EVALUATION OF PROPAGATION TECHNIQUES AND ROOTSTOCK STUDIES OF MANGO (*Mangifera indica* L.)

by

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(2016-22-001)

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DECLARATION

I, hereby declare that this thesis entitled **"EVALUATION OF PROPAGATION TECHNIQUES AND ROOTSTOCK STUDIES OF MANGO (*Mangifera indica* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
Bp	-	Base pairs
CD	-	Critical difference
CRD	-	Completely Randomized Design
cv.	-	Cultivar
DAS	-	Days after sowing
DAG	-	Days after grafting
DNA	-	Deoxyribonucleic acid
DPX	-	Distyrene Plasticizer Xylene
EST	-	Expressed Sequence Tags
et al.	-	Et. alia and (others)
ETBR	-	Ethidium bromide
Fig.	-	Figure
GA_3	-	Gibberlic Acid
GI	-	Germination index
ISSR	-	Inter Simple Sequence Repeat Markers
KNO_3	-	Potassium Nitrate
MGT	-	Mean germination time
Mm	-	Milli molar
NS	-	Non-significant
OD	-	Optical density
PCR	-	Polymerase Chain Reaction
PVP	-	Poly Vinyl Pyrrolidone

RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
RH	-	Relative humidity
rpm	-	Rotation per minute
RWC	-	Relative water content
SDS	-	Sodium Dodecyl Sulfate
SE (md)	-	Standard error of mean difference
SSR	-	Simple Sequence Repeats
TBE buffer	-	Tris- Borate- EDTA buffer
TE buffer	-	Tris EDTA Buffer
viz.	-	*Videlicet* (namely)

Introduction

1. INTRODUCTION

Mango (*Mangifera indica* L.) is the most important fruit crop grown in India and is revered as the 'king of fruits' due to its delicious flavour and high nutritional and therapeutic values. India is the largest producer of mango in the world, occupying an area of 2.25 million ha with a production of 21.82 million tonnes (NHB, 2018). In Kerala, even though mango is not considered as a commercial crop, trees are inevitable components of every homestead. The state has a rich collection of traditional mango varieties. Currently, mango is cultivated in Kerala in an area of 79,496 ha with a production of 4.2 lakh tonnes (FIB, 2019).

The importance of mango cultivation is increasing due to great demand for fresh fruits and processed products in the international markets. The area under mango cultivation in India is increasing, but the pace of development is not perceptible.

Mass multiplication of rootstocks from sexually developed seedlings, which are highly heterozygous has caused non uniformity in mango orchards (Srivastava *et al.*,1977). Moreover, mango is propagated commercially by grafting using random rootstocks of unknown pedigree. This practice has resulted in loss of desirable characters over continuous years of cultivation and selection. Use of vegetatively propagated clones from desired parents as rootstock could thus help to overcome such issues. The growth potential and overall performance of different rootstocks may be easily predicted at nursery stage itself by observing various vegetative and growth parameter, which in turn helps to screen the rootstocks at very early stage (Bhajan, 1987).

The introduction of polyembryonic rootstocks in the area of propagation is of great importance since they produce one zygotic and several nucellar plantlets. There are large number of polyembryonic mango varieties in Kerala (Naik *et al.*, 2000; Radha and Manjula, 2000).

In polyembryonic genotypes, the seedlings developed from nucellar embryos are clones of the mother plant (true-to-type), regardless of the pollen parent genotype. Hence they give more uniformity to the orchard, whereas this

zygotic plantlets showing variability are mainly preferred for breeding programmes.

Identification of nucellar seedlings is usually done based on vigour. However, some studies suggest that zygotic seedlings need not always be less vigorous. In general the zygotic seedlings are smaller and weaker in nature than the nucellar seedlings. Different methods *viz.,* flow cytometry, rootstock colour test, examination of morphological traits, biochemical markers and isoenzyme pattern analysis could not be employed commercially to discriminate both types due to their varying degrees of reliability. Among various marker systems, the codominant simple sequence repeats (SSRs) are quick and more efficient to differentiate the zygotic and nucellar seedlings.

Standardisation of polyembryonic mango rootstocks for fruit quality, dwarfness, stress tolerance and yield is important in view of the emerging issues related to climate change and severe dearth of land area for cultivation. The screening of seedlings at very early stage of growth can be confirmed through certain selection criteria introduced by Majumdar *et al.* (1972). The various vegetative and physiological parameters to predict vigour include leaf area, stomatal count, chlorophyll fractions, phenolic content and anatomical peculiarities especially with regard to xylem vessels. But the efforts in this regard are limited. However, attempts have been made using indigenous mango varieties of north and central Kerala to study the dwarfing potential (Marie, 2001; Shenoy, 2016) as well as histological and biochemical characterisation (Babu, 2005).

Mango seeds are in general recalcitrant. Moreover, they are available only during April- May months which are the drier parts of the year. So stone germination and plant vigour are very low. Pre-sowing treatments could be the best method to induce early germination, boosting growth, enhancing the seedling vigour and reducing mortality (Rao and Reddy, 2005a; Shaban, 2010a).

Being a highly heterozygous and cross pollinated perennial crop, vegetative propagation methods like grafting have to be resorted to get true-to-

type progenies and accomplish precocity in bearing. The main grafting methods used are veneer grafting, side grafting, stone grafting, soft wood grafting and inarching.

It is important to study the success of these methods in various commercially grown mango cultivars under different agro climatic conditions. Nonetheless, its adoption and success would differ from cultivar to cultivar, as well as region to region depending on the climatic conditions prevailing on a particular area and plant factors *viz.*, stock, scion etc.

Under Kerala Agricultural University, several attempts have been made to standardize the various aspects of propagation by epicotyl grafting (Dhungana, 1984; Ratan, 1985 and Radhamony, 1987), softwood grafting (Geetha, 1993) and veneer grafting (Dhungana, 1984). However, efforts made to evaluate the performance of different propagation techniques in various plant propagation structures are limited.

Under these circumstances, the present investigation entitled "Evaluation of propagation techniques and rootstock studies of mango (*Mangifera indica* L.)" were accordingly taken up with following objectives:

- To screen local mango varieties/ collections for polyembryony.
- To study the pre-sowing treatments, sowing position and age of stone after extraction from fruit on germination of mango stones.
- To screen local mango varieties for use as dwarfing rootstocks.
- To study the effect of two propagation methods in three modified environments on three varieties of scions.

Review of Literature

2. REVIEW OF LITERATURE

Mango has a long history of cultivation in India for over 4000 years. India's heritage and culture is so much associated with mango that it has earned the reputation of being the 'Apple of the tropics'.

Globally India leads in mango production as well as consumption. However the potential of mango for its commercial production has not been fully exploited. Selection of quality planting materials of improved varieties, adoption of scientific practices right from planting to harvest including adoption of pre sowing treatments, selection of appropriate vegetative propagation methods and high density planting utilizing dwarfing rootstocks would serve to improve the productivity. Ensuring uniform plant stand in commercial orchards is yet another pre-requisite for optimizing productivity, for which polyembryonic rootstocks can be utilized.

