

The trees are dioecious in nature and the seedling progenies segregate into male and female in the ratio of 1:1 (Nichols and Pryde, 1958). Several attempts were made to identify the sex at the seedling stage, on the basis of leaf form and venation (Prestoe, 1948), colour of young sprouts, seedling vigour, chromosome morphology (Flach, 1966), shape of calcium oxalate crystals on leaf epidermis (Nayar et al., 1977) and chemical methods (Phadnis and Choudhari, 1971, Zachariah et al., 1986 and Krishnamurthy et al., 1992). But none of these methods seems to be reliable. On account of the uncertainity in predicting the sex in nutmeg seedlings, it can be determined only after flowering by about 7-8 years.

In this context the vegetative methods for propagating female plants have a distinct advantage and different methods were standardised by several workers. But the commercial coverage through vegetative propagules are very much limited due to the unavailability of orthotropic shoots for large scale multiplication and seeds continue to be the major propagating material even now.

In a nutmeg plantation, for successful pollination and fruit set one male tree need be maintained for every ten female trees. Expecting a 1:1 segregation into male and female, 50 per cent of the total trees in any nutmeg plantation raised through seedlings will be males. Cutting down of excess male trees will result in irregular spacing and economic loss to the growers. Top working has got its own significance in the case of dioecious perennial trees like nutmeg, since this permits the transformation of unproductive males to desired females with precocious bearing. There are sporadic reports of successful top working in nutmeg but the method is not universally adopted by farmers due to problems such as low success percentage and delayed and erratic response. But the method of top working is well standardised in cashew and the technique is being practised commercially to rejuvenate unproductive trees. In view of the reported success of top working in other perennial trees an attempt is made in the present study to standardise the methods for top working in nutmeg and to probe into the problems encountered.

Review of Literature

REVIEW OF LITERATURE

Literature pertaining to sex problem and top working through budding and grafting and the factors influencing their success in nutmeg and some of the tree crops are briefly dealt in this chapter.

2.1 Sex problem

Deinum (1931) found that on an average 55 per cent of the mature seeds of nutmeg was female, 40 per cent male and 5 per cent bisexual. The sex of tree cannot be determined with any degree of certainity until flowering has commenced seven to eight years after planting.

Prestoe (1948) claims to have determined the sex of young seedlings of nutmeg, less than 30 cm high, by means of leaf form and venation. Leaves of female trees should be nearly elliptical with more or less straight veins, where as male trees should possess nearly obovate leaves with their veins rounded to the more pronounced point of the leaf. This method was tested by planting and observing until fruiting and was found to be 85 per cent accurate. However the method has not been used, but Flach (1966) thinks it worth investigating since he found a slight difference in tree size between female and male tree while studying secondary sex character among four year old seedlings.

Nichols and Pryde (1958) after reviewing the literature on the subject came to the conclusion that female and male trees were produced approximately in equal numbers. The variation in sex expression was investigated and concluded that there are both a female only flowering sex and a male flowering sex segregated in a 1·1 ratio. The latter is subdivided into four different groups i.e., unisexual males, bisexual males, true bisexuals and bisexual females (Flach, 1966).

Flach (1966) has postulated a hypothesis on sex determination in nutmeg which includes the possibility of sexing the young seedling by a root tip method. Flach and Cruickshank (1969) suggested that controlled crosses between highly producing mother trees and various types of male flowering trees held possibilities of diminishing the percentage of male trees in the offspring.

Phadnis and Choudhari (1971) in their trials using 0.1 g of leaf sample and ammonium molybdate reagent found the colour difference to be effective in differentiating the sex. They also observed that the colour differences were more marked in the fifth leaf collected from old flushes than the second leaf from the new flush.

Nayar et al. (1977) postulated a method for distinguishing the sex by the shape of the calcium oxalate crystals in the lower epidermal cells of the leaves of plants at least two years of age.

Krihnamoorthy et al. (1992) studied the suitability of some of the earlier reported methods to detect the sex at the nursery stage. The study of crystal patterns in the epidermal cells of the leaves revealed that the crystal differentiation was very clear in the mature leaf in almost 70 per cent of male and 78 per cent of female plants, whereas in the case of seedlings the crystal pattern was not definite and so the sex could not be differentiated in seedlings based on crystal study. The study of the seedling morphological traits like sprout colour, days for germination, leaf shape, size, venation etc. led to the conclusion that none of these characters can be taken as a guide to sex the nutmeg plants. Out of a number of colour tests carried out on the leaf exudates or alcoholic leaf extracts of male and female trees, the ammonium molybdate alone gave satisfactory differentiation between male and female nutmeg trees. This test gave a 'faint green' colour with the male and a 'sea green' colour with the female plant. This was reproducible in the case of all the trees tested but not in the seedlings.

2.2 Budding on hard trunk

In cacao, top working trials were carried out by budding on hard trunk but success rate was very low and even after repeated budding only three out of six plants could be successfully budded (CCRP, 1989-90).

In nutmeg, budding on hard trunk was reported to be successful (Manithottam, 1992). He observed that patch budding was quite successful during July and August. Further, it was also successful on 18 to 24 month old *Myristica beddomei* rootstocks.

2.3 Methods of propagation

2.3.1 Budding

Different types of budding tried in various tree crops are briefly reviewed here.

Nutmeg

Postma (1935) observed 30 per cent success with budding on rootstock of *Myristica succedana* in Indonesia. Nichols and Cruickshank (1964) reviewed the available methods of propagation in nutmeg. They reported that budding was successful in nutmeg and this technique offered the advantage that other species of *Myristica* could be used as rootstocks.

In New Guinea, budding was tried with *Myristica argentea* as rootstock but they were not successful in the long run (Flach, 1966). Mathew and Joseph (1979) tried forkert method of budding in *Myristica fragrans* and *Myristica beddomei* rootstocks and observed that percentage of success was very low (4 per cent) in both rootstocks.

In further trials on forkert budding of nutmeg, Mathew (1979) observed that buds remained green under mist but none survived when taken out. Mathew *et al*. (1984) opined that forkert method of budding would be done on *Myristica fragrans* and *Myristica beddomei* rootstocks with good amount of success. They also observed that buds remained alive even for one year without sprouting.

Cacao

Magalhaes (1974) opined that cacao can be shield budded with an initial success of 60 per cent but final survival when field planted was 40 per cent. Giesberger and Coster (1977) in their trials with green budding and grafting found that for budding, the modified forkert method has a successful take of 90 to 100 per cent. Kumaran and Nair (1982) observed that the modified forkert method of budding gave best bud take (60 to 73 per cent) than other methods like patch and T-budding. Subsequent attempts yielded 90 to 95 per cent success with this method.

Kadje and Ndjama (1982) in their trials on budding with shield and inverted 'T' methods found that take varied from 52 to 92 per cent. Prawoto (1984) observed that budding done on one year old seedling in the field gave a take of 98 per cent compared to 28 per cent success in the nursery. Keshavachandran and Nair (1985) found that forkert method of budding gave highest success ranging from 60 to 100 per cent depending on the season.

Nagabhushanam and Nair (1988) conducted trials on asexual propagation of cacao and found that patch budding gave only 35 per cent success. In top working of cacao, budding on new chupon growth gave cent per cent success almost in all seasons (CCRP, 1987-'88, 1989-1990).

Rubber

Double working or crown budding was first tried out in Java in 1926. Possibility of forming a tree with improved root-stock, trunk and crown components was also envisaged by this method (De Vries, 1926).

Green budding of rubber was first developed in North Borneo (Hurov, 1961). For this, only the buds from the leaf axils of the terminal and the two preceding whorls of leaves of a shoot, as well as the scale buds, could be used without any significant difference in budding success or in rate of emergence and growth of scion (Gener, 1966, Samaranayake and Gunaratne, 1977, Leong and Yoon, 1979). Ooi et al. (1976) reported 90 per cent success in this method

Leong and Yoon (1979) reported that six different types of buds could be used for green budding and they observed only little difference in the budding success or the subsequent scion growth in terms of height, diameter and dry matter production.

Sena Gomes (1984) tried brown and green budd ng n the field and nursery and found that any of the four methods as suitable for rubber planting in Southern Bahia But field budd ng on brown tissue gave the best results Top work ng was also tr ed to reduce the wind damage (Samaranayake et al 984)

Gan and Chew (1988) reported that young budd ng on two month old seedlings and cutting back after one month ga e on 48 per cent survival but they further observed that delav ng budding to four months and cutting at two or three months as more successful

In rubber brown budding was the earliest developed method of vegetative propagation Ax llary buds taken from the mature parts of shoots with the bark turning from green to b o n were used for budding Brown budding was done on seedling sto ks of similar age and size to the bud st cks earl er in the eld or n nursery for the production of budded stumps (Webster and Baulkwill 1989)

Cashew

Phadnis et al (1971) reported that budding was more successful on one year or older seedl ngs than graft ng Fe az et al (1974) opined that patch budding on eight month old seed lings gave the best results Palaniswamy and Hameed (1976) also reported higher percentage of success (71 per cent) with patch budding and they observed that bud sprouting vas early and plants grow vigorously

Nagabhushanam and Rao (1977) in their trials on patch budding on 15 to 20 days and six month old seedl ngs reported very poor success Similar trend was observed by Rao and Nambia (1977) Nagabhushanam (1985) from Vittal observed that patch budding in situ gave better results as compared to that n poly bags

Rao (1985) reported that patch budding gave the best esult with 41 per cent success among different methods of budding unde Baptla conditions The works done at Vriddhachalam gave 75 per cent success in patch budding during July (Palaniswamy et al 1985) Khan et al (1986) from Ullal have reported that fo top working patch budding gave very low (18 3 per cent) success and growth of sprouts was not rapid and so they considered th s technique not promising for top working

Other crops

Preliminary trials on the budding of mango in Kodur showed that shield budding gave highest success in comparison to the other six different methods tried (Naik 1941) followed by patc budding Singh and Srivastava (1962) observed that forkert method of budding was found to be the best method with 10 per cen success This method was also cheaper and less t me consum ng compared to inarching

Srivastava (1962) observed that in guava both the forkert and patch budding methods gave a complete take and also found that plants propagated by forkert method grew better than those by the patch method Further studies in clonal propagation of guava showed that forkert method of budding gave better results than shield or patch budding (Chandra 1965)

Singh et al (1982) compared patch and T budding n jack and found that patch budding gave highest take of about 90 per cent Konhar et al (1990) from Bhubaneswar reported that patch budding gave the highest percentage of success (80 per cent followed by chip budding and flute budding

In tamarind a comparison of patch and modif ed ring budding performed on nine month old seedlings showed that patch budding was the best method for large scale multiplication (Pathak et al 1992)

2 3 2 Effect of physical treatments on bud take and sprouting

Girdling the removal of complete ring of bark (essentially the phloem) is done to check the downward passage of food which gets collected above the girdle It also effect temporary changes in the balance of nitrogen and possibly growth substances to induce flowering and better fruiting

Mathew and Joseph (1979) used 0 5 per cent urea solution along with gradual defoliation of the stock in nutmeg and observed that bud sprouting was induced in 15 days They also reported that partial decapitation of stock above the bud union was found to be the best to induce the bud sprouting

Mathew *et al* (1984) tried various treatments in nutmeg such as notching ringing decapitation and bending of rootstocks at budded portion along with GA_3 application They observed that among these treatments bending of rootstocks at bud union point was found to give cent per cent sprouting within 30 days They also observed that notching GA_3 application application of liquid manure and ringing were not effective in inducing bud sprouting but decapitation tended to kill the budded plants

In citrus to break apical dominance and to force inserted bud into growth several mechanical and chemical measures were undertaken by Samson (1986) Bending combined with topping gave significantly better results than topping alone and the best place for bending or topping was directly above the bud while half ring enhanced the effect of bending

2 3 3 Effect of age of rootstock scion and season

Nutmeg

Mathew (1979) opined that one year old seedlings which are uniform in growth can be used as rootstock and the budwood (scion) from one season old shoots of vigorous female trees having unsprouted dormant buds can be used for forkert budding Budding during July gave maximum take Similar results were obtained during March July and August (Mathew and Joseph 1979)

Mathew and Joseph (1980) further noted that maximum bud take was in May which coincided with the flushing season in nut meg Rainy season (July) was found to be ideal for budding (Mathew et al 1984)

Cashew

Phadnis et al (1971) opined that budding should preferably be done from September to November on one year or older seedlings to get better success Patch budding on eight month old seedlings gave the best budding results when carried out during the dry season at Pernambuco (Ferraz et al 1974) But Palaniswamy and Hameed (1976) observed that July budding gave higher percentage of success followed by March April September and October

Ramana and Reddy (1991) reported that patch budding gave good results at Vengurla and Vridhachalam when carried out on 2_2 years old seedlings growing in nursery beds or containers Best time was one month after the begining of flowering (March) In Maharashtra September to November appeared to be the best time

Cacao

In Cacao four to five month old seedling rootstocks of 1 to 1 5 cm diameter could be successfully budded by shield method as reported by Magalhaes (1974) Experiments on green budding and grafting on two to six week old seedling rootstocks gave best take (Giesberger and Coster 1977) Kumaran and Nair (1982) found that forkert budding on six month old Forastero seed lings gave good bud take (60 to 73 per cent) Bud taken from pre cured budwood gave better results (73 per cent) than intact budwood (60 per cent)

For patch budding vigorous four month old seedlings were used as rootstocks and scion material was taken from fan branches of a single clone Success percentage was highest in June and lowest in September (Nagabhushanam and Nair 1988)

Other crops

In mango trials conducted at Basti indicated that one year old seedlings was the ideal rootstocks for budding and also observed that budding in March was superior over April and May and better results were reported during June and July respectively (Teaotia and Maurya 1970) Similar results were obtained in two year old seedlings (Prasad et al 1973)

Patch budding on one year old seedlings of guava from April to September showed that success was highest in May followed by June (Mehrotra and Gupta 1984) Similar results were obtained by Kaundal et al (1987) In tamarind patch budding on nine month old seedlings gave highest percentage of success during June (Pathak et al 1992)

2 4 Top working

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

2 4 1 Beheading the tree

Conversion of undesirable trees of apple and pear after making a frame have been reported from India and abroad (Maney 1939 Roberts 1936 Garner 1944 and Srivastava 1965a b)