Unlike in other fruit crops, not much research work has been done to screen the local mango varieties for polyembryony and to standardize rootstocks and appropriate vegetative methods of propagation. Although Kerala, the land of diversity, has a rich assemblage of local mango germplasm (Naik, 2000; Jyothi, 2000 and Simi, 2006) including polyembryonic genotypes, only meagre attempt has been made so far to evaluate these types for their efficiency to be used as potential rootstocks. Review of research works on polyembryony, germination, rootstock studies and propagation techniques in mango and some other fruit crops has been presented in this chapter.

2.1 Polyembryony in mango

It is a common trait in mango cultivars derived from South-Eastern Asian ancestry (Singh, 1960) and is characterized by the development of more than one embryo in a single seed, and all may be nucellar (Degani *et al.*, 1993) or one may be zygotic and the others, nucellar (Ravishankar *et al.*, 2004).

The phenomenon of polyembryony was first discovered by Anton Van Leeuwenhoek in 1719 in citrus (Batygina and Vinogradova, 2007). Occurrence of polyembryony in mango has been reported by many authors from different

countries (Sturrock, 1968; Schnell and Knight, 1992; Aron *et al.,* 1998; Radha and Manjula, 2000).

Polyembryony may arise due to the formation of embryos from embryo sac cells other than egg, pro-embryo cleavage, activation of some sporophytic cells of the ovule or formation of more than one embryo sac within the same ovule. In polyembryony system, the most common way for formation of polyembryonic embryo is by activation of some sporophytic ovular cells. The adventive embryos are mainly arising from sporophytic maternal tissue i.e. outside the embryo sac. In nucellarembryony, the embryos are developing from nucellus tissue, which is the most common feature in many of the angiosperm families of greater horticultural significance (Aleza *et al.,* 2010).

The polyembryony is genetically controlled, and in mangoes, it is due to a single dominant gene (Aron *et al.,* 1998). In polyembryonic genotypes, the plants which are developing from nucellar embryos are clones of the mother plant (true-to-type), regardless of the pollen parent genotype. Being true-to-type as the mother parent, it will supposedly give more uniformity to the orchard whereas zygotic plantlets are mainly preferred for breeding programmes as they do not maintain the same genetic constitution as that of mother plant (Abirami *et al.*, 2008). In polyembryony, the additional embryos arise as a result of the differentiation and development of various zygotic and maternal tissues associated with the ovule of seed. Earlier this phenomenon in angiosperms was regarded as an abnormal feature but now it is considered as one of the desirable features in fruit crops like mango, citrus, rose apple, jamun, etc. to obtain true-to-type planting materials (Raymond and Robert, 1999).

Introduction of polyembryonic rootstocks in mango has got more relative advantages. The fact is that very meagre research have been attempted to standardize the polyembryonic mango rootstock for fruit quality, vigour management, biotic and abiotic stress tolerance and yield. The nucellar seedlings of citrus and mango provide better clones of orchard rootstock than cuttings (Bhojwani and Bhatnagar, 2000) and provide more consistent results in fruit production.

The introduction of polyembryonic rootstocks may extend the mango cultivation to those areas where the external stresses limit production and potential yield. Also, many polyembryonic varieties can be used as potential dwarfing rootstocks in high density orcharding system. In general, the polyembryonic mango varieties are heavy bearers than the monoembryonic varieties. Hence proper understanding and exploitation of this behaviour of a particular variety will help to improve the germplasm pool of commercial as well as local landraces and the yields of these varieties could be increased (Sturrock, 1967).

The mango stone germination and seedling growth of polyembryonic cultivars were studied by Srivastava *et al.* (1980b). They found the highest germination (75.93 %) in Olour and the lowest (40.57 %) in Nekkare. The number of plantlets per stone ranged from 1 to 7 whereas, the number of embryos varied from 2 to 10. Vellaikolamban and Muvandan recorded more number of seedlings per stone followed by Bappakai, Kurukkan and Olour. Earliness in germination (16 days) was observed in Vellaikolamban, while cv. Pahutan recorded the highest number of days (26 days) for germination. Vigorous growth was observed in cv. Muvandan and Bappakai, while less vigorous nature with shorter internodes was observed in cv. Chandrakaran, Goa, Olour and Kurukkan.

Geeta (1993) reported that the germination percentage of polyembryonic varieties, Puliyan, Chandrakaran, Olour, Tholikaipan and Muvandan were comparatively higher than the monoembryonic variety, Banglora. Among the different polyembryonic varieties studied, Tholikaipan recorded the highest germination percentage of 60.66 % followed by Puliyan (51.00 %). The Chandrakaran recorded the lowest per cent of germination (29.33 %). The monoembryonic variety Banglora recorded least germination percentage (5.67 %) among all the varieties studied. The largest number of seedlings per stone (1.74) was noted in Muvandan and the lowest (1.11) in Chandrakaran.

Singh and Reddy (1990) noted wide variations in the extent of polyembryony among various polyembryonic mango cultivars and reported the highest number of seedlings per stone in cv. Peach and Kurukkan. Khobragade *et*

al. (2000) reported the highest number of plantlets per stone in mango cv. Kitchner (3.66) and the lowest in Nekkare (1.14). The extent of polyembryony in different mango rootstocks at nursery stage was evaluated by Rao and Reddy (2005b). They observed 338.00 per cent polyembryony in cv. Peach, followed by EC 959862 (296.00 %) and 138.00 % in cv. Kurukkan.

The comparative study of seed germination in monoembryonic var. Haden and polyembryonic var. Manila done by Barbosa *et al.* (2009) revealed that var. Haden germinated faster than var. Manila and Haden showed high germination percentage (75.00 %). At all the growth stages, the survival rate of Haden was superior to that of Manila.

If a cultivar has more than 80 per cent polyembryony, the possibility of obtaining nucellar seedlings increases and making it possible to have uniform rootstocks (Santos *et al.*, 2010). Ochoa *et al.* (2012) studied the occurrence of polyembryony in mango cultivars Ataulfo and Manila to distinguish the zygotic and nucellar plantlets and found 97 per cent polyembryony in Manila and 95 per cent in Ataulfo with an average of 3.4 and 3.2 embryos per seed, respectively. Both the cultivars had 2 to 4 embryos in more than 80 per cent of their stones.

Abirami *et al.* (2011a) conducted an experiment to compare the fruit characters, germination behaviour and seedling growth of twelve polyembryonic and ten monoembryonic genotypes in mango. The results of the studies on germination behaviour revealed that the monoembryonic genotypes were superior over polyembryonic ones. The monoembryonic varieties Mallika and Amrapali required the least number of days for germination (17.00 days), 50 per cent germination (27.30 and 27.70 days), the highest rate of germination (0.032), earliness index (1.53 and 1.52) and germination index (1.72 and 1.69). The number of plantlets per stone ranged from 1.2 to 2.8 among the various polyembryonic genotypes. The highest extent of polyembryony was observed in Peach, followed by Kurukkan and the least was in Vellaikolamban. Based on the nursery evaluation, among the polyembryonic genotypes, Nekkare recorded the highest seedling height (38 cm) and Starch recorded the least (13.10 cm). The significantly higher fresh (30.50 g), dry weight of seedlings (14.80 g), vigour

index based on growth (9477.00) and weight basis (2295.00) were recorded in Nekkare, followed by Bappakai and Kerala 5, whereas the least fresh weight (15.10 g), dry weight of seedlings (4.40 g), vigour index based on growth (1489.00) and weight basis (358.00) were in starch. Among monoembryonic genotypes, the highest seedling height (44.6 cm), fresh and dry weight (30.70 g and 15.10 g), vigour index based on growth and weight basis (9807.00 and 2697.00) were observed in Bombay Green and the least fresh weight (20.80 g), dry weight of seedlings (24.80 g), vigour index based on growth (2395.00) and weight basis (806.00) were in Amrapali.