Srivastava et al (1975) observed that plum trees headed back within 1 to 1 5 m from the main crotch during the dormant season produced a number of new shoots on the stumps during the growing season (spring) In mango inferior trees were headed back to 60 to 90 cm length and the wounds were coated with some disinfectant like Bordeaux paste immediately (Kanwar and Jawanda 1983)

Nahlawi et al (1983) reported that in pistachio trees grafting was most successful when done in February with trees cut back to 20 cm and 60 cm height Beheading the trees 0 75 to 1 00 m above ground level was found to be ideal in cashew from the point of view of sprouting and after care of the successful graft and trees (Khan et al 1986)

In top working trials of cacao plants were cut back at jorquette height and also at 30 cm height from ground level (CCRP 1987 1988) Kar et al (1989) in an attempt to top work wild pomegranate with the commercially cultivated pomegranate trees were cut back to a height of 90 cm from the ground level which could produce enough shoots for budding and grafting

2 4 2 Age of tree and month of beheading

In a study on top working 30 year old Ralls Janet apple trees with Golden Delicious at a planting distance of 7 3 m x 7 3 m the best growth and yields were obtained by grafting at 30 points for tree and removing the fruiting branches on the original variety (the interstock) in the fifth year after grafting (Kon et al 1970) Ristevski (1972) opined that reworking four year old Red Delicious trees on M 4 rootstocks with Golden Delicious during September produced a rapid union and in the first year of growth a total shoot length of 85 5 m per tree of 9 5 m per scion was observed

Srivastava et al (1975) found that 25 year old unprodutive plum trees could be top worked by budding with new plum cultivars and they came to bearing within two years Rejuvenation of peach debiliated by over production was attempted by Casini et al (1982) Eight year old Cardinal peaches were cut back severely between early July to mid August and they produced new crowns and resumed a mean yield of 160 q/ha with a large saving in management costs

Hirobe et al (1983) conducted studies on the top grafting of Satsuma citrus variety with Aoshima mandarin He found that to obtain stable yields and good fruit quality 15 scions per tree should be grafted when renewing 12 year old trees Various methods were used to top work Okitsu Early Satsuma onto 10 year old Hayashi Unshiu trees on trifoliate orange (Poncirus trifoliata) stocks Simultaneous replacement of the whole canopy by bark and side grafting proved most successful and was better than simple replacement of the main branches by bark grafting (Kin et al 1987)

Top working old pistachio trees indicated that eight year old trees cut back to 20 cm and 60 cm above ground level and grafting in February was most successful as reported by Nahlawi et al (1983) Top working trials were also carried out in nutmeg on five year and 13 year old male trees by slotted side grafting which was a failure (Mathew et al 1984)

Khan et al (1986) attempted top working in cashew trees in the age group of 11 to 26 years by heading back at monthly interval between September 1983 and August 1984 They found that the most suitable time for heading back was between December and February Similar results in cashew were also reported by Prasad et al (1988) and Pugalendhi and Shah (1991)

2 4 3 Time taken for sprouting and number of sprouts

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

2 4 4 Effect of age of rootstock scion and season

Nutmeg

Mathew (1979) obtained successful take using 1_2 to 2 year old rootstock for approach grafting But for side grafting eight to twelve month old seedling and nine month old scion was most suited and best season was found to be July Wedge grafting done on one year old seedlings as rootstock gave maximum success during March

Mathew and Joseph (1982) in their trials on epicotyl grafting observed that rootstock of first leaf stage having a thick stem (diameter of 0 4 cm or more) with sufficient length to give a cut of 5 0 cm long and the scions collected from the female grafts having a stem diameter of 0 3 to 0 5 cm gave more success

Cashew

Patch budding on six month old seedlings and on one year old shoots gave best results and ideal months were April May and June (Nagabhushanam 1985) Similar results were obtained by Khan et al (1986) Valsalakumari et al (1985) reported that highest success in patch budding was obtained between May or June and September or October

For top working 90 to 110 days old shoots were used for softwood grafting using scion material of 0 8 to 1 0 cm girth 10 to 12 cm length bearing mature leaves and having sprouted apical buds from the previous seasons growth (Prasad et al 1988) Monsoon period (June to October) was found to be the best for commercial multiplication as the per cent of success was quite high (above 60 per cent) when compared to other months (Swamy et al 1990)

Pushpalatha et al (1990) opined that 25 to 30 days old rootstock seedlings could be used for softwood grafting which was successful during the months of March to May Trials at Baptla showed that one month old seedlings when used as root sock with 10 cm long scion precured 10 days prior to grafting gave highest success Suitable months for grafting were August September and January (Sarada et al 1991)

Ramana and Reddy (1991) while reviewing the literature on propagation of cashew found that for side grafting two to three year old seedlings used as rootstocks in the month of July gave best results But for epicotyl grafting tender cashew seedlings of 10 to 15 days were used as rootstocks and best season was observed to be during February to May

Rubber

Lins and Castro (1979) observed that patch budding on 28 month of seedlings as rootstocks gave highest take when mature buds were used Sena Gomes (1984) in their trials on green budding on six and eight month old seedlings and budding on 12 to 16 month old rootstocks observed good percentage of take For green budding five to six month old seedlings with the bark at the base of the stem turning from green to brown is used for budding (Webster and Baulkwill 1989)

Coffee

Cleft grafting on six month old suckers gave good take during October to November (Vossen et al 1977) Raghuramalu and Purushotham (1987) also reported best success with cleft grafting on sixty days old seedlings

2 4 5 Factors influencing bud take and sprouting

2 4 5 1 Bud dormancy

Dormancy of buds is defined as cessation of observable growth Buds of temperate zone woody plants alternate from active growth during the warm season to a dormant state during the cold season When seasonal shoot growth ceases, the plants first enter reversible phase of inactivity called prerest quiescence or predormancy (Romberger 1963)

Bud dormancy is regulated by interactions between endogenous growth promoters and inhibitors At least three major and several minor phases of bud development are recognized each controlled by balance of growth hormones Michniewicz (1967) considered bud dormancy to involve an interaction of the influence of substances inhibitory to growth and the promotive influence of gibberellins

Growth inhibitors accumulate in bud scales bud axills and leaves within buds bringing about dormancy (Tinklin and Schwalae 1970) Three lines of evidence emphasize the importance of endogenous inhibitors in the development of bud dormancy

(i) Under short day conditions the leaves inhibit growth of the shoot tip

(ii) Generally more inhibitors are found in leaves and buds under short day than long day conditions and

(iii) When growth inhibitors such as ABA are extracted from leaves of a dormant woody plant and reapplied to plants of the same species that were not dormant prior to appli cation of the inhibitor shoot elongation stopped and sequential development towards the dormant state was initiated (Wareing 1965)

Ramsay and Martin (1970) suggested that release from bud dormancy was regulated by a delicate balance between growth promoters (gibberellins) and inhibitors

Growth promoters other than gibberellins also appear to play a role in release from bud dormancy Kinetin like activity was absent in dormant buds of white birch and balsam poplar After bud dormancy was broken kinetin like activity increased progressively until shortly after buds opened and it decreased thereafter (Domanski and Kozlowski 1968)

Cytokinins increased in both xylem sap and buds during chilling and bud burst of Populus x Robusta The levels of cytokinins often decreased in the xylem sap during bud swelling However this may have been due to dilution of the sap after transpiration became rapid (Hewett and Wareing 1973a b)

Browning (1973) found that gibberellin levels in coffee flower buds increased and ABA levels did not change when bud dormancy was released by rain or irrigation Taylor and Dumbroff (1975) showed increase in cytokinin activity but no significant changes in inhibitors during dormancy release in sugar maple buds

2 4 5 2 Effect of growth regulators on bud take and sprouting

Parups (1971) in experiments on green house grown roses used 0 5 per cent 6 benzyl amino purine (BA) and 0 5 per cent adenine in lanolin and could obtain bud break and shoot develop ment in 34 to 60 per cent of the treated buds

Green house grown macadamia seedlings with the apex either removed or intact were sprayed weekly one to four times with 6 (benzyl amino) 9 (tetrahydropranyl) 9H purine (PBA) at 250 to 1 000 ppm inducing sprouting of axillary buds on 42 to 50 per cent of the plants (Boswell and Storey 1974)

Brome and Zimmerman (1976) reported a technique for forcing the buds of 12 to 18 month old tea crab apple seedlings BA (6 benzyl amino purine) at 2 000 ppm produced the best results in which dimethyl sulphoxide was an excellent solvent for the cyto kinins which slightly enhanced their effect

Rehm et al (1978) observed in six month old arabica coffee seedlings grown in greenhouse that apical dominance was broken and growth of orthotropic shoots was induced from secondary or serial buds by GA_3 alone Except for BA all growth substances tested reduced the shoot elongation induced by GA_3 Nauer and Boswell (1981) opined that application of BA to quiescent buds on single leader citrus plants increased the percentage of buds initiating active growth

Kathiravetpillai and Kulasegram (1981) conducted trials to activate axillary shoots of tea cv TRI 2025 IAA (0 5 per cent) in lanolin applied to decapitated stumps and BA (50 250 ppm) induced the plants to remain active and produce more and longer shoots However IAA suppressed the production of side shoots

Jaafar (1982) observed that the bud patches of budded stumps of rubber sprayed with BA (2 000 ppm) or dikegulac sodium (2,000 ppm) showed that former induced more bud emergence than dikegulac-sodium but later this trend was reversed

Works conducted on bud break of roses proved that smearing BA (0 25 per cent) in lanolin paste on the cut surface 0 5 cm above the axillary bud completely broke bud dormancy and hastened growth (Ohkawa 1984)

In rubber bud break could be accelerated by treating the bud patches with a formulation containing 2500 ppm Atrinal (dikegulac sodium) 5 per cent dimethyl sulfoxide and 10 per cent Triton X 100 in 50 per cent ethanol which was sprayed or painted six times on alternate days (Jaafar 1984) Semeniuk and Griesbach (1985) observed that BA suspended in lanolin at concentrations of 1 5 25 or 50 mg/ml induced either the initiation of bud growth or the development of lateral branches in poinsettia Bud application of 150 kg/ml t cinnamic acid alone or in combination with BA had no effect in promoting bud activity

In trials conducted on the effect of some plant growth regulators and urea on the growth of walnut grafts showed that GA at 250 ppm followed by urea at 0 5 per cent gave good shoot growth of 50 8 cm and 47 9 cm respectively (Joshi et al 1986)

The effect of 0 to 6 000 ppm IBA and 0 to 60 ppm GA_3 on bud sprouting and growth of budded stumps in *Hevea* showed that IBA increased the sprouting percentage of the buds plant height stem diameter and root numbers while GA_3 increased the sprouting percentage of the buds and plant height alone (Hushny et al 1986)

Halim et al (1990) reported that buds of orange cv Valencia budded on rootstock of *Poncirus trifoliata* remain dormant for variable periods The results of soaking in the buds in solution before budding killed some buds especially when GA_3 was used Under moist conditions BA promoted earlier bud burst

2 4 6 Grafting

Different methods of grafting followed in various crops are briefly reviewed here under

Nutmeg

Sundararaj and Varadarajan (1956) have obtained 40 to 80 per cent success by approach grafting Myristica fragrans on the rootstock of M malabarica and M beddomei Nichols and Cruick shank (1964) reported 90 per cent success in approach grafting Shanmugavelu and Rao (1977) also reported 60 to 100 per cent success in approach grafting in all the three rootstocks Myristica malabarica M beddomei M fragrans Mathew (1979) tried different methods of grafting and found that approach grafting gave higher percentage of success (95 per cent) followed by side and wedge grafting (40 per cent)

Epicotyl grafting in nutmeg using Myristica beddomei and M malabarica as rootstocks was reported (CPCRI 1981) Cleft grafting using leafy scion gave 48 per cent and 40 per cent take with M beddomei and M malabarica respectively

Mathew and Joseph (1982) reported epicotyl grafting in nutmeg with 48 per cent success with M beddomei as rootstock and cleft grafting using leafy scions

Cashew

Rao *et al* (1957) found that side grafting could be success fully done in cashew with 70 per cent take by placing moist moss above and below in union Nagabhushanam (1983) reported about 60 to 68 per cent success in epicotyl grafting with suitable scions But a modified method was reported with two bottom leaves intact in the rootstock which gave nearly 45 per cent success (Seshadri and Rao 1985)

Das and Mishra (1985) reported that side grafting was more successful than T budding and they observed that the best months for grafting in decreasing order of success (80 to 60 per cent) were January February September July and June Similarly veneer grafting by defoliating the scion shoots gave 36 per cent success but during the rainy season 92 per cent success was obtained (Dhandar 1985) Wedge grafting recorded 75 per cent success on eight month old seedlings grafted in March (Valsalakumari *et al* 1985)

The highest success in softwood grafting was reported during April (39 6 per cent) on shoots of trees beheaded in January and February followed by grafting during August (33 3 per cent) on trees beheaded in May (Khan *et al* 1986) They also found that rate of grafting success was increased (80 to 100 per cent) by increasing the age of the rootstock and by using fresh scion shoots (Khan et al 1989) Similar results were obtained in top working (Pugalendhi and Shah 1991 and Pushpalatha et al 1991)

Coffee

Top grafting in Coffea arabica L was successfully done by cleft grafting single noded scions with one pair of leaves on six month old suckers which gave 85 per cent success (Vossen et al 1977) Couturon and Berthaud (1979) described a technique for grafting embryos of C arabica C canephora and C liberica onto C canephora seedlings at collar level and the plants were kept in damp sawdust in a saturated atmosphere and potted after 4 to 6 weeks recording 95 per cent success.