Sane *et al.* (2015) studied the implications of polyembryony in germination and growth of seven open pollinated mango cultivars. They reported the highest germination percentage (75.85 %) in Bappakai, followed by Vellaikolamban (73.80 %) and Kurukkan (73.70 %). The highest germination index (0.87) was noted in cv. Nekkare. Bappakai recorded the highest seedling vigour index (2000.00) on growth basis. The least mean germination time (1.49) was in Kurukkan. The lowest germination percentage (35.44 %), germination index (0.46), seedling vigour index (500.00) and least mean germination time (2.68) was noted in cv. Peach. Percent polyembryony (84.39 %) was the highest in Olour followed by Moreh and less than 30 per cent polyembryony was noted in both in Peach and Nekkare. The polyembryony was moderate (59-64 %) in Vellaikolamban, Bappakai and Peach.

Deepak *et al.* (2018) evaluated the germination characters of different polyembryonic mango rootstocks. The results revealed that the lowest number of days for initiation of germination (21.33 days) and 50 per cent germination (31.78 days) as well as the highest percentage of germination (43.78 %) and rate of germination (0.016) were observed in rootstock Olour. The cv. Vellaikolamban recorded more time period for initiation of germination (29.11 days) and for 50 per cent germination (40.44 days) and recorded the least germination percentage (36.89 %) and rate of germination (0.014).

Extent of polyembryony in salt tolerant mango rootstocks *viz.*, Olour, Kurukkan and 13-1 were investigated by Kumar *et al.*, (2018). The highest per

cent of germination (80.09 %) in cv. Kurukkan followed by Olour (75.10 %) and the lowest in 13-1 (27.00 %). The extent of polyembryony was more in Kurukkan. Out of 80.09 per cent stones germinated, 74.43 % were polyembryonic. However, 28.57 % stones produced two plantlets and 33.08 per cent produced three plantlets per stone. Remaining 10.52 % stones produced four plantlets and 2.25 per cent stones produced five plantlets per stone, while, 25.56 % stones gave rise to only one plantlet. Hence the extent of polyembryony in Kurukkan was 74.43 per cent followed by rootstock 13-1 (51.85 %) and the lowest in Olour (33.15 %). Hence Olour is identified as weak polyembryonic rootstock.

2.2 Molecular characterization of polyembryony

Proper identification of sexual embryo from each hybrid seed is necessary in order to preserve only the nucellar seedlings, which would help to maintain the rootstock's genetic characteristics as well as to overcome the major constraints in the area of fruit breeding especially in hybridization programme by eliminating the nucellar ones to advanced generations (Kumar and Rani, 2013).

Contrasting reports exists regarding the vigour of zygotic seedlings of polyembryonic mango genotypes. Srivastava *et al.* (1988) described the zygotic plantlet as being the weakest in polyembryonic mango stones because it probably degenerates due to competition with nucellar plantlets. Most polyembryonic mango cultivars occasionally produce morphologically off-type plants that presumably are zygotic in origin (Schnell and Knight, 1992).

Cordeiro *et al.* (2006) revealed that the zygotic one was the most vigorous plantlet and he later confirmed this fact with RAPD marker. According to Rocha *et al.* (2014) the zygotic (sexual) seedling need not always be weak. In certain cases the sexual seedling will be vigorous and grow healthy along with vegetative seedlings.

It is necessary to identify/ distinguish the zygotic seedling from the nucellar population at an early stage, for which, various methods *viz.,* rootstock colour test (Furr and Reece, 1946), thin layer chromatography (Tatum and Berry, 1974), gas chromatography (Tatum *et al.*, 1974; Weinbaum *et al.*, 1982),

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biochemical markers (Schnell and Knight, 1992; Truscott *et al.*, 1993), isoenzyme pattern analysis (Moore & Castle, 1988 and Degani *et al.*, 1993), examination of morphological traits (Hearn, 1977, Bhat *et al.*, 2010) and flow cytometry (Tusa *et al.*, 2002) have been tried. But none of these could be employed commercially due to lack of reliability.

Morphological markers may be used to differentiate zygotic and nucellar seedlings but the extent of accuracy is questionable. In general the zygotic seedlings are smaller and weaker in nature. The plant height, leaf size or thickness of stem alone could not be treated as a standard criterion for selection. The lateral position and irregular shape of embryo can be taken into account to discriminate nucellar seedlings from the zygotic one (Xiang and Roose, 1988; Cordeiro *et al.*, 2006).

Various molecular markers have been employed in many fruit crops for distinguishing the zygotic and nucellar seedlings (Rodriguez *et al.*, 2004; Rao *et al.*, 2008). Discrimination of zygotic and nucellar seedlings obtained from single seed can be emphasised through various marker systems such as ISSR (Tusa *et al.* 2002, Krueger and Roosse, 2003, Shareefa *et al.*, 2009; Golien *et al.*, 2011), RAPD (Rodriguez *et al.* 2005; Srivastava *et al.*, 2010), Expressed Sequence Tag (EST)-SSR (Rao *et al.*, 2008) and SSR (Ruiz *et al.*, 2000; Yildiz *et al.*, 2013; Begum *et al.*, 2013).

Investigations were carried out by Valenzeula *et al.* (1997) to identify embryo type of fifteen mango cultivars using RAPD markers. Bulk segregant analysis of polyembryonic and monoembryonic cultivars detected a specific RAPD marker for polyembryony. In most cases the use of microsatellites is a more efficient and simpler means to distinguish the sexual origin of citrus seedlings and resulted in higher degree of polymorphism compared to isozymic markers. Among various marker systems, the simple sequence repeats (SSRs) are quick and more efficient to discriminate the zygotic and nucellar seedlings from both selfing and interspecific cross (Ruiz *et al.*, 2000).

The fact is that the zygotic seedlings from most of the citrus rootstocks in open-pollination appears to have arisen from self-pollination (Moore & Castle, 1988). When the level of heterozygosity is low, it is very much efficient to use SSR markers to distinguish the zygotic seedlings which were derived either by open-pollination or self-pollination of autogamous rootstocks.

Microsatellite/ SSRs consists of 1-6 bp long monomer sequence that is repeated several times (Joshi *et al.*, 1999).The strength of SSRs include the co-dominant nature of alleles, high genomic abundance, random distribution throughout the genome and polymorphic nature. They are very much simple to handle and are characterised by high degree of reproducibility. SSR markers can contribute to 'direct allele selection', if they are shown to be completely associated or even responsible for a targeted trait. The use of co dominant SSR marker would be a more reliable way to differentiate the zygotic and nucellar seedlings (Rafalski *et al.,* 1995).

Golein *et al.* (2011) recognized 67 hybrids and 160 nucellar seedlings among 227 plantlets by using ISSR markers and concluded that ISSR analyses were more efficient and reliable than other markers. Six elite landraces of mango in Andra Pradesh were characterized for their genetic distinctiveness and relationships with five of the choicest juicy cultivar at molecular level using 109 mango specific microsatellite markers (SSRs). Microsatellite SSR- 84 was able to differentiate all of the 11 genotypes under study (Begum *et al.,* 2012).

Among 18 stones of polyembryonic mango cv. Uba, the most vigorous zygotic seedlings in six stones were obtained using ISSR primers by Rocha *et al.* (2014) and concluded that the most vigorous seedlings are not always nucellar one. Out of 14 SSR primers analysed, 9 primers had given uniform profiles similar to the mother plant indicating the nucellar origin of seedlings in mango cv. Moreh. The primers IIHR 11, IIHR 31 and IIHR 34 generated profiles characterized by loss of band compared to the mother plant indicating the polymorphism in 10 per cent of mango seedlings (Sane *et al.*, 2015).

Yildiz *et al.* (2013) conducted an investigation to differentiate nucellar and zygotic individuals evolved from several crosses of citrus using SSRs primers. They found that nucellar seedlings had showed similar banding pattern as that of mother plant. The mandarin cv. Fremont and Robinson produced 36.91 and 31.09 per cent nucellar seedlings respectively. The occurrence of spontaneous sexual polyembryony in 24 olive cultivars were characterized by Trapero *et al.* (2014). The microsatellite analysis of DNA profiles showed that polyembryonic seedlings in olive had sexual origin because of the identical and distinguishable profiles from the mother plant and this might be due to monozygotic cleavage after normal fertilization.

2.3 Seed germination in mango and other fruit crops

Being a recalcitrant seed, the viability of mango stone is comparatively low. There is only about 12-50 per cent germination when sown within a month after extraction (Gill *et al.*, 1985). The availability of fruits are confined to mainly one season. So the stones which are available during a particular season need to be properly utilized and exploited in an effective way for raising strong, healthy and actively growing rootstocks.

Usually the mango stones are available during April- May months i.e. drier part of the year. Therefore the stone germination and plant vigour are critically very low. In our country, mainly non-descriptive monoembryonic seedlings are utilized for rootstock purpose (Patel *et al.*, 2016). Hence there is a great variation in stone germination, vigour and further seedling development depending on the location and region, where the rootstocks are raised.

Generally the stones begin to germinate 12 to 15 days after sowing, but may take about a month or even more to complete the germination. The sporadic and slow germination in mango is due to the stony endocarp and consequently seedlings take more time to attain graftable size. It is necessary to improve stone germination and enhance seedling growth for synchronization, rapid seedling emergence and healthy rootstocks within a short period of time (Patel *et al.*, 2017). To achieve a perceptible difference in enhancing germination, rapid emergence, boosting up of growth and reducing mortality, sowing positions (seed

orientation), age of stone after extraction from fruit and various pre-sowing treatments need to be taken into consideration.

2.3.1 Effect of sowing positions

Sowing of seeds at proper position and depth is one of the most important nursery operations as it affects the germination and subsequent growth of the plant. The planting of cashew with the stalk end upward position and inclined at an angle 45^0 to the soil surface was best and resulted in high germination rate (Garner and Chaudhri, 1976).

Good germination and vigorous seedlings plays a vital role in determining the successful establishment of an orchard. The energy required to complete the germination process (emergence of radicle and plumule) is highly influenced by genotypes and seed orientation in seed bed. This is because of the quantity of stored nutrient as well as the positioning of micropyle. The stalk-end upward position helps to place the micropyle in the most suitable position and resulted in less requirement of germination energy for the emergence of radicle from the embryo. It also enhance the accessibility of oxygen for the initial metabolic process that produces energy for radicle emergence (Bewley, 1997). While the inappropriate seed orientation could deny the oxygen needed for emerging embryo which could lead to high production of pyruvate and ethanol in the plant system and finally leads to the death of the emerging embryonic plants. It ultimately leads to the poor germination and quantitative plant vigour.

Sowing of mango stones with plumule up position offered greater advantages in germination than other methods of sowing *viz.,* plumule down, suture up, suture down and flat (Vijaya and Satyanarayana, 2004). Plumule-up sowing position resulted the least number of days taken for germination (35.90 days) and the highest germination percentage (63.85 %) in mango (Chaudhari and Patel, 2012). Hammed *et al.* (2014) reported that the Brazilian cashew cultivars with medium sized nuts sown on both nut-side and with stalk-end up position had higher germination percentage (86.70 % and 100.00 % respectively) and quantitative plant vigour.

With regard to seed orientation in seed bed, sowing of mango stones in vertical position with convex edge upward markedly increased the germination percentage over other methods of sowing *viz.*, vertical position with convex edge downward and the horizontal position with flat side. The positive results might be due to the straight growth of seedling without any curvature when planted on convex edge upward in vertical position but the curvature was produced when planted on the convex edge downward in horizontal position (Abbas *et al.,* 2015).

The seed orientation on the seed bed significantly influenced the seedling emergence. The seedling emergence was quicker and more when the seeds were sown in flat position, on their sides and with the radicle pointed downward in litchi (Zhang *et al.,* 2015).

2.3.2 Effect of age of stones after extraction from fruit

As the age advances, rate of germination became progressively slower (Corbineau *et al.*, 1986). Moisture content is a conclusive factor in maintenance of viability and quality of recalcitrant seeds (Mc Donald, 2007).

The freshly harvested seeds had higher moisture content (85 % on dry weight basis) that might be the probable cause for higher germination percentage. Loss of critical moisture content during seed storage can cause alterations in a series of metabolic processes which led to accumulation of free radicals and resulted in onset of deterioration process (Patil and Krishna, 2016).

Teaotia and Singh (1971) noted cent per cent germination in mango cultivars Desi and Dashehari for freshly harvested stones. Chandra (1980) noted that the open storage treatment of mango stones for 10 days after extraction of pulp resulted in 85 per cent germination. The germination was drastically reduced to 66 per cent at 45 days after harvest from pulp.

Krishnaswamy (1990) found 100 per cent germination in jackfruit seeds when stored in plastic tray and kept at room temperature for shade drying for 9 days. Kadam *et al.* (1994) studied the effect of various storage conditions and periods of storage on seed viability and germination in Rangpur lime. More viability and higher germination percentage was obtained when the seeds were sown after fifth week of storage at room temperature. Chiesotsu *et al.* (1995)

noted highest percentage of germination, survivability and least number of days taken for germination in fresh seeds of jackfruit over seeds stored for 15 days.

The fresh seeds of mandarins exhibited highest seedling vigour as compared to 30 days old stored seeds (Doijode, 2003). Chaudhari and Patel (2012) opined that there was decreasing percentage of germination in mango stones with increasing sowing duration. The least number of days (24.67 days) and highest germination percentage (74.72 %) were reported in freshly harvested stones than stones sown 10 days and 20 days after extraction of pulp.