Purushotham (1982) reported that four month old leafy suckers of arabica coffee could be grafted on four month old nrooted robusta leafy suckers It was observed that rooting and healing of the graft union occurred simultaneously within four months and resulted in 60 per cent take Reghuramulu and Prushotham (1987) observed that sixty days after sowing young coffee seedlings could be cleft grafted or wedge grafted The rootstock used were Coffea arabica and C canephora and the scion components were C arabica alone Best grafting success (95 to 100 per cent) and better survival of grafted plants (67 to 90 per cent) were observed on C arabica rootstock

Cacao

Cacao could be grafted by saddle and wedge graft methods but they have little commercial application But to detect virus infection in budwood it is necessary to graft the budwood into a susceptible stock for which a rapid and reliable method suggested was side graft technique (Soderholm and Shaw 1965) Giesberger and Coster (1975) observed nearly 90 to 100 per cent success in side grafting cacao seedlings

Rubber

Comparison between whip grafting and green budding showed that the former gave better results with regard to the production of upright plants in short time especially in unfavourable weather conditions (Moraes and Muraes 1978) Pereira et al (1979) in their studies on the reduction of the immature phase (7 to 8 years) of rubber trees shoot apices (3 to 4 cm long) of the cvs IAN 717 IAN 873 and FX 3899 were cleft grafted onto seeds two to five days after epicotyl emergence The best take was obtained when the two lateral cotyledonary buds were allowed to grow until a good callus union was formed

Other crops

Haldankar et al (1987) observed that by softwood grafting the promising genotypes of kokam a dioecious plant exhibiting a vast variation in quality and quantity of fruits could be rapidly multiplied for commercial purposes They further observed that survival was nearly 70 to 90 per cent

In tamarind softwood and veneer grafting were undertaken for comparison and the results indicated that percentage graft take in softwood grafting was (68 per cent) than in veneer grafting (49 per cent) (Purushotham and Narasimharao 1990) Similar results were obtained in pomegranate also (Kar et al 1989)

Nazeem et al (1984) tried several methods of propagation in jack namely grafting budding and layering and best success was obtained by air layering ringed shoots Epicotyl grafting in jack gave maximum sprouting of 83 3 per cent when compared to softwood grafting which was very poor (Jose and Valsalakumari 1991)

Kar et al (1974) compared several methods of top working in olive and found that patch budding followed by T budding and side veneer grafting was equally effective

2 5 Anatomical studies of bud union

Walter (1932) reported that mango bud was unique in its method of union with stock plant He also observed that cohesion took place only under the bark not at the sides The bud was thus found to be carried upward on a cushion of rapidly dividing cambium cells

Mendel (1936) carried out anatomical studies of the bud union in citrus The six stages described in the healing of citrus T-budding were stage 1 (24 hours) cell division stage 2 (5 days) callus bridge, stage 3 (10 days) differentiation in the callus of bark flaps and callus of shield, stage 4 (5 days) occurrence of xylem tracheids in the callus of shield stage 5 (25 30 days) lignification of callus completed in the bark flaps stage 6 (30 40 days) lignification of callus completed in the bark flaps and in the bark shield Callus formation began in all the tissues adjacent to wound Lignification occured gradually in the course of differentiation

In walnut, the stages of union of budding was reported by Nedev (1969) Grafted buds began to form callus in 5 to 10 days In those buds the isolation layer in the first few days a deep brown line began to be reabsorbed in some places after budding Vascular elements began to form 20 to 25 days after budding The differentiation of scar tissues was most active close to the two sides of the bud shield and the vascular tissue differentiated more slowly inside the callus and complete union was obtained in 60 to 65 days

Wagner (1969) reported that in Malus within 15 minutes of the bud being set the grafted material had developed a necrotic plate of material released from mechanically ruptured cells Callus parenchyma cells began to develop from the rootstock xylem rays after two days and broke through the plate after four days Callus cells from the scion similarly ruptured the necrotic region but at this stage there was no cytoplasmic connections between the rootstock and scion

Soule (1971) conducted detailed studies on the anatomy of bud union in mango by chip budding method He observed the four important stages in formation of bud union Stage 1 (four days after budding) only a wound periderm formation stage 2 (8 days after budding) callus proliferation from tissues near the cambium resulted in firm attachment of components Stage 3 (12 days after budding) completion of cambial bridge and vascular tissues were differentiated within 48 days and completion of healing union Stage 4 (6 to 8 months after budding) several cylinders of new tissues developed lateral shift of scion to align with the stock had begun

Soule (1971) reported in mango excessive growth of parenchymatous tissue between stock and scion distortion of xylem elements and blockage of vessels in Langra budlings examined 6 and 18 months subsequent to inarching It was noted in transverse and radial sections of chip buds that excessive callus development and impregnation of large areas of tissue by wound gum did occur however where cambial layers were not in close alignment or tissues adjacent to the cuts were crushed or other wise damaged to a depth of several layers of cells Difficulties of this nature may induce a poorly healed or uncongenial union resulting in sickly lived trees

Batchellor (1973) found budding of ornamental Eucalyptus fiafolia on rootstocks of E calophylla to be unsuccessful because of excessive callus formation on the cut surface

Sinsor (1982) revealed that in incomplete stock and scion combination of apple the xylem and phloem within the graft union were greatly reduced Breakage and failure within the graft union of certain stock scion combination was associated with abnormal ray development Skene et al (1983) has suggested that infilling of callus and improper development of cambium in between the stock and scion was another reason for graft failure in certain apple varieties viz Cox s Orange Pippin

Jose (1989) found four distinct stages in the formation of graft union in jack Proper graft union was obtained 90 days after the grafting operation callus proliferation was mostly from the pith cells In the unsuccessful graft failure was due to the absence of callus formation between stock and scion forming a wide gap formation of a thick necrotic layer between stock and scion excessive callus production without any differentiation to cambium leading to failure of grafts Cut surface of bark and pholem some times come into each other which resulted in the lack of proper callus formation and consequent drying of grafts The same reasons for graft failure in Garcinia indica was attributed by Nazeema (1992)

Materials and Methods

MATERIALS AND METHODS

The present study was conducted at the Department of Plantation Crops and Spices College of Horticulture Vellanikkara during 1991 to 1993 The nutmeg trees available at State Seed Farm Mannuthy and farmers fields at Mannuthy Ettumuna in Thrissur district and Neduvannur in Ernakulam district were utilised for the investigations The selected localities enjoy a suitable environment for the growth of nutmeg plants with high rainfall and warm humid climate throughout the year with less fluctuations in daily temperature The altitude of the place is 22 25 m above mean sea level at 10° 32 N latitude and 76° 16 S latitude The methodology followed in various experiments to standardise the methods for top working of nutmeg are described below

3 1 Standardisation of budding on hard trunk

3 1 1 Selection of buds

Healthy and high yielding female trees were selected for collecting budwood The budwood (scion) was taken from orthotropic shoots with bark thickness that could match more or less the peeled bark of the stock From the selected budwood only viable buds were taken which could be identified by the scar inside Length and girth of budwood and the number of viable and nonviable buds on a budwood were recorded

3 1 2 Effect of methods of budding on bud take

3 1 2 1 Patch budding

A rectangular patch of about 10 to 15 cm length and 5 cm width was marked deep on the trunk Budwood was selected as detailed in 3 1 1 From budwood bud piece was removed by cutting a rectangular patch and inserted on the stock in two ways

(i) Compact bud piece exacting the patch of bark removed from the stock

(ii) Loose bud piece lesser in size so that when inserted some space is left on four sides

Then the patch of bark was removed from the stock and the bud piece taken as above was inserted taking care that the bud is not inverted Then it was tied tightly with polythene tape of 200 gauge thickness (Plate 1 and 2)

3 1 2 2 Forkert budding

A transverse incision of about 5 cm was made in the bark of the rootstock At the two ends of the transverse incision two parallel cuts of about 10 to 15 cm were made and then the bark was peeled carefully upwards Then the bud piece was inserted as outlined in 3 1 2 1 and the bud was covered with bark flap and the whole operated portion was secured tightly with gunny thread (Plate 3 4 and 5)

Plate 1 Patch budding Bud inserted into the rootstock

Plate 2 Patch budding Bud union after securing with a polythene tape

Plate 3 Forkert Budding Bark of the rootstock lifted for inserting the bud

Plate 4 Forkert budding Bud inserted into the stock and covered with flap

Plate 5 Bud union after tieing with gunny thread

3 1 2 3 Modified forkert budding

The operations were done as in forkert method described above except for the partial retention of the flap (Plates 6 and 7)

The budd ng was done during the months of November and December In all the above three methods the binding was removed one month after budding and flap was lifted and removed After the lapse of one more month the bud was examined for initial success by scratching The retention of green colour indicated success and this was expressed as initial success percentage

3 1 3 Effect of different types of bud on bud take

These studies were conducted during October to December in order to find out the best type of bud The treatments include six different types of buds as given below

(i) bud wood full brown with leaf

(ii) budwood full brown with fallen leaf

(iii) budwood turning brown with leaf

(iv) budwood turning brown with fallen leaf

(v) budwood green with leaf

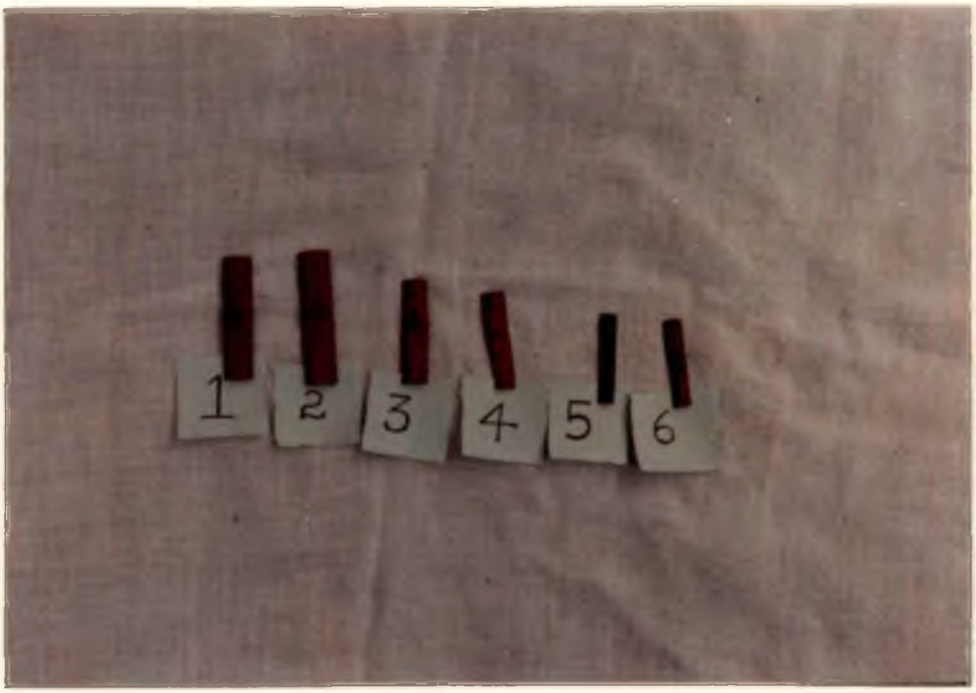
(vi) budwood green with fallen leaf

In the case of budwood with leaf the leaf was removed prior to budding (Plate 8) For each treatment four trees were

Plate 6 Modified forkert Bud flap of rootstock cut to half length and bud inserted into the stock

Plate 7 Modified forkert Bud union after securing with polythene tape

Plate 8 Types of bud utilised for budding after removing the leaves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budded by the forkert method as outlined in 3 1 2 2 and the initial success was recorded as mentioned in 3 1 2 3

3 1 4 Effect of physical treatments on sprouting of buds

In order to force the buds to sprout physical treatments as outlined below were tried on the scion

(i) precuring scion wood by defoliation 10 days before collection

(ii) bending the scion wood two weeks before collection

(iii) stumping the scion wood two weeks before collection

Since these treatments were not effective another set of treatments as outlined below were tried on the stock also

(i) full ringing 20 cm above the budded portion

(ii) stumping of trees 20 cm above the budded portion

(iii) no stumping or ringing (control)

The initial success was ascertained as outlined in 3 1 2 3 After these physical treatments the bud was observed for sprouting The percentage of sprouting number of days for sprouting and growth parameters of sprouts like girth length and number of leaves were recorded

3 1 5 Effect of age of plant

This trial was conducted to find the optimum age of plant for getting maximum success The plants in the age group of one three four five ten and eighteen years were used as rootstocks The trees available at the farmers field in Neduvannur in Ernakulam district were utilised for the study

The plants were budded during August by forkert method using uniformly brown bud with fallen leaf and leaving space on all the four sides The plants showing initial success were stumped 20 cm above the bud union two months after budding and the observations on days for sprouting percentage sprouting and growth parameters like length and girth of sprout and number of leaves produced were recorded

3 1 6 Influence of season on budding

With a view to standardise the season budding was done during June August November and December on ten year old plants Method of budding selection of scion and stumping were similar as in 3 1 5 and observations exacting 3 1 5 were recorded

3 2 Top working

3 2 1 Influence of height and month of beheading on sprouting

The experiment was conducted to standardise the height and month of beheading for maximum production of sprouts For practical consideration the treatments were fixed as

(i) the trees cut above the first tier and

(ii) the trees cut below the first tier

To prevent desiccation and disease infestation the cut surface was smeared with Bordeaux paste (10 0 per cent) and sealed with molten wax To protect from the entry of water it was covered with polythene paper Two trees per treatment were beheaded at monthly interval from April 1991 to October 1991

Observations

The beheaded trees were closely watched and the following observations were recorded

(i) days for first sprouting

(ii) number of sprouts produced at monthly interval upto four months from the day of sprouting After four months new sprout production was observed at an interval of six months

(iii) growth parameters which include length and girth of sprouts

On these newly produced sprouts budding and grafting were done

3 2 2 Standardisation of budding on new sprouts

Budding was tried during a period of nine months starting from November 1991 to October 1992 Age of stock could not be kept constant and the stock in the available age were budded each month

Selection and preparation of buds were similar to that of 3 1 1 except that the thickness of the budwood was less

Methods such as patch forkert and modified forkert budding exacting 3 1 2 were tried (Plate 9) Here the bud was covered with polythene tape of 250 gauge thickness (Plate 10)

Observations

The binding was removed after three weeks and the flap of bark was gently lifted and cut off The budpiece was examined for success by gentle scratching and the initial success was indicated by the retention of green colour The initial success was recorded 30 days after budding

3 2 3 Effect of different types of buds on bud take

Inorder to standardise the type of buds to be used for budding in sprouts four different types of buds were tried viz

(i) budwood full green with leaf

(ii) budwood full green with fallen leaf

(iii) budwood full brown with fallen leaf

(iv) budwood turning brown with fallen leaf

For each treatment four sprouts were budded during November December and February Method followed was forkert budding and the bud was kept loose by leaving space on all four sides

3 2 4 Influence of season and age of sprouts

The experiments were conducted to find out the best season

Plate 9 Forkert budding in new sprouts Insertion of bud into the stock

Plate 10 Forkert method Bud union secured after insertion of bud

and the age of sprout for budding Budding was done during November 1991 and from February 1992 to October 1992 excepting April Regarding the age of sprouts five, seven, eight, twelve and eighteen month old sprouts were utilised for budding Forkert method of budding with brown bud devoid of leaf and loosely kept by leaving space on all four sides was followed

3 2 5 Effect of physical and chemical treatments on survival

As the bud remained alive without sprouting, different treatments were tried to force the buds to sprout

Chemical treatments

(a) Urea 0 5%

(b) Dimethyl sulphoxide (DMSO) 5%

(c) Dimethyl sulphoxide (DMSO) 10%

Physical treatments

(a) Stumping

(b) Half ringing

(c) Full ringing

Chemical and physical treatments

(a) DMSO (5%) + stumping

(b) DMSO (5%) + full ring

(c) DMSO (5%) + half ring

(d) Urea (0 5%) + stumping

(e) Urea (0 5%) + full ring

(f) Urea (0 5%) + half ring

(g) Control

These chemical and physical treatments were given once in two weeks upto two months

3 3 Grafting

3 3 1 Effect of different types of scion material age of stock and season on grafting success

For grafting the scions were taken from healthy semi mature orthotropic shoots from high yielding female trees Shoots having 15 to 17 cm length and nearly of same thickness as that of stock with and without leaves were used as scion Eight different types of scions were utilised (Plate 11)

(i) Full brown with leaf

(ii) Full brown without leaf

(iii) Green with mature leaf

(iv) Green without leaf

(v) Turning brown with leaf

(vi) Turning brown without leaf

(vii) Tender with leaf

(viii) Tender without leaf

In case of scion without leaves, the leaves were removed on the day of grafting keeping the petioles intact on the scion shoots These scion shoots were severed from mother trees on the day of grafting They were wrapped in banana leaf or poly thene till grafting was done

Different age group of stock plants were also utilised which included one two three and four month old sprouts The effect of season for grafting was studied from March to August In each treatment four sprouts were grafted

3 3 2 Method of grafting

The grafting operations were done by wedge method of grafting The stock was decapitated about 8 cm from the emerging point and a vertical incision was given upto 4 to 5 cm from the top of the stock The scion was then prepared like a wedge giving the same length as that of stock with slanting cut on the opposite sides of basal end of scion with a very sharp knife Then the scion was inserted into the prepared wedge in the stock and the graft joint was firmly tied with polythene strip of nearly 200 gauge thickness (Plate 12 and 13) Humid cap was provided with polythene bag on the top portion covering the grafted part

3 3 2 1 After care of grafts

The trees were watered regularly in summer months The sprouts produced down the graft joint were pinched off Once

Plate 11 Types of scion shoot used for grafting in sprouts

Plate 12 Insertion of scion in the wedge cut


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Plate 13 Graft joint after tieing with polythene tape

in two days the humid cap was wetted with water to ensure high humidity

3 3 2 2 Percentage of initial and final success

Number of days taken for initial (30 DAG) and final success (45 DAG) were recorded Sprouting of grafts were observed at fortnightly intervals and the final survival was recorded The growth parameters of the grafts were recorded at monthly interval from the time of sprouting

3 3 2 3 Extension growth of scion

The growth of scion was measured in centimetres from the point where the scion put forth new extension growth at monthly interval

3 3 2 4 Girth of rootstock and scion

The girth of rootstock and scion of the graft was measured one centimetre below and above the graft joint

3 3 2 5 Number of leaves

Number of leaves newly produced at monthly interval was also recorded

3 4 Anatomical studies of bud union

Anatomical studies were conducted to unravel the anatomical changes contributing to budtake and bud sprouting Samples were collected from successful budding which remained unsprouted and the buds which are dried

3 4 1 Collection and preservation of samples

Representative samples of the green, unsprouted buds and dried buds were taken for anatomical studies Two samples each were collected for each treatment Immediately after collection the samples were processed and preserved as detailed below

The samples were killed and fixed using FAA solution (850 ml of 70 per cent alcohol + 100 ml of 40 per cent formaldehyde + 50 ml of glacial acetic acid) The specimens were kept in FAA solution for 72 hours and then transferred to 70 per cent alcohol The samples were removed using sterilised forceps and washed in running water for 30 minutes and finally with distilled water Uniform sections of 40 μ thickness were taken using Reichert sliding microtome as per standard microtomy suggested for hard wood (Cutler, 1978)

The schedule followed for cleaning and staining the sections were as follows (Johansen 1940)

Sections

↓

1% alcoholic safranin (5 to 10 minutes)

↓

30% alcohol (2 minutes)

↓

50% alcohol (2 minutes)

↓

70% alcohol (2 mintues)

↓

80% alcohol (2 minutes)

↓

90% alcohol (2 minutes)

↓

95% alcohol (20 seconds)

↓

Acetone (1 minute)

↓

Xylene (1 minute)

↓

Mounted using DPX mountant

↓

Microscopic examination

A second stain was used for selected sections The procedure followed is furnished below

Sections

↓

0 5% toludene blue O (10 15 minutes)

↓

30% acetone (2 minutes)

↓

50% acetone (2 minutes)

↓

70% acetone (2 minutes)

↓

95% acetone (2 minutes)

↓

Acetone (1 minute)

↓

Xylene

↓

Mounted using DPX mountant

↓

Microscopic examination

3 4 2 Photo micrography

The slides were carefully examined through Carl Zeiss binocular research microscope fitted with objective of magnification ranging from 6 2 to 12 and eyepiece of 10x magnification

Photomicrographs of selected sections were taken using photomicrography system (Zeitz Dialux 20EB Germany) and Kodak Gold Colour film of 100 ASA

3 5 Statistical analysis

Difference among the treatments with regard to the per cent sprouting were tested by Friedman two way analysis of variance of ranks as described by Siegel (1956) When the number of rows and or columns is not too small it can be shown that Xr^2 is distributed approximately as chisquare with df K 1 where

$$Xr^2 \quad \frac{12}{NK \ (K+1)} \sum_{J \quad 1}^{K} (RJ)^2 \ 3N \ (K+1)$$

where N Number of rows

K Number of columns

RJ Sum of ranks in the J^{th} column

$\sum_{J \quad 1}^{K}$ Directs one to sum of the squares of the sums of ranks over all the K conditions

Correlation between age of trees initial success per cent sprouting were compared using Spearman rank correlation coefficient (r_s) The most convenient formula for computing r_s is as follows (Siegel 1956)

$$r_s \quad 1 - \frac{6\, d_i^2}{N^3 - N}$$

d_i Difference between the two ranks

r_s Spearman rank correlation coefficient

N Number of sprouts

Kruskal Wallies one way analysis of variance by ranks

This analysis was done inorder to compare the number of trees sprouts, length of sprout and number of leaves produced in different age groups The Kruskal Wallies test was defined by formula (Siegel 1956)

$$H \quad \frac{12}{N\ (N + 1)} \sum_{j \quad 1}^{k} \frac{R_j^2}{n_j} - 3\ (N+1)$$

R_j Number of samples

n_j Number of cases in j^{th} sample

N n_j the number of cases in all samples combined

R_j Sum of ranks in j^{th} sample (column)

$\sum_{j \quad 1}^{k}$ directs one to sum over the K samples

H is distributed approximately as chisquare with df k-1 for sample sizes (n_j s) sufficiently large

Results

RESULTS

The results of the investigations conducted are given below

4 1 Standardisation of budding on hard trunk

Budding was done in some sex determined male trees and also in juvenile trees to convert them to productive female trees

4 1 1 Selection of buds

The viable and non viable buds available on a budstick were considered as this has a pronounced influence on the success of budding (Table 1) The viable buds were identified by the presence of the scar inside and the non viable buds by the absence of scar and browning From a mean length of 129 cm 19 3 buds could be obtained out of which viable buds and non viable buds were 14 8 and 4 5 respectively Highest number of buds was found when the length was maximum As length decreased the total number of buds also decreased

4 1 2 Influence of different methods of budding on bud take

The influence of different methods of budding such as patch modified forkert and forkert methods on budtake are presented in Table 2 Different methods of budding such as patch forkert and modified forkert method were tried in two ways by keeping the scion bud compact and loose It was found that two

Table 1 Number of buds per budstick obtained for budding on hard trunk

Sl No	Length of budsticks (cm)	Girth of budsticks (cm)	Total number of buds	Number of buds per unit length	Number of viable buds	Number of non viable buds
1	250	5 2	55	0 22	41	14
2	220	5 1	24	0 10	18	6
3	200	5 0	25	0 13	19	6
4	135	4 8	20	0 14	15	5
5	110	4 6	15	0 13	12	3
6	120	4 8	17	0 14	14	3
7	85	3 2	14	0 25	10	4
8	75	3 1	10	0 13	9	1
9	55	3 0	7	0 13	5	2
10	40	4 5	6	0 15	5	1

Table 2 Comparison of different methods of budding on hard trunk

Sl No	Month of budding	Age of trees (years)	Number of trees buded and placement of buds						Initial success (percentage) 2 MAB					
			Patch		Modified forkert		Forkert		Patch		Modified forkert		Forkert	
			Com pact	Loose	Com pact	Loose	Com pact	Loose	Com pact	Loose	Com pact	Loose	Com pact	Loose
1	Nov 91	18	3	3	3	3	3	3						33 33
2	Dec 91	18	3	3	3	3	3	3						66 66

MAB Months after budding

months was needed even to assess the initial success Hence in the preliminary experiments better treatments were selected based on the initial success only Forkert method with the bud placed loose alone was successful during the two months tried November and December with 33 33 per cent and 66 66 per cent success respectively (Plate 14)

4 1 3 Effect of different types of buds on budtake

In order to standardise the best type of bud six different types were tried for forkert budding The buds were inserted by leaving space The data on the influence of different types of buds on budtake revealed that brown budwood with fallen leaf when used for budding alone was effective on budtake (50 per cent) (Table 3)

4 1 4 Influence of physical treatments on sprouting of buds

Physical treatments tried on the scion such as precuring of scion wood bending and stumping of scion wood were not effective in inducing sprouting of buds Three treatments such as stumping, ringing and no stumping (control) were tried on the stock The data presented in Table 4 revealed that stumping (2 MAB) showed highest sprouting per cent (50 per cent) Ringing did not impart a favourable influence on sprouting percentage (25 per cent) and there was no difference from control It was also observed that stumped trees sprouted early (70 days) and

Plate 14 Successful bud union Initial stage of sprout formation

Plate 15 A stumped rootstock producing vigorous sprouts

Table 3 Effect of different types of buds on budtake

Sl No	Types of buds used	Number of trees budded	Initial success (2 MAB)	
			Number	Percentage
1	Budwood green with leaf	4		
2	Budwood green with fallen leaf	4		
3	Budwood turning brown with leaf	4		
4	Budwood turning brown with fallen leaf	4		
5	Budwood brown with leaf	4		-
6	Budwood brown with fallen leaf	4	2	50

MAB Month after budding

Table 4 Effect of physical treatments on sprouting of buds

Treatments	Age of trees (years)	Number of trees beheaded	Sprouting (%)	Days to sprout	Girth of sprouts (cm)	Length of sprouts (cm)	Number of leaves
Stumping	18	4	50	70	1 0	8 0	2
Ringing	18	4	25	173	1 0	6 5	1
Control	18	4	25	295	0 8	5 0	2

stumping substantially reduced the time for sprouting when compared to other treatments (Plate 15) Though the per cent sprouting (25 per cent) was similar in ringing and control the days for sprouting differed The trees which were ringed sprouted in 173 days whereas trees which were not stumped took 295 days Growth of sprouts showed that length of sprout was maximum (8 0 cm) in stumped trees followed by trees where ringing was done (6 5 cm) Girth of sprouts (1 0 cm) was not altered by stumping and ringing and it was less in control (0 8 cm) The leaf product ion on the sprouts was not influenced by the treatment and it remained same in stumped trees and control

4 1 5 Effect of age of plant on budding success

The observations recorded on the biometric details of trees at different age groups (Table 5) indicated that as age of tree increased the height and tier at which budding was done and girth of tree increased Eighteen year old trees the oldest among the treatments recorded the maximum height (89 00 cm) tier at which the budding was done (4 40) and girth of trees (43 80 cm) In youngest group of plants which were one year old budding could be done at a lower height (19 00 cm) and tier (1 00) with least girth of plants (3 9 cm)

The effect of age of plants on sprouting of buds were assessed (Table 6) Friedman s two way analysis of variance

Table 5 Biometric details of trees of different age group

Sl No	Age of trees (years)	Height at which budding done (cm)			Girth of trees (cm)			Tier at which budding done
		Minimum	Maximum	Mean	Minimum	Maximum	Mean	
1	1	17	21	19 00	3 9	4 00	3 90	1 0
2	3	17	58	35 28	6 5	10 00	7 30	1 4
3	4	23	106	65 79	7 0	14 00	10 57	2 4
4	5	48	98	70 14	8 5	18 00	12 92	2 7
5	10	64	80	71 90	18 0	23 00	20 45	3 4
6	18	75	103	89 00	43 8	50 00	43 80	4 4

Table 6 Effect of age of plant on sproutıng of buds

Sl No	Age of trees (years)	Number of trees budded	Inıtıal success (2 MAB)		Per cent sproutıng				Buds remaınıng green (3 FAS) (%)
			Number	Per cent	1 FAS	2 FAS	3 FAS	Total 3 FAS	
1	1	5	4	80 0	0	0	0	0	0
2	3	15	15	100 00	46 70	13 30	33 30	93 30	6 70
3	4	35	32	91 40	31 40	20 00	25 70	77 10	14 30
4	5	7	5	71 40	28 60	42 80	0	71 40	0
5	10	11	8	72 70	9 10	27 20	0	36 30	36 40
6	18	5	3	60 00	20 00	20 00	0	40 00	20 00

FAS Fortnıght after stumping

showed that age of trees did not have any influence on per cent sprouting in three fortnights after stumping The percentage of initial success was positively correlated with per cent sprouting As the percentage of initial success decreased the percentage of sprouting also decreased The per cent of initial success and sprouting were not significantly related to the percentage of buds that remained green