2.3.3 Effect of pre sowing treatments

For successful graft union, the selected rootstocks should be very healthy, strong and actively growing in nature. Hence for producing healthy and vigorous rootstocks within a short period of time, it is essential to concentrate much on improvement of stone germination and enhancement of seedling growth. Being recalcitrant in nature, mango stones are characterized by low viability. Besides, mango stones have stony endocarp which slows down the germination and makes it take more time to attain appropriate graftable size. Hence the pre-sowing treatments could be the best way to induce early germination, boosting the growth, enhancing the seedling vigour and reducing mortality (Rao and Reddy, 2005a; Shaban, 2010a).

Soaking the stones in cow dung slurry, water and aqueous solutions of plant growth regulators for 12-36 hours has been proved to be effective for enabling quick germination, enhance percentage of germination and rapid seedling emergence and growth (Muralidhara *et al.*, 2015). These treatments shorten the period of emergence, protect the seeds from various biotic and abiotic stresses and remove the obstruction in embryo growth. Synchronization in seedling emergence and uniform crop stand can be achieved through such treatments (Patel *et al.*, 2016).

The chemicals like Gibberellins and Potassium nitrate had been successfully employed for breaking the seed dormancy in numerous species and also proved to be effective in accelerating the seed germination of non-dormant types (Rao *et al.*, 2006 and Kumar *et al.*, 2007). Lot of evidences are available to

substantiate the effect of pre-sowing treatments (including non-chemical treatments) that can make significantly higher differences in germination and subsequent growth of seedlings.

The pre-soaking treatments of GA_3 increased endogenous auxin content and might have altered the enzymatic reactions, conversion of starch to sugars involved in the germination and protein synthesis (Paleg, 1960). Application of GA_3 also stimulates vegetative growth by increased uptake of osmotic nutrients, cell multiplication and elongation (Shanmugavelu, 1966). GA_3 also has known effects to overcome all sorts of dormancies *viz.,* thermo-dormancy, photo dormancy, dormancy imposed by incomplete development of embryo, as well as certain mechanical barriers and presence of germination inhibitors (Diaz and Martin, 1971).

The *de novo* synthesis of proteolytic enzymes like ribonuclease and α-Amylase were induced by Gibberellic acid. Enzyme amylase hydrolyses the endosperm starch, make available the essential sugars for growth initiation and mobilization of endosperm reserves and also liberate chemical energy which is essential for the activation of embryo (Copeland and Mc Donald, 1995). The stem elongation is achieved by inducing the cell wall extensibility, stimulating the synthesis and reducing the cell wall rigidity. More growth obtained from GA_3 application was attributed to increased cell division and synthesis of IAA. The cumulative effect of higher shoot length, root length and germination percentage might have resulted in higher vigour of the seedling (Pawshe *et al.,* 1997; Padma and Reddy, 1998).

Pillewan *et al.* (1999) revealed that stones of Neelum treated with tap water for 24 hours followed by soaking in 100 ppm GA_3 for 24 hours proved to be the best in terms of vegetative growth and survival percentage of mango seedlings. Higher germination percentage (90.00 %), early germination (12.15 days) and highest seedling emergence index (21.95) were obtained by pre-soaking of sapota seeds in GA_3 and ethrel each at 400 ppm concentration (Pampanna and Sulikeri, 2001). Osmopriming in khirni seeds with 300 ppm GA_3 recorded the highest germination percentage. The highest seedling growth and vigour was

obtained by 300 ppm GA_3 treatment in khirni. Significant correlations were obtained between germination percentage, growth and vigour of seedlings (Reddy and Khan, 2001). Kagzi limepre treated with 80 ppm GA_3 for 12 hours resulted in better germination, significantly higher seedling height as well as more number of leaves (Kalalbandi *et al.*, 2003).

Pre-soaking of mango stones of var. Alphonso with 100 ppm GA_3 resulted in the highest seedling height, girth and seedling vigour, whereas the significantly higher fresh and dry weight was in var. Totapuri. (Rao *et al.*, 2006). Kumar *et al.* (2007) reported that pre-soaking of mango stones with GA_3 at 100 ppm exhibited the highest germination index and seedling height, which were on par with 3 % Panchagavya, Water soaking, 3 % Amrit Pani and 1 % KNO_3. Ber seeds treated with 250 ppm GA_3 recorded the highest plant height and intermodal length (Rajwar *et al.*, 2007).

The significantly higher seedling growth was obtained from pre-soaking treatment of 1.0 mM GA_3 in papaya (Rodriguez *et al.*, 2008). Wankhede *et al.* (2008) reported that khirni seeds treated with 50 ppm GA_3 resulted in highest germination (92.31 %) followed by 75 ppm GA_3 (89.76 %). Papaya seeds treated with 2 mM GA_3 for 60 minutes recorded the highest germination percentage (Ashmore *et al.*, 2009). Sehrawat *et al.* (2010) found fresh seeds as well as 24 hours accelerated aged seeds of papaya treated with GA_3 at 1000 ppm resulted in significantly higher seedling length, dry weight and vigour indices on both growth and weight basis followed by GA_3 at 500 ppm.

GA_3 at 200 ppm for 12 hours reduced the time taken for completion of 50 percent germination in papaya (Anburani and Shakila, 2010), whereas 500 ppm GA_3 for 12 hours recorded the least number of days for completion of germination in papaya hybrid Mayuri (Barche *et al.*, 2010). Husked stones of mango rootstocks *viz.*, Zebda, Sukkary, Sabre and 13-1 treated with 200 ppm GA_3 for 48 hours exhibited significantly better results with regard to germination and growth parameters (Shaban, 2010a). The highest values for germination percentage, the highest seedling length, seedling diameter and survival of saplings were obtained for pre-treatment with 500 ppm GA_3 for 40 hours in sweet orange (Pal and Dhaka,

2010). The growth of seedlings in terms of height and number of leaves were the highest for GA_3 treatment at 200 ppm in mango (Munde and Gajbhiye, 2010).

Extracted mango kernels pre-treated with aqueous solution of 500 ppm GA_3 for 12 hours resulted in significantly higher seedling height and internodal length. This might be due to the triggering action of GA_3 on cell multiplication and cell elongation in the cambium tissue of the internodal region (Aatla and Srihari, 2013).

Stones pre-treated with 100 ppm of GA_3 exhibited the highest germination, plant height, number of leaves and seedling vigour indices (growth and weight basis) in mango. Pre-soaking treatments with chemicals help to reduce the time taken for initiation of germination and also removes the obstruction in embryo which hampers the seedling emergence whereas alterations in growth attributes by gibberllins mainly relates to its stem elongation properties (Muralidhara *et al.*, 2015). Mango stones dipped in aqueous solutions of 100 ppm GA_3 for 24 hours prior to sowing required the least number of days to germinate and resulted in the highest percentage of germination (Patel *et al.*, 2016).

Kolekar *et al.* (2017) found significant difference among various pre-soaking treatments on stone germination and growth attributes of seedlings in mango. Soaking of mango stones in 100 ppm GA_3 for 12 hours required least number of days (12.53 days) for initiation of germination, the higher germination percentage (85.67 %) and germination vigour index (4.05). The noticeable effect of GA_3 on triggering the seed germination might be due to the significant role of GA_3 in activating alpha amylase enzyme which converts starch into simple carbohydrates and liberate chemical energy which is essential for embryo activation. The growth parameters like seedling height, number of leaves and leaf area were also the highest in pre-soaking treatment with 100 ppm GA_3.