The analysis of age of trees against per cent of initial success and sprouting showed a negative correlation It was found that as age of trees increase the per cent of initial success and sprouting decrease (Plate 16 to 19) The data indicated that initial success was highest (100 per cent) in three year old plants followed by four year old plants (91 40 per cent) The minimum per cent of initial success (60 00 per cent) was for eighteen year old trees Three year old plants which recorded highest initial success had also given the maximum sprouting (93 3 per cent) in three fortnights after stumping One year old plants failed to sprout and barring this treatment the lowest sprouting (36 30 per cent) was obtained in ten year old trees which was on par with eighteen year old plants (40 per cent)

The influence of age of trees on growth of buds are presented in Table 7 Kruskal Wallis one way analysis of variance showed a significant effect of the age of trees on the growth of buds or sprouts The number of sprouts was maximum (2 6) in

Plate 16 A sprouted bud in three year old plant

Plate 17 A sprouted bud in four year old plant

Plate 18 A sprouted bud in five year old plant

Plate 19 A sprouted bud in ten year old plant

Table 7 Influence of age of trees on growth of buds

Sl No	Age of trees (years)	Number of trees budded	Per cent sprouting	Number of sprouts	Length of sprouts (cm)	Number of leaves
1	3	15	93 30	1 30	8 09	3
2	4	35	77 10	1 05	5 74	2
3	5	7	71 40	2 60	3 29	2
4	10	11	36 30	1 25	2 20	-
5	18	5	40 00	1 00	3 50	

Kruskal Wallis one way ANOVA

five year old trees and minimum (1 0) in eighteen year old trees Vigorous sprouts as indicated by maximum length (8 09 cm) and number of leaves (3) were produced in three year old plants This was followed by 5 74 cm length and two leaves in four year old plants Minimum length (2 2 cm) of sprouts was obtained in ten year old trees The sprouts developed on ten and eighteen year old plants failed to produce leaves within three fortnights after sprouting

4 1 6 Influence of season on budding success

The results indicated that season of budding had a pronounced effect on the budding success (Table 8) Spearman rank correlation coefficient (r_s) indicated that initial success was highly correlated with per cent of sprouting and as the per cent of initial success and per cent of sprouting increased the time taken for sprouting decreased or vice versa July was found to be the best season for budding on hard trunk as budding during this month gave highest initial success (80 per cent) and also highest sprouting per cent (50 per cent) The least sprouting per cent (10 per cent) was observed during December

Considering the time taken for sprouting budding during June July and August were equally effective and the buds sprouted within the minimum time (1 month) Sprouting was very much delayed (5 months) when budding was done in December followed

Table 8 Effect of season on budding success

Sl No	Month of budding	Age of trees (years)	Number of plants budded	Percentage of initial success (2 MAB)	Sprouting (MAS)	
					Per cent	Time for sprouting
1	June	10	10	70	50	1
2	July	10	10	80	50	1
3	August	10	10	70	40	1
4	November	10	10	40	20	4
5	December	10	10	30	10	5

MAB - Months after budding
MAS Months after stumping

by November (4 months)

Seasonal influence on growth of buds are presented in Table 9 The analysis showed that the length of sprouts and number of leaves produced were positively correlated The length of sprouts was maximum (6 5 cm) during June followed by July (6 2 cm) Budding during July recorded maximum number of leaves (1 66) Eventhough least sprouting and maximum time for sprouting was during December, vigour of sprouts as indicated by length and number of leaves was comparable to the best month (July) Length of sprouts (2 5 cm) and number of leaves were lowest in August

4 2 Top working

4 2 1 Influence of height and month of beheading on sprouting

Observations recorded on the effect of height and month of beheading on days for sprouting are furnished in Table 10 Beheading the trees above first tier during May recorded the minimum time for sprouting (53 days) where as trees beheaded during October took the maximum time for sprouting (90 days) Likewise for the trees cut below first tier it took maximum of 90 days for sprouting during October and minimum (57 days) during July The mean height of beheading the trees above and below first tier were 92 5 cm and 86 3 cm, respectively Behead ing the trees above first tier was found to be beneficial in

Table 9 Effect of season on growth of buds

Sl No	Month of budding	Average length of sprouts (cm)	Number of leaves produced
1	June	6 50	1 50
2	July	6 20	1 66
3	August	2 50	0 00
4	November	3 25	1 50
5	December	5 00	1 50

0 7

Table 10 Influence of height and month of beheading on days to sprout

Sl No	Month of beheading	Height of beheading (cm)		Days for first sprouting	
		Above first tier	Below first tier	Above first tier	Below first tier
1	April	113 5	85 5	60	69
2	May	80 0	50 0	53	67
3	July	99 0	103 0	55	57
4	August	90 0	91 0	59	69
5	October	105 0	102 0	90	90
	Mean	97 5	86 3	63 4	70 4

reducing the time taken for sprouting i e 63 4 days as against 70 4 days in the case of trees cut below first tier

Data on the sprouts produced at monthly intervals are presented in Table 11 During all the months observed beheading above the first tier produced more number of sprouts except during July Considering the number of sprouts produced initially (IMAS) beheading during April and August were found to be ideal which recorded five sprouts A gradual increase in the product ion of sprouts was observed as time advanced Beheading during August produced maximum number of sprouts (23 Nos) followed by trees beheaded during April (11 Nos) 24 months after sprout- ing in trees cut above Ist tier (Plate 20) Likewise sprouts produced was maximum (10 Nos) in August beheaded trees and minimum (5 Nos) in May beheaded trees for those cut below first tier

The observations on length and girth of sprouts are tabulated in Table 12 The trees beheaded above first tier produced longer sprouts in all the month except May With regard to the length of sprouts one month after sprouting, beheading above first tier during August was most effective (7 20 cm) followed by beheading during April (6 50 cm) This trend was retained after 24 months also Beheading during August produced maximum length of sprouts (29 00 cm) followed by April (28 60 cm) at four months after sprouting

Plate 20 Production of sprouts in beheaded male tree

Table 11 Number of sprouts produced at monthly intervals

Sl No	Month and age of trees beheaded	Number of trees beheaded	Tier	Number of sprouts produced at monthly interval						
				1 MAS	2 MAS	3 MAS	4 MAS	6 MAS	12 MAS	24 MAS
1	April (10 years)	4	Above Ist tier	5	7	8	8	9	9	11
			Below Ist tier	4	5	5	6	6	6	7
2	May (18 years)	4	Above Ist tier	3	5	6	6	6	6	6
			Below Ist tier	1	1	3	3	3	4	5
3	July (18 years)	4	Above Ist tier	2	3	3	3	4	4	4
			Below Ist tier	1	3	4	5	5	6	8
4	August (18 years)	4	Above Ist tier	4	8	12	15	19	22	23
			Below Ist tier	3	4	6	9	9	10	10
5	October (10 years)	4	Above Ist tier	3	3	3	4	4	5	5
		4	Below Ist tier	3	3	4	4	4	4	4

MAS Months after sprouting

Table 12 Growth increment of sprouts

Sl No	Month of beheading	Number of trees beheaded	Tier	Length of sprouts (cm)				Girth of sprouts (cm)			
				1 MAS	2 MAS	3 MAS	4 MAS	1 MAS	2 MAS	3 MAS	4 MAS
1	April	4	Above Ist tier	6 50	14 27	22 50	28 60	1 90	2 40	3 00	3 40
			Below Ist tier	4 50	13 13	16 20	19 50	1 50	2 00	2 50	2 90
2	May	4	Above Ist tier	4 16	10 60	15 90	20 60	1 20	1 80	2 10	2 60
			Below Ist tier	8 00	10 20	13 10	17 80	1 60	1 81	2 00	2 40
3	July	4	Above Ist tier	6 30	13 00	18 60	22 80	1 60	2 00	2 60	2 80
			Below Ist tier	4 20	10 10	16 30	19 60	1 30	1 80	2 00	2 30
4	August	4	Above Ist tier	7 20	16 30	23 00	29 00	1 70	1 90	2 40	2 90
			Below Ist tier	4 50	13 00	17 50	21 50	1 20	1 50	1 90	2 30
5	October	4	Above Ist tier	4 20	10 90	17 30	23 00	1 20	1 80	2 20	2 70
			Below Ist tier	3 70	8 50	13 50	19 80	1 10	1 60	1 90	2 30

MAS   Months after sprouting

Minimum girth of sprouts (1 10 cm 1 MAS) was observed in October beheaded trees throughout the period and it was 2 30 cm four months after sprouting in trees beheaded below first tier Maximum girth (1 90 cm 1 MAS and 3 40 cm 4 MAS) was obtained in tree beheaded in April followed by beheading in August (1 70 cm in 1 MAS and 2 90 cm in 4 MAS) in trees beheaded above first tier Beheading above the first tier recorded more girth of sprouts in all the months tried except May Sprouts on October beheaded trees produced the minimum girth

4 2 2 Standardisation of budding in new sprouts

The number of buds per budstick suitable for budding on new sprouts were recorded (Table 13) The average length of budstick was 101 10 cm with 4 54 cm girth Total buds per budstick was 15 out of which 11 80 were viable buds and 3 20 were non-viable The data on the comparison between different methods of budding namely patch modified forkert and forkert by two methods of bud placement are presented in Table 14 From the table it is evident that among the different methods tried forkert method with the buds placed by leaving space on four sides alone was successful

4 2 3 Influence of different types of buds on budtake

Four types of buds were tried to record its influence on budtake (Table 15) Highest success percentage (100 per cent)

Table 13 Number of buds per budstick obtained for budding on new sprouts

Sl No	Length of budsticks (cm)	Girth of budsticks (cm)	Total number of buds	Number of viable buds	Number of non viable buds
1	80	6 5	10	8	2
2	76	5 0	12	8	4
3	110	4 5	11	11	
4	200	5 1	24	18	6
5	85	3 8	15	12	3
6	65	2 9	8	7	1
7	90	3 1	12	9	3
8	150	5 2	25	20	5
9	80	4 8	18	13	5
10	75	4 5	15	12	3
Mean	101 1	4 54	15	11 8	3 2

Table 14 Comparison of different budding methods on new sprouts

Months	Age of sprouts (months)	Number of sprouts budded and placement of buds						Initial success 30 DAB (%)					
		Patch		Modified forkert		Forkert		Patch		Modified forkert		Forkert	
		Compact	Loose	Compact	Loose	Compact	Loose	Compact	Loose	Compact	Loose	Compact	Loose
Nov 91	5	4	4	4	4	4	4						50
Dec 91	5	4	4	4	4	4	4						75
Jan 91	5	4	4	4	4	4	4	-	-				50

DAB Days after budding

Table 15 Effect of different types of buds on budtake

Sl No	Types of buds used	Number of sprouts budded	Initial success (30 DAB)	
			Number	Percentage
1	Budwood green with leaf	4		
2	Budwood green with fallen leaf	4	-	
3	Budwood turning brown with fallen leaf	4	1	25
4	Budwood full brown with fallen leaf	4	4	100

DAB Days after budding

was obtained from budding done with budwood full brown with fallen leaf followed by budwood turning brown with fallen leaf (25 per cent)

4 2 4 Influence of season and age of sprouts on success

The data on the seasonal influence and age of sprouts on success of budding are presented in Table 16 The age of sprouts could not be kept constant and so the sprouts available in different age groups were tried One year old sprouts recorded maximum initial success (90 per cent) followed by five month old sprouts (80 per cent) Minimum success (30 per cent) was observed in eight month old sprouts

Similarly while observing the girth of rootstock an average girth of 5 25 cm was found to give maximum success (90 per cent) Among the different months compared July was found to be the best season for budding (90 per cent) followed by June and November (80 per cent) The buds remained green for variable periods It was found that the retention of viability was highest (6 months) in one year old sprouts budded in July

4 2 5 Effect of physical and chemical treatments on sprouting of buds

Influence of physical and chemical treatments on sprouting of buds are presented in Table 17 Three sets of treatment viz

Table 16 Effect of season and age of sprouts on success

Sl No	Month of budding	Age of sprouts (months)	Girth of sprouts (cm)			Number of sprouts budded	Initial success (1 MAB)		Retention of viability (MAB)
			Minimum	Maximum	Mean		Number	Percentage	
1	November 91	5	3 0	3 5	3 25	10	8	80	Dried (4 MAB)
2	February 92	7	4 0	4 2	4 1	10	5	50	Dried (3 MAB)
3	March 92	8	4 3	4 5	4 4	10	3	30	Dried (3 MAB)
4	May 92	12	4 9	5 5	5 2	10	6	60	Dried (4 MAB)
5	June 92	12	4 9	5 4	5 15	10	8	80	Dried (4 MAB)
6	July 92	12	5 0	5 5	5 25	10	9	90	Green upto 5 months
7	August 92	18	5 2	5 6	5 4	10	7	70	Green upto 5 months
8	September 92	18	5 2	5 9	5 55	10	4	40	Dried (4 MAB)
9	October 92	18	5 3	6 1	5 7	10	4	40	Dried (3 MAB)

Table 17 Influence of physical and chemical treatments on sprouting of bud

Sl No	Treatments	Treatment intervals	Number of buds treated	Retention of viability (MAB)
1	Urea (0 5%)	Weekly	4	Dried (2 MAB)
2	DMSO (5%)	Weekly	4	Green (7 MAB)
3	DMSO (10%)	Weekly	4	Green (6 MAB)
4	Full ring		4	Dried (2 MAB)
5	Half ring	-	4	Dried (2 MAB)
6	Stumping		4	Dried (2 MAB)
7	DMSO (5%) + Stumping	Fortnightly	4	Green (6 MAB)
8	Urea (0 5%) + Stumping	Fortnightly	4	Dried (3 MAB)
9	DMSO (5%) + full ring	Fortnightly	4	Green (8 MAB)
10	Urea (0 05%) + full ring	Fortnightly	4	Dried (3 MAB)
11	DMSO (5%) + half ring	Fortnightly	4	Dried (8 MAB)
12	Urea (0 5%) + half ring	Fortnightly	4	Dried (4 MAB)
13	Control	-	4	Dried (4 MAB)

MAB - Months after budding

chemical physical and combination of both were compared Of the chemicals used dimethyl sulphoxide (5 per cent) was found best than other two Urea at 0 5 per cent caused dryness of buds after two months of budding Similarly DMSO (10 per cent) resulted in low retention of viability than that of DMSO at five per cent Similarly in physical treatments none was found successful in inducing sprouting These treatments caused early death of the buds (2 MAB)

Among the physical + chemical treatments application of DMSO + full ring and DMSO + half ring was found equally effective in retention of viability Alone or in combination with physical treatments urea was found to kill the buds after short periods when compared to control

4 3 Grafting

4 3 1 Influence of different types of scion and age of stock on graft success

Different types of scions were tried and the results indicated that precuring of scion was not beneficial and did not give any grafting success and such grafts wilted within 15 days The tender scion with and without leaf when grafted was found to wilt in five days itself though provided with humid cap The cutting of wedge was found to be difficult as age of stock increases and two month old stock was found to be the optimum for getting grafting success Among different types of scions tried full green with mature leaf alone gave success Among different months tried grafting during March alone was successful giving 50 per cent success (Table 18)

4 3 2 Growth parameters of grafted sprouts

Growth increment of grafted sprout is presented in Table 19 and depicted in Plate 21 Growth of the sprout was found to be gradual and in six months time the increase in length was 6 8 cm with an added leaf production of 18 numbers The first tiering of sprouts were from four centimetres length of scion and after four months of sprouting High number of side sprouts (seven) was also observed

4 4 Anatomical studies

4 4 1 General anatomy of bud

A mature bud is an embryonic shoot or part of a shoot bearing at the tip the apical meristem from which it originated Most lateral buds initiate in leaf axils and arise from relatively superficial tissues The initiation of bud involves cell division of cell layers in the leaf axil to form a bud protrusion as well as the organisation of the apical meristem Vegetative buds vary greatly in maturity More commonly however they contain a small mass of meristematic tissue nodes internodes and small rudimentary

Table 18 Influence of different types of scion and age of stock on graft success

Month of grafting	Age of sprouts grafted	Types of scion	Initial success (20 DAG) percentage			
			Full brown	Turning brown	Full green	Tender
March	1 MAS	With leaf				
		Without leaf		-		
	2 MAS	With leaf			50	
		Without leaf				
	3 MAS	With leaf				
		Without leaf				-
	4 MAS	With leaf	-		-	
		Without leaf				

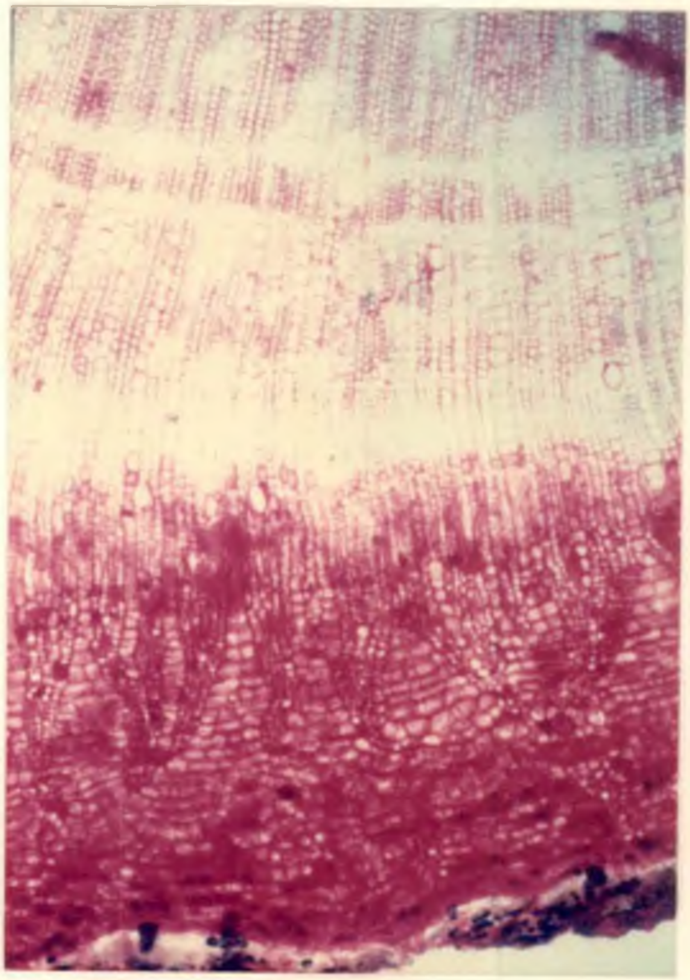
DAG Days after grafting
MAS Months after sprouting

Table 19 Growth parameter of grafted sprout

Sl No	Months after grafting	Increase in girth		Extension length of scion (cm)	Number of leaves produced
		Stock	Scion		
1	January	3 0	2 0	13 00	2
2	February	3 5	2 3	14 70	5
3	March	3 8	2 9	16 30	9
4	April	3 9	3 3	17 10	12
5	May	4 1	3 8	19 00	17
6	June	4 3	4 2	19 80	20

Plate 21 A successful graft six months after grafting

Plate 22 Anatomy of the stem



leaves with buds in their axils all enclosed in bud scales In buds leaf promordia appear in upward succession Hence larger and older leaf primordia are located at the bud base and the smaller rudimentary leaves occur towards the growing point

4 4 2 General anatomy of the stem

In order to understand the type of cells from which callus formation and bud union occurs the structure of mature stem was studied in detail (Plate 22)

Transverse sections from mature stems of the unbudded seedlings revealed a comparatively wide pith which is paranchymatous In the secondary xylem solitary or occasional groups of two to three multiple vessels were found arranged in radial rows Closely spaced uniserial homogenous rays often plugged with gummy infilteration seperated by two to three rows of fibre tissues could be traced A distinct cambial zone and many small tanniferous cells, sieve tubes and companion cells constituted secondary phloem A well defined hypodermis and epidermal layer capped with cuticle could also be seen

Most of the cells in the secondary xylem except those adjacent to the cambium were lignified Vessels are often plugged with tylosis (plugging material) Scalariferous perforations present in the plate of vessels revealed the basic primitive feature of the family Profuse occurrence of tannin containing vessels is a remarkable feature of the anatomy of this species

4 3 3 Anatomy of bud union

Anatomical studies of early union formation showed the necrotic or inactive tissues exposed when stock and scion were wounded Live cells of wood rays in the most recently formed xylem, cambial layers and most recently formed phloem could be traced New xylem tissues which were produced from stock proliferate towards the scion During budding bark side of the stock will be wounded The dark necrotic line is the wounded portion of the stock The gap will be filled by the callus tissues produced from the bark side of the stock itself in due course (Plate 23)

The Plate 24 reveals the orientation of the cells towards the scion It could also be seen that the stock cells formed subsequent to budding are shifted circumferentially to the wounded portion

The undifferentiated callus tissue extends radially from the stock which was found orienting towards the scion near the bud portion This could be seen clearly in Plate 25 The gap which is found in between will be filled by the newly produced cells from the scion A clear cambium (cambial initials) which

Plate 23 Anatomy of bud

Plate 24 Orientation of cells from stock towards the scion portion

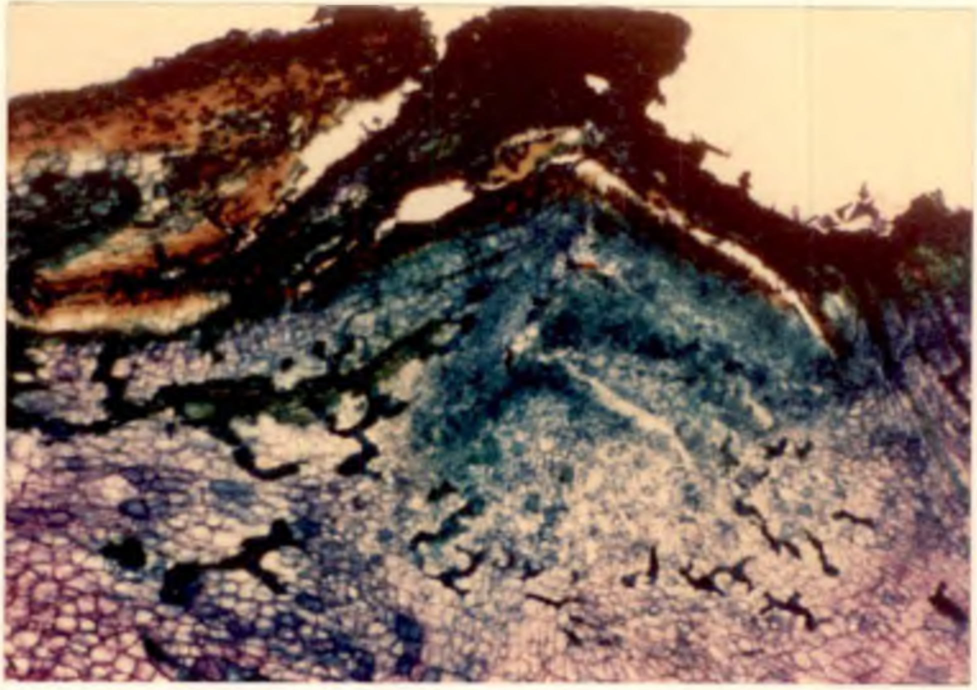
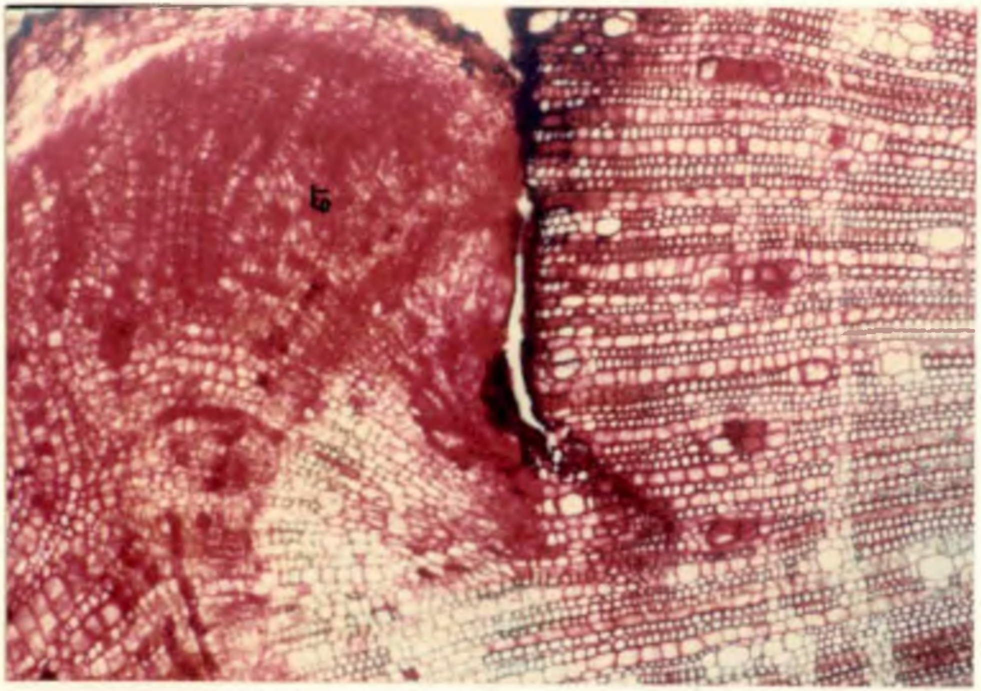
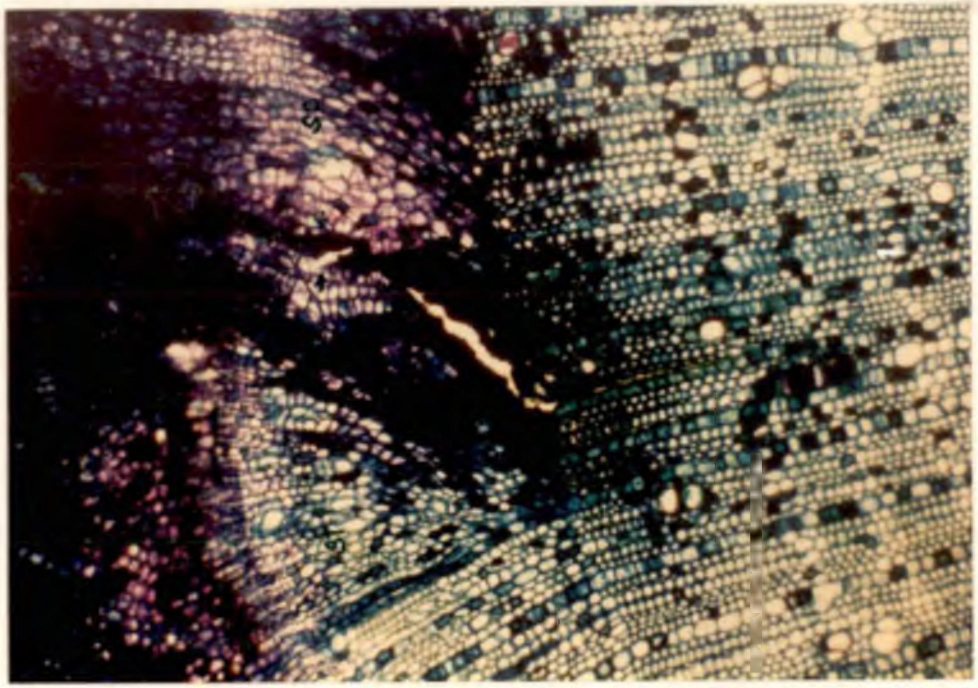


Plate 25 Formation of callus from the stock towards the scion

Plate 26 Cambium producing secondary xylem and phloem





produces secondary xylem inside and second phloem outside could be seen in Plate 26 The callus production from both stock and scion took place which results in good union (Plate 27)

During union formatioon the cells proliferate from the cambial layers (secondary tissue) which was found directed towards the bud which results in a good union The bud scar along with the orientation of secondary xylem cells towards the bud is visible in Plate 28

In later stages of union formation the wound was completely healed by the callus production from both stock and scion This is probably with closer matching of the scion tissue to the root stock stem (Plate 29)