Enhanced enzymatic activities and suppression of germination inhibitors along with RNA synthesis as stimulated by KNO_3 resulted in better germination. KNO_3 also promote various physiological processes that accelerate the translocation of food reserves in the tissue which ultimately leads to the production of new leaf primordia thus resulted in more number of leaves. Also the

rapid accumulation of these food materials resulted in an increased seedling diameter. Pre-soaking treatment of stones and kernels with 1 per cent KNO_3 for 24 hours recorded the highest seedling height whereas those treated with 0.5 per cent KNO_3 for 24 hours recorded the largest number of leaves per seedling in mango (Padma and Reddy, 1998).

Treatment combination of *Azospirillum, Phosphobacteria*and KNO_3 at 0.5 per cent concentration for 8 hours in fresh amla seeds recorded the highest percentage of germination, highest shoot length, dry matter production, root length and seedling vigour (Rajamanickam and Anbu, 2001). Dubey *et al.* (2003) stated that Khasi mandarin seeds treated with KNO_3 at 2.5 % for 12 hours resulted in the highest germination, seedling height and number of leaves per seedling. The highest percentage of germination, root length and seedling vigour index in amla was secured on treatment with 0.5 % KNO_3 (Rajamanickam *et al.*, 2004).

The synergistic effect between K^+ and NO^{3-} ions brings up the uptake of both ions by roots. The K^+ ions play a vital role in many metabolic processes in plant cell and act as an osmoregulator (Aatla *et al.*, 2014). The higher seed germination and survival was obtained with KNO_3 at 1 per cent concentration for 18 hours in aonla (Purbey and Meghwal, 2005).

The stimulated effect of KNO_3 on vigorous shoot growth of mango seedlings might be due to more production of photosynthates as well as improved translocation of assimilates through phloem to the root zone contributing to increased root length. At the time of seed germination, there might be an increased rate of the oxidation of nicotinamide adenine dinucleotide phosphate during the process of respiration, led to more production of lengthy roots. The significantly higher production of functional leaves as well as improved root length might be resulted in overall assimilation and redistribution of photosynthates within the plant system which in turn lead to improved seedling dry weight and vigour (Kumar *et al.*, 2007). Ber seeds treated with a combination of Ca $(NO_3)_2$ and 100 ppm KNO_3 were the best to increase the germination capacity (Laamouri *et al.*, 2009).

Mango stones osmoprimed with 0.5 per cent KNO_3 was the best as it resulted in the highest germination percentage (64.00 %) and seedling vigour in mango. The enhanced germination might be due to the simulative effect of KNO_3 by increased enzymatic processes and suppression of chemical inhibitors for germination. Due to enhanced uptake of water and nutrients, greater seedling emergence, germination percentage and photosynthetic rate could be achieved, thus ensuring better root and shoot growth which ultimately contributed to highest seedling vigour in mango (Aatlaand Srihari, 2013).

Mango stones pre-treated with 2 per cent KNO_3 recorded the least number of days (29.00 days) for 50 per cent germination, resulted in 14.90 per cent more germination and the highest seedling height (23.61 cm) compared to control. The increased plant height may be due to the synergistic effect of KNO_3 on IAA synthesis bringing about stem elongation. Besides, more growth could be achieved through KNO_3 by inducing cell wall extensibility and loosening, increasing cell wall synthesis and cell division as well as by reducing cell wall rigidity (Reddy and Reddy, 2017).

In addition to synthetic chemicals, the naturally available bio products of organics are also enriched with vital plant growth substances meant to enhance the seed germination and boosting up plant growth. The role of bio-regulators are well known in enhancing seed germination and seedling growth in numerous plant species (Pampanna and Sulikeri, 2001).

Soaking of seeds in cow dung slurry for 24 hours resulted in higher seed germination (66.83 %) compared to other pre-soaking treatments *viz.,* 2 % thiourea (47.25 %) and water (49.83 %) in Khirni (Shirol *et al.,* 2005).

The pre-soaking treatments with cow dung slurry resulted in rapid seedling growth, which might be due to the presence of efficient water, growth promoting substances (auxins), nutrients (both macro and micro nutrients) and bio digestible enzymes. The treatment ultimately prompted softening of seed coat there by effecting the radical protrusion in *Carissa inermis* (bush plum) (Prashanth and Prakash, 2009).

The mango stones soaked in cow dung slurry for 24 hours required the least number of days for germination (34.78 days) and highest germination percentage (64.33 %) (Chaudhari and Patel, 2012). The significantly earliest germination (15.24 days), the highest germination percentage (66.11 %) and rapid growth of seedlings were obtained from 12 hours soaking of Khirni seeds in a combination mixture of cattle urine and cow dung slurry. The presence of essential plant nutrients (N, P, K, Ca, Mg, S and other micronutrients), minerals, plant protection substances and beneficial microbes in cow dung slurry may be the probable cause for better seedling growth, more survival percentage and vigorous growth (Shinde and Malshe, 2015).

Water soaking treatment is a very simple and inexpensive method which hastens germination. The dead stones which floated on water could be discarded before sowing thereby saving time and labour requirement. The critical soaking duration is different for each crop and it should be less than the safe limit. The early germinated seedlings produce deep root system and it facilitates better field establishment of that particular crop (Harris *et al.,* 2000).

If the seeds are normally slow to germinate, water soaking prior to sowing may shorten the time for seedling emergence as the seeds get triggered to germinate in presence of water. The process of seed germination starts with imbibition of water by testa or seed coat. The imbibed water either triggers germination process directly by reacting with chemicals in the endosperm or gradually washes away germination inhibitors which block the passage of water channels to the endosperm (Hartmann *et al.,* 1997). The highest seedling diameter was obtained from water soaking of mango stones for 24 hours (Padma and Reddy, 1998).

Pillewan *et al.* (1997) noted that mango stones of cv. Neelum and Totapuri pre-soaked in water for 24 hours resulted in highest percentage of germination followed by 150 ppm GA_3 treatment. Eight hours water soaking of jackfruit seeds (Prakash, 1998) and 24 hours soaking of stones of local Nagpur mango variety (Pillewan *et al.,* 1999) proved to be best for improving germination, growth and survival percentage of seedlings.

Singh *et al.* (2001) communicated that ber seeds soaked in water for 48 hours recorded highest percentage of germination both in seed bed (78.50 %) and polythene tubes (51.25 %). The significantly higher germination rate was obtained from one year old aonla seeds which were pre-soaked in cold water for 24 hours (Rajamanickam *et al.,* 2002). The highest germination percentage was obtained from de-coated seeds of jackfruit soaked in a combination of tap water for 12 hours, kinetin at 100 ppm for 7 minutes and GA_3 at 10 ppm for 10 minutes (Abd-El-Zaher, 2008).