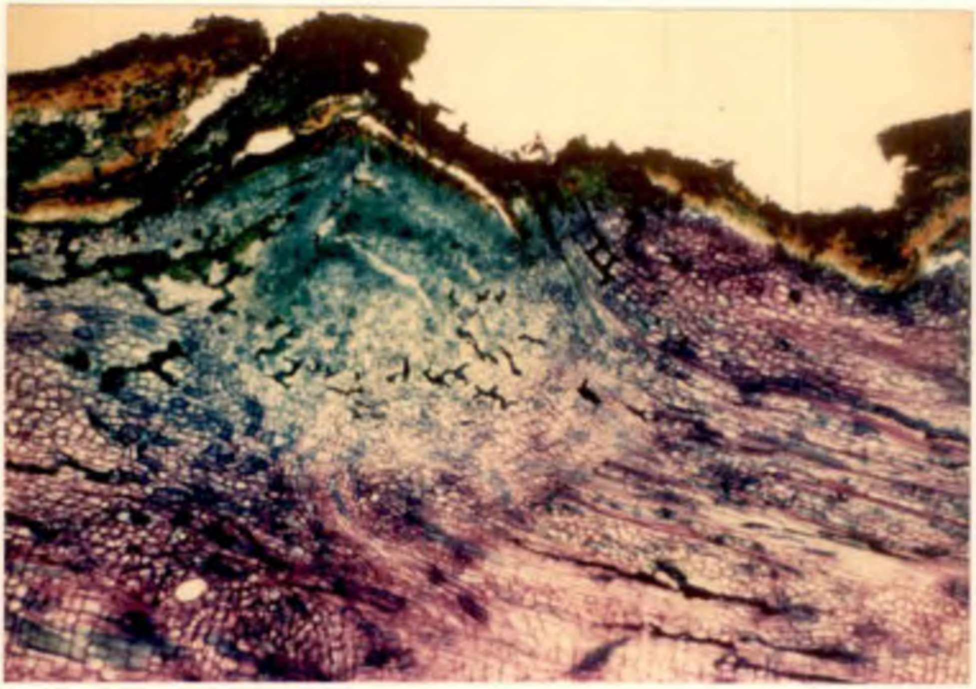
Tissues on the stock opposite the scion had normal configur ation of cells The differentiation of apparently normal secondary xylem and phloem from cambial layers in the callus was observed This shows the properly healed union of the scion with stock (Plate 30)

4 4 3 Failure of bud union

In specimens showing poor union least callus developed beneath the bark flap and the largest woody bridge which accounted for low take It was also observed that there was very

Plate 27 Completed bud union

Plate 28 United portion of stock and scion



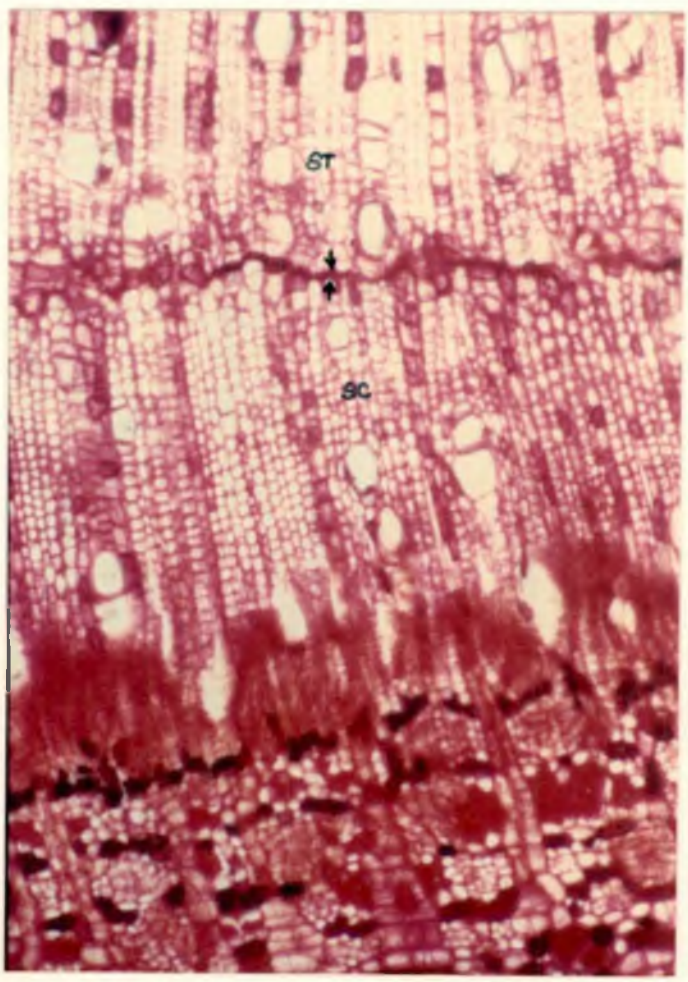

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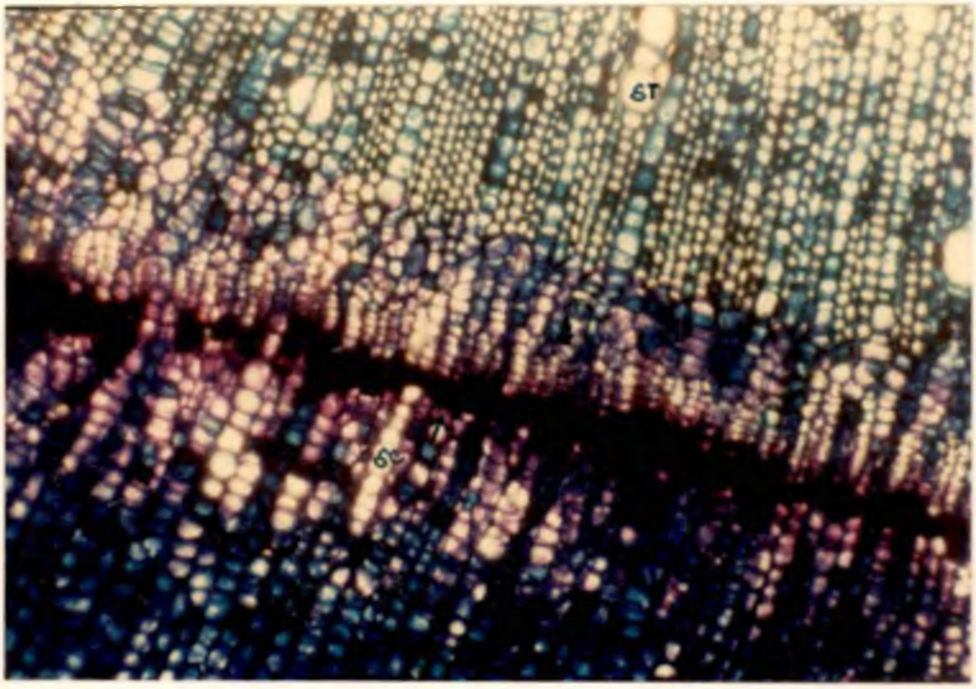
Plate 29 A good union of stock and scion with cambial cells

Plate 30 United bud with good cambial cell formation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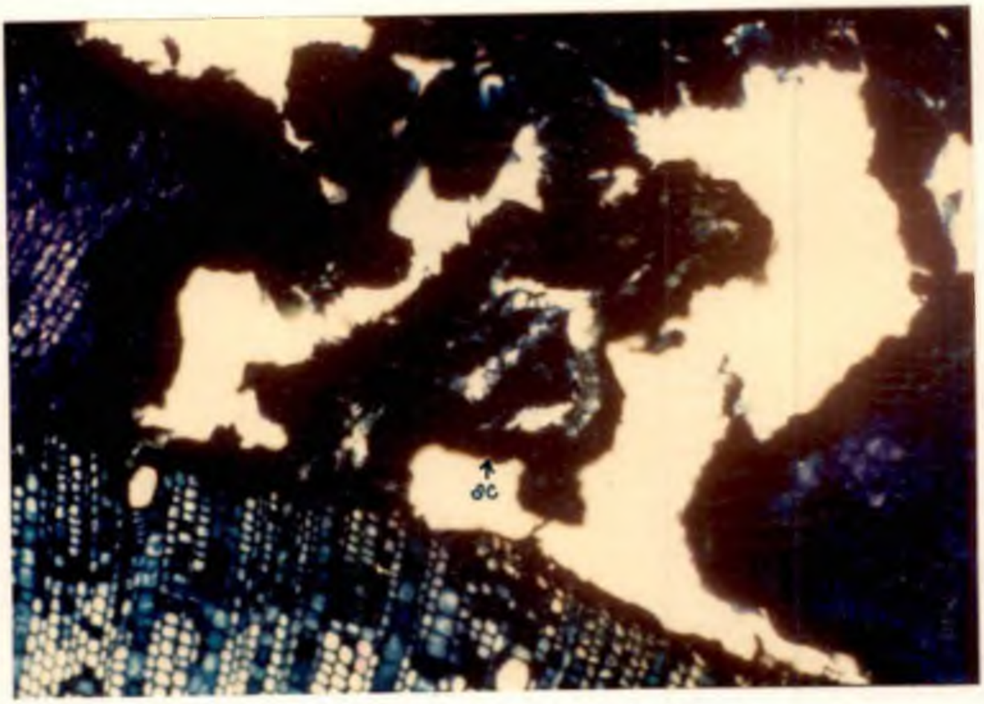
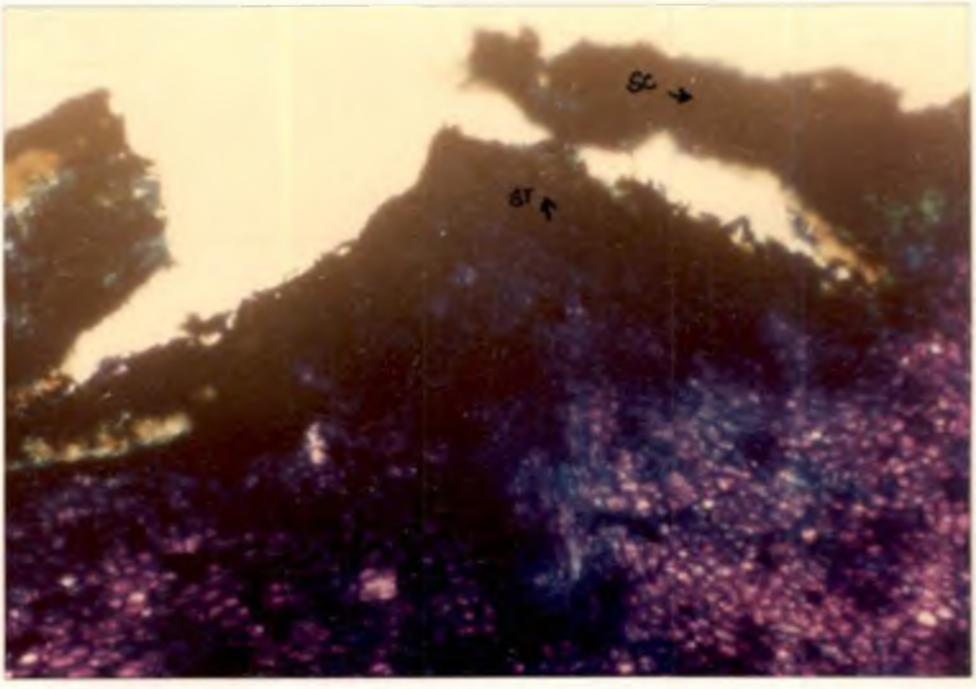
sparse development of new xylem tissues in the bud shield giving a poorer union The dried bud will split off in due course (Plate 31)

In Plate 32 the original stock wound scar and innerface of the original scion are present as lines of necrotic cells The lower end of the callus tapered to a pocket lined with necrotic cells including those representing the old stock flap

Plate 31 Splitting of bud portion

Plate 32 Necrotic cells contributing to bud failure





Discussion

DISCUSSION

Nutmeg (Myristica fragrans Houtt) is usually propagated through seeds but the seedling progenies have a long pre bearing period and exhibits dioecious nature and hence there will be segregation of the sex into male and female usually in 1 1 ratio The absence of a reliable technique to identify the sex in the seedling stage warrants the maintenance of the unproductive male trees upto the flowering stage which takes 5 to 7 years The destruction of the male trees at this stage involves great economic loss to the growers Top working is claimed to be a successful method to transform unproductive male trees to productive female trees in nutmeg But a proper method of top working is not standardised in nutmeg so far Top working is tried with advantage in cashew to rejuvanate unproductive trees In the present study the worth of top working to transform male nutmeg plants to productive females is being probed

5 1 Standardisation of budding on hard trunk

5 1 1 Selection of buds

Selection of buds is the most important factor in budding It affects the success of budding as well as sprouting of buds In nutmeg the presence of non viable buds demand the selection of proper buds to ensure budding success The non-viable buds are indicated by browning and absence of bud scar on the inner side of the bark The viable buds on the other hand have a deep scar of the bud inner to the bark The importance of bud selection was stressed by Manithottam (1992) and opined that sleeping buds or non viable buds should be avoided while taking buds In the present study the average number of viable buds obtained for budding on hard trunk was 14 8 out of 19 3 buds available on a budstick having an average length of 129 cm Thus it is confirmed that both viable and non viable buds are present in nutmeg and so selection of viable buds is warranted in budding

5 1 2 Influence of different methods of budding on hard trunk

The three different methods of budding tried with two methods of bud placement indicated that forkert method with the bud placed loosely leaving space on all the four sides alone was successful The initial budtake observed was 33 33 per cent and 66 66 per cent during November and December respectively In compact placement of buds drying was observed which may be due to the exudation of phenols from the wounded portion of the bark which accumulate at the closely placed cut ends The rainwater sweeping from the trunk also get absorbed at the closely placed cut ends which causes decay and ultimate bud failure For the same reason the forkert budding where the flap of bark is retained above the bud is advantageous in draining out the water and keeping the bud compact which lead to good bud take

Though in contrast with the findings of Manithottam (1992) that patch budding on hard trunk of nutmeg trees to be more successful when compared to other methods the present result can be considered as the further improvement of earlier works Because earlier works indicated that top working of nutmeg trees were not at all successful (CPCRI 1984) However success of forkert method of budding was observed in certain fruit crops like guava (Srivastava 1962 and 1964 Chandra 1965)

5 1 3 Effect of different types of buds on bud take

Of the six different types of buds tried for forkert budding, dark brown coloured bud in the axil of fallen leaves gave maximum success of 50 per cent This observation corroborates the report of Mathew (1979) that dormant buds from mature budwood of one season old were ideal for budding in nutmeg Precuring of budwood to enhance budtake of cacao was reported by Parades, 1949 Kumaran and Nair 1982 Thus the present study revealed the importance of natural curing and mature budwood with ample food reserves for successful budding in nutmeg