2.3 Dwarfing potential of mango rootstocks

One of the most important constraints in mango production is the huge size of the trees (Campbell, 1991). The high cost of maintaining the tree size through various canopy management techniques can reduce the fruit production and have severe impact on profitability of fruit production. In the recent past, realisation of significance of dwarf trees has increased tremendously with the introduction of the concept of high density planting system (Urrutia and Elisea, 1997). The possibility of establishment of the high density/ ultra-high density orcharding system mainly rely on the availability of low vigour cultivars. The introduction of dwarfing rootstocks provides the possibility of reducing tree size by checking excessive vegetative growth and facilitate early net returns without increasing the input costs (Vaio *et al.,* 2012).

Dwarfing trees have the potential to curtail the tight competition between the developing fruits on one side and the requirements for the growth and development of other parts of the plant. Hence much of plant's energy is stored in fruits, which ultimately leads to better harvesting index. The net assimilation rate and fruit quality can be improved by better utilization of solar radiation and the quick translocation of assimilates from source to sink leads to higher productivity (Tyagi, 1986). Besides, the dwarf trees also facilitate easy horticultural operations *viz.,* training, pruning, plant protection measures, etc. and thereby reduce the labour cost. Harvesting can be easily employed from such trees with lesser injury and better postharvest life could be achieved. There are several advantages for

using dwarf rootstocks, such as easiness for harvest and fungicide spraying as well as the reduction of fruit loss at postharvest stage (Ramos *et al.*, 2004).

The field level investigations for the purpose of identifying low vigour (dwarf) plants fitted for high density planting system require extreme long duration for cultivation tests, large sized plots and laborious tasks. The mechanism behind the vigour control by rootstocks were not clearly understood or well explained though many hypotheses have been proposed (Webster, 2004).

Dwarfing effect may be attributed to limited water supply, partial compatibility between stock and scion, production and translocation of hormones, or the hydraulic conductivity of xylem vessels and other peculiar anatomical features of the vascular system (Atkinson *et al.*, 2003; Solari *et al.*, 2006; Zach *et al.*, 2010).

The screening of seedlings at very early stage of growth can be confirmed through certain selection criteria introduced by Majumdar *et al.* (1972). Besides, various vegetative and physiological parameters were suggested to predict vigour *viz.,* stomatal count (Srivastava *et al.*, 1980a), phenolic content (Babu *et al.*, 1985, Murti and Upreti, 2003; Abirami *et al* 2011b), leaf area (Rao and Reddy, 2005b), chlorophyll fractions (Abirami *et al.,* 2011b), and anatomical peculiarities especially with regard to xylem vessels (Majumdar *et al.,* 1972; Tombesi *et al.*, 2011; Hegazi *et al.,* 2013).

2.3.1 Germination characters

Abirami*et al.* (2011b) conducted an experiment to study the relationship between seedling vegetative and physiological parameters with vigour in some monoembryonic and polyembryonic mango genotypes. Based on the nursery evaluation, among polyembryonic genotypes vigorous rootstock Nekkare recorded the highest seedling height (38.00 cm) and Starch recorded the least (13.10 cm). The highest fresh and dry weight of seedling (30.50 g and 14.80 g) and vigour index based on growth and weight basis (9477 and 2295) were recorded in Nekkare, followed by Bappaka and Kerala 5, while the least fresh weight (15.10 g), dry weight (4.40 g), vigour index- I (1489.00) and vigour index –II (358.00) was in Starch. Among monoembryonic genotypes, the highest

seedling height (44.6 cm), fresh and dry weight (30.70 g and 15.10 g) and vigour index based on growth and weight basis (9807.00 and 2697.00) were observed in Bombay Green and the least seedling height (20.80 cm), fresh weight (24.80 g), dry weight (10.40 g), vigour index- I (2395.00) and vigour index –II (806.00) was in Amrapali.

2.3.2 Vegetative and growth characters

Vigorous rootstocks Goa, Kurukkan and Chausa resulted in the highest stem growth, whereas Bombay Green and Totapuri Red Small showed the least stem growth rate in mango (Majumdar*et al.*, 1972). Based on canopy volume, Kurian and Iyer (1992) grouped the nine year old mango trees into least vigorous (<14 m^3), medium vigorous (14-25 m^3) and most vigorous (>25 m^3) on growth basis and found positive correlation between length of new flush and vigour.Kurian and Iyer (1997) conducted an experiment for the identification of morphological traits related to vigour management in mango and revealed that leaf area was a good indicator for imparting dwarfness.

According to Iyer and Subramanyam (1972), plant height has positive correlation with first extension growth and number of internodes. Iyer and Subramanyam (1986) found a positive correlation between internodal length and dwarfness. The less vigorous cv. Creeping showed the shortest internodal length (2cm) in mango. Plants with shorter internodal length had dwarf stature in papaya (Lim and Hawa, 2007).

Murti and Upreti (2003) pointed out the highest plant height (42.40 cm) and number of leaves (24.20) in vigorous rootstock Muvandan and highest stem girth (3.30 cm) in Bappakai. The least plant height (17.00 cm), number of leaves (7.40) and stem girth (1.80 cm) were recorded in less vigorous Vellaikolamban, followed by Kurukkan and Chandrakaran. They found a positive correlation between number of leaves and plant height with vigour at nursery stage and revealed that it is a potential tool for assessment of vigour at early growth stage. The variations in vegetative behaviour of rootstocks might be attributed to

vigorous growth, genetic factors and leaf producing capacity which enhance light reception in a better way by plants which in turn accelerate leaf production.

The number of secondary roots might be used as a good indicator of dwarfness (Mukherjee and Das, 1976). Shaban (2010b) reported a positive correlation between the root length and plant height in polyembryonic mango rootstocks.

Singh *et al.* (1986) reported that dry matter content of shoot and root could be a useful tool to assess the vigour of mango seedlings at nursery stage and they exhibited positive correlation.

Abirami *et al.* (2011b) reported that the highest leaf area (34.50 cm^2) and root length (17.20 cm) were observed in vigorous polyembryonic rootstock Nekkare, followed by Bappakai whereas the lowest values were observed in Starch (10.70 cm^2 and 4.40 g respectively). Among the various monoembryonic rootstocks, the highest value was recorded in Bombay Green (39.60 cm^2 and 18.60 cm respectively) followed by Dashehari whereas, the lowest value was recorded in Amrapali (19.60 cm^2 and 13.10 cm respectively). The difference in values might be attributed to the genetic characters.

Shenoy (2016) conducted an experiment to identify morphological, physiological and anatomical features associated with dwarfness in mango varieties. She noticed the highest plant height (94.10 cm), internodal length (10.27 cm), more number of leaves (98.00), leaf length (21.99 cm), leaf width (5.53 cm) and total leaf area (3051.43 cm^2) in Kalapady at 12 MAS. More number of roots were produced in Creeping (93.67). The root length (100.33 cm) and total dry matter production (215.00 g) were the highest in Rumani. The Vellaikolamban exhibited the least plant height (37.13 cm), internodal length (3.20 cm), number of leaves (17.00), leaf length (13.58 cm), leaf width (3.70 cm), total leaf area (68.85 cm^2), root length (20.67 cm) and dry matter production (66.67 g). A high positive correlation existed between internodal length, number of leaves, leaf length and width, total leaf area, number of roots, root length and total dry matter content with plant height. Based on the findings, the Vellaikolamban was grouped under low vigour (dwarf).