5 1 4 Influence of physical treatments on sprouting of buds

In nutmeg as reported elsewhere and observed in the present study buds remained green without sprouting for long periods Attempts on precuring of scion wood by defoliation ten days before collection bending the scion wood and stumping of scion wood two weeks before collection were not effective in inducing the sprouting So the physical treatments such as stumping and ringing were tried on the stock to induce the buds to sprout These treatments were given two months after budding when initial success could be ascertained The results showed that stumping was most effective in bringing the buds to sprout, with 50 per cent sprouting Stumping was also found to be beneficial in reducing the time for sprouting of buds (70 days) as against 173 days in rigning and 295 days in control The sprouts produced from stumped trees were also vigorous as indicated by the length and girth of sprouts and leaf production

In confirmity with the present finding Manithottam (1992) also observed that sprouting of buds could be induced by stumping nutmeg trees one month after the removal of the polythene tape In fruit trees like mango and citrus decapitation is a common practice to force the growth of an inserted bud (Bhan et al 1969 Samson 1986) The beneficial effect of stumping can be explained by the fact that when apical dominance is disturbed the buds below are activated and they start to sprout In contrast with the present finding partial decapitation of stock above the bud union was reported to be the best to induce bud sprouting in nutmeg and full decapitation tended to kill the budded plants and buds remained alive even for one year without sprouting

(CPCRI 1979 and 1984) This type of relationship was also reported in cacao In cacao seedlings, modified forkert budding was found superior and snapping back about 10 cm above the bud two weeks after budding was practiced to encourage the bursting of buds (Kumaran and Nair, 1982)

5 1 5 Effect of age of rootstock on success of budding

In order to standardise the age of rootstock for budding, six different age groups from one year to eighteen year were selected Three years old plants gave maximum initial success and sprouting (100 per cent and 93 3 per cent, respectively) and minimum initial success was observed for eighteen year old trees and minimum sprouting (36 3 per cent) in ten year old trees As age of plant increases the success percentage and sprouting percentage were also found to decrease Height and girth of the tree at which budding was done had also good influence on budding success As the age of plant increases there is every chance for the height and girth of tree to increase and the success percentage decreases

In the present study budding made on one year old seedlings failed to sprout suggesting that a minimum girth requirement is essential for bud sprouting This may be the reason for getting budding success with two year old rootstock as reported earlier (CPCRI, 1979 1980 1984 Manithottam, 1992) Top working in thirty year old trees of apple (Kon *et al*, 1970), 25 year old plum (Srivastava *et al* 1975) twelve year old Satsuma mandarin (Hirobe *et al* 1983) and 11 to 26 year old cashew (Khan *et al* 1986) were reported to be successful, but the success percentages were found to differ It is to be presumed that the type and age of the tree influence the success percentage as indicated by the present study also

Budding done on younger plants was also found to be advantageous in getting early sprouting and vigorous growth of sprouts In three year old plants 46 7 per cent of the plants sprouted within the first fortnight against 9 1 per cent in ten year old plants Vigorous sprouts as indicated by maximum length (8 09 cm) and number of leaves (3) were produced in three year old plants followed by four year old plants Added advantage of budding in younger plants was that it could be done at a lower height which reduces the chance for wind damage through branch snap at the bud union

5 1 6 Influence of season on success of budding

Budding success was found to be influenced by season The best season for budding on hard trunk was found to be July which recorded highest initial success (80 per cent) and also highest sprouting per cent (50 per cent) The initial success was found to be highly correlated with sprouting The time taken for sprouting was also influenced by the season of budding The budding done during June July and August were equally effective and the buds sprouted within the minimum time (1 month) Time for sprouting was found to be enhanced when budding was done in December (5 months) Trees budded during June recorded maximum length of sprouts (6 5 cm) followed by July (2 5 cm) budded trees Budding during July recorded maximum number of leaves (1 66)

This observation is in confirmity with Mathew (1979) and Mathew et al (1984) who found that budding done during July gave maximum take in nutmeg In guava and cashew also July was found to be the best season (Srivastava 1962 Palaniswamy and Hameed 1976) In cocoa budding success was highest in June followed by September in four month old seedlings as reported by Nagabushanam and Nair (1988) The favourable influence of rainy months over dry months on budding success as reported by several workers in different crops could be visualised in this study also

5 2 Top working

5 2 1 Influence of height and month of beheading on sprouting

The trees were beheaded above and below first tier to find out the comparative sprout production It was observed that trees beheaded above first tier produced more sprouts (23) and also less time (63 4 days) for first sprouting in contrast to 10 sprouts and 70 4 days for first sprouting for the trees cut below first tier As the height of beheading increases the sprout product ion also increases Trees beheaded during August produced more sprouts (23) and those during October produced minimum sprouts (5 sprouts) Considering the number of sprouts produced trees beheaded during August was found superior with maximum sprout production in trees cut above and below first tier Beheading during August was also beneficial in getting vigorous sprouts as indicated by maximum length at 24 months after sprouting Regard ing the growth of sprouts, maximum length (29 00 cm) and maximum girth (2 90 cm) were observed in August beheaded trees Minimum length (20 60 cm) and girth (2 60 cm) were observed in trees beheaded during May

For top working, heading back at different heights was followed in other tree crops In plum trees heading back within 1 to 1 5 cm from the main crotch and top working by budding gave good yield (Srivastava et al 1975) Cashew trees headed back to about 0 75 - 1 5 m at monthly interval between September 1983 and August 1984 resprouted in 30 35 days (Khan et al 1986)

5 2 2 Standardisation of budding on new sprouts

In the case of budding on new sprouts also the number of viable buds per budstick were accounted A budwood measuring

101 cm length with a girth of 4 54 cm gave an average of 15 buds out of which 11 80 were viable and 3 20 were non viable Experiments conducted with different methods of budding on new sprouts n nutmeg showed that forkert budding with bud kept by leaving space on all the four sides gave highest success percentage In confirmity with the present finding Mathew (1979) has also reported success in forkert budding of one year old nutmeg seedlings Of the four types of buds tried for forkert budding highest success percentage (cent per cent) was obtained from budding done with budwood full brown with fallen leaf followed by budwood turning brown with fallen leaf (25 per cent) Green buds were found unsuitable for budding which failed to give any response

Studies on the influence of season and age of sprouts on budding success showed that the sprouts which were one year old recorded maximum initial success (90 per cent) with a minimum (30 per cent) in eight month old sprouts Preferential influence of July budding on budding success was also reported from CPCRI (1984) in nutmeg and Singh and Srivastava (1962) in mango Age of rootstock was also found to be a factor determining budding success as observed by Prasad et al (1973) in mango

5 2 3 Influence of physical treatments on budding

The buds remained alive for months together without sprouting and so physical and chemical treatments were tried to force the buds to sprout None of the treatments was effective in inducing bud burst In rubber dimethyl sulphoxide (5 per cent) was found to induce bud break (Jaafar 1984) but this beneficial effect was not noticed in the present study However dimethyl sulphoxide (5 per cent) was found to be the best which helped in the retention of viability for a longer period (7 months) Urea (0 5 per cent) and stumping the rootstock above the bud union were least effective in inducing sprouting and this is in confirmity with the result of budding trials in nutmeg reported from CPCRI (1979 and 1984)

5 3 Grafting

The experiments on scion selection showed that precuring of scion and removal of leaves prior to grafting were detrimental for graft success Retention of leaf on the scion was found to be essential and scion shoot which were full green with mature leaf alone gave a successful graft union The different age group of sprouts tried for grafting showed that two month old stock was optimum for getting grafting success As age of stock increased cutting of wedge was found to be difficult and have a negative influence on success Among the different months tried success was obtained only during March Compared with budding on hard trunk grafting was found to be less successful corroborating the report from CPCRI (1982) in top working nutmeg

Seasonal influence on grafting in different crops was found to be variable In cashew, for top working, softwood grafting done during April gave highest success followed by March (Khan et al 1986) Propagation done during the month of March to May was found to be ideal for cashew under Madakkathara conditions (Pushpalatha et al 1991) Epicotyl grafting in cashew at Ullal produced maximum take during February to May (Murthy et al , 1985)

The growth parameters of the grafted sprouts in nutmeg showed that in six months the increase in length was 6 8 cm with an added leaf production of 18 numbers High number of side sprouts (seven) was also observed

5 4 **Anatomical studies**

The anatomy of the stem revealed that the vessels were blocked with tyloses i e the plugging materials The gummy exudate mainly consists of phenols which cause deleterious effect on the newly inserted bud and hinder the union The long time for healing the union is because callus production initiates from the bark side of stock which orients towards the scion and quick and proper union can be ensured if there is callus production from the scion side which appear to be a rather slow process in nutmeg

During union formation the cells proliferate from the cambial layers which was found directed towards the bud which results in a good union as observed in mango and citrus (Soule 1971 Skene et al 1983)

Differentiation of callus to new xylem tissues also initiate from the stock side and proliferates towards the scion The gap was found to be filled by the callus tissues produced from stock

The anatomical observations of the dried up buds suggest that the failure of bud union can be attributed to poor callus development and very sparse differentiation of xylem tissues in the bud shield even after several days of budding which lead to a large woody gap between stock and scion Formation of thick necrotic cells on the wounded surface of the stock and scion which hinder callus development is another probable reason for bud failure

When the stock was cut back to force the scion bud into active growth new callus will be proliferated to heal the union which will not get united with the old callus probably due to the slow production of initial callus Failure can also be attributed to the above reason which is in line with the finding of Jose (1989) in jack and Nazeema (1992) in Garcinia cambogia

The results of the present study bring out the usefulness of _in situ_ budding even before sex determination as a reliable technique to assure femaleness and productivity in nutmeg _In situ_ plants with the vigorous root system enable faster growth of the inserted bud This increased growth rate has an edge over the observed slow growth of grafted plants In a crop like nutmeg, which exhibits dimorphic branching, availability of orthotropic shoots is a limiting factor for commercial production of vegetative propagules Viewing from this angle also budding which enable production of more number of propagules from a budstick is preferential over grafting Further work to standardise the _in situ_ budding methods for plants younger than three years is warranted

Summary

SUMMARY

Investigations on top working in nutmeg were carried out at the Department of Plantation Crops and Spices College of Horticulture Vellanikkara during the period 1991 93 For the study nutmeg trees at State Seed Farm Mannuthy farmers field at Mannuthy and Ettumuna in Thrissur district and Neduvannur in Ernakulam district were used The following experiments were undertaken during the course of investigation

(i) Budding on hard trunk

(ii) Beheading the male trees and then budding and grafting on the emerging sprouts

(iii) Anatomical studies of the bud union

The salient findings of the study are summarised below

The two types of buds namely viable and non viable could be distinguished by the presence of bud scar at the inner side of the bark for the viable buds and absence of scar and browning in the non viable buds The viable buds alone produced good union

Standardisation of the methods of budding on hard trunk revealed that forkert budding was most successful when compared to patch and modified forkert methods For successful union the buds should be placed by leaving space on all four sides For obtaining maximum success the buds should be selected from brown budwood with fallen leaves

The trials conducted to study the effect of physical treat ments on sprouting of buds indicated that stumping the plants two month after budding was the most effective in achieving a quicker bud burst Stumping had the added advantage of producing vigorous sprouts Ringing 20 cm above the bud union also had a favourable influence on sprouting when compared to control

The observations on the biometric details of trees at different age groups showed that as age of tree increases the height and tier at which budding can be done and girth of the trees also increases Budding done on different age groups of trees were compared and the results showed that as age of trees increases the budding success and sprouting percentage decrease

Budding done on three year old plants were found to be the best which recorded cent per cent initial success and maximum sprouting (93 3 per cent) with good vigour of sprouts Budding done on younger plants had the added advantage of lowering the budding height and reduced bark thickness which enable easy peeling and good matching with the inserted bud resulting in good bud take and sprouting

July was observed to be the best season for budding on hard trunk which recorded highest initial success (80 per cent) and sprouting (50 per cent) followed by budding in June Budding during dry and cold month of December was the least effective and sprouting was very much delayed

Heading back the male trees above first tier was found to be beneficial in reducing the time taken for sprouting and for producing vigorous sprouts as indicated by maximum length and girth Beheading during August was found to be superior with regard to the production of sprouts and vigour of sprouts closely followed by April

Among the three methods of budding tried on new sprouts forkert method with buds placed by leaving space on all four sides gave highest percentage of success The best type of bud was identified to be full brown with fallen leaf Forkert budding on new sprouts revealed that one year old sprouts as stock recorded maximum initial success and July was found to be the best season Phys cal, chemical and physical + chemical treatments tried to induce bud break were not effective

Cleft grafting technique tried in sprouts emerging on beheaded trees indicated that for successful graft union the scion shoots should have mature leaf with full green stem and the optimum age of the stock was two months Grafting showed the least response among the various methods tried and success was observed only during March

The anatomical reasons identified for failure of bud union were, poor callus development beneath the bark flap leaving a large woody bridge very sparse development of new xylem tissues in the bud shield giving poorer union presence of necrotic tissues which hinder the formation of callus and lack of union between old callus and the callus proliferated, when the stock was cut off

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ABSTRACT

The present investigations on Standardisation of top working in nutmeg (Myristica fragrans Houtt) were conducted at the Department of Plantation Crops and Spices College of Horticulture Vellanikkara Thrissur during 1991 1993 For the purpose of the study nutmeg trees available at State Seed Farm Mannuthy and farmers field at Mannuthy and Ettumuna in Thrissur district as well as Neduvannur in Ernakulam district were utilised

The presence of two types of buds on the bud stick could be distinguished by the bud scar at the inner side of the bark for the viable buds and absence of scar and browning in the non-viable buds

Among the different methods tried in situ budding on hard trunk proved to be most successful Forkert method with bud selected from brown bud wood with fallen leaves and the buds inserted by leaving space on all the four sides gave maximum success In order to achieve a quicker bud burst stumping the plants two months after budding was most effective

Budding on hard trunk could be done with maximum success in three year old plants and July was found to be the best season

Trials on topping of male trees indicated that heading back the trees above the first tier during August was found to be the

best with regard to sprout production and reducing the time for sprouting

Forkert budding on new sprouts gave only initial success which failed to sprout even with physical and chemical treatments

Grafting on new sprouts showed least response among the methods tried Successful graft union was obtained during March with scion shoots having mature leaf and full green stem and stock having two months growth

The anatomical studies revealed low callus development and sparse differentiation of vascular tissues in the bud shield presence of necrotic tissues hindering the formation of callus and the lack of union between old and new callus as the probable reasons for bud failure

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