Deepak *et al.* (2017) evaluated the vegetative growth performance of three different polyembryonic rootstocks *viz.,* Nekkare, Olour and Vellaikolamban at nursery stage.The highest seedling height (31.17 cm) and internodal length (14.21 cm) were observed in Nekkare. The rootstock Olour recorded the highest number of leaves (22.56), leaf length (22.8 cm), leaf width (5.77 cm) and leaf area (90.33 cm^2). The lowest values for seedling height (23.72 cm), internodal length (8.42 cm), number of leaves (15.04), leaf length (20.39 cm), leaf width (5.46 cm) and leaf area (79.59 cm^2) at 265 DAS were recorded in Vellaikolamban. They concluded that the rootstock Vellaikolamban was less vigorous in growth, Olour was semi vigorous and Nekkare was vigorous.The variation in growth potential of different rootstocks might be attributed to stone characters as well as stone germination rate. The stone weight of Nekkare and Olour was more than Vellaikolamban, which might have led more vigorous growth of seedlings.

2.3.3 Physiological and anatomical characters

More distribution of stomata was found in vigorous rootstocks than the dwarf ones. The young leaves were characterized with higher number of stomata than the mature leaves (Eckerson, 1908). Chakladar (1967) adopted the stomatal density technique for the first time in mango for assessment of vigour at nursery stage and reported that this parameter might be a useful tool for easy forecasting of growth potentials at early stage.

It has been evident from the research by Pathak *et al.* (1977) that the photosynthesis and stomatal density are mutually dependant and may increase the photosynthetic efficiency resulting in more accumulation of photosynthates which might be the probable reason for high plant vigour in plum rootstock. The highest number of stomata was found in vigorous rootstock Dashehari while the least number was in dwarfing rootstock Kalapady (Srivastava *et al.*, 1980a). Higher phenolics in apical buds seem to be associated with reduction in vigour and dwarfing in mango (Iyer, 1991). Based on the evaluation of stomatal density in different species of guava, a dwarf type *Psidium chinensis* resulted in the lowest stomatal density (Saroj *et al.*, 1997). The phenolic content, bark percentage and

chlorophyll fractions were found to be very useful in predicting vigour of mango rootstocks at nursery stage (Chadha, 1998).

Pandit *et al.* (2004) conducted an experiment with 21 different species of apple to assess the plant vigour at nursery stage by leaf stomatal density technique. The observations were recorded from both young and matured leaves and found that vigorous rootstocks had more stomatal distribution than the dwarf ones. According to the stomatal distribution, they grouped the rootstocks into vigorous, semi- vigorous, semi dwarfing and near dwarfing. Among different apple rootstocks, *Malus baccata* was categorized as vigorous; *M. baccata* and *M. sargenti* were classified as semi-vigorous; *M. eseltine, M. baccata, M. sikkimensis, M. kindsomex, M. micromalus, M. floribunda, M. simcoe, M. mandshurica* and *M. purpuria* were classified as semi dwarfing and *M. crimson, M. robusta, M. seibolbii* and *M. orientale* were grouped as near-dwarfing.

Abirami *et al.* (2011b) conducted an experiment to find the relationship between physiological parameters and seedling vigour. The highest stomatal density was recorded in vigorous polyembryonic mango rootstock Kurukkan followed by Nekkare and Bappakai. The lowest stomatal density was found in Starch. Among different monoembryonic genotypes, the highest stomatal density was in Bombay Green followed by Pusa Arunima and Dashehari whereas the least was in Amrapali. They opined that stomatal density could not be a useful criterion to determine the vigour of mango seedlings because the ultra-dwarf mango variety Amrapali did not show any critical difference in stomatal distribution from very vigorous rootstocks like Chausa and Langra. They found a non-significant correlation between relative water content and seedling vigour in mango. The polyembryonic vigorous rootstock Bappakai had more relative water content than the others. Among various monoembryonic genotypes, the highest relative water content was recorded in Bombay Green and the lowest value was recorded in Amrapali. High relative water content could be a useful criterion to determine the drought tolerant mechanism. A negative correlation between bark percentage and plant height was also observed. The least vigorous polyembryonic mango rootstock Starch had more bark percentage than the most vigorous rootstock

Nekkare. The lowest bark percentage was recorded in the least vigorous monoembryonic genotype, Amrapali.

Mendel and Cohen (1967) conducted an experiment to study the starch content in the trunk of citrus as a measure of graft incompatibility between scion and rootstock. Seven different rootstock varieties each budded with scion Shamouti orange and unbudded rootstocks were considered for the purpose of comparison. They found a negative correlation between starch level in bark and wood of stock with the tree vigour. But no correlation was found between the starch content in scion varieties and vigour in citrus.

Gaudillere *et al.* (1992) conducted an experiment to determine the effect of carbon partitioning in relation with tree vigour in young prunes. Three rootstocks *viz.,* Ishtara, Marianna GF8.1, and St. Julien Pixy 2879 which induce dwarfness on vigorous graft combinations with two *Prunus domestica* scion genotypes were considered for evaluation. After three years of planting, dry matter partitioning and carbohydrate content were estimated from different parts of the plant just before the bud burst stage. The relationship between carbohydrate and vigour mainly depend upon the type of carbohydrate and the season. No relation was obtained between the carbohydrate reserves of perennial plant parts with induced vegetative vigour.

Reduced cell growth and metabolism are the general characteristics shown by dwarfing rootstocks after grafting before any visible changes apparent in either scion or rootstock. Usually lipid, amino acid and cell wall biosynthesis are found to be down regulated, while degradation pathways of these compounds are up regulated. In dwarfing rootstocks, cellulose and lignin biosynthesis pathways are highly down regulated, accordant with reduced cell wall synthesis. The imbalanced allocation of carbon highly influences the growth and development, generally the starch reserves of roots are found to be catabolized when carbon for metabolic pathways are limiting. More accumulation of starch in stem and roots as well as reduced levels of glucose and fructose was found in apple dwarfing rootstock M 9 relative to Royal Gala (Foster *et al.*, 2017).

Babu *et al.* (1985) found a negative correlation between total phenol content and vigour. They classified mango rootstocks based on the phenol content. The cultivar Rumani had the highest phenol content whereas, the lowest was in Chinnarasam.

Total phenol content of dormant apical bud of 24 varieties of mango and chemically induced dwarfness by soil drenching with Paclobutrazol in cv. Alphonso were assessed by Kurian *et al.* (1994). According to them the plant height was inversely proportional to the phenol content of apical bud. Murti *et al.,* (2000) stated that total phenols plays very significant role in vigour determination of mango. The highest phenol content of leaves (59.10 mg/g) was recorded in less vigorous polyembryonic cv. Vellaikolamban followed by Kurukkan (50.24 mg/g) whereas, vigorous cultivars Bappakai (9.46 mg/g), Muvandan (22.46 mg/g) and Alphonso (19.50 mg/g) had the least phenol content. Murti and Upreti, (2003) found a significantly negative correlation between the total phenol content and plant height.

The leaves and stem bark of dwarf genotypes of Iranian mahaleb (*Prunus mahaleb* L.) had higher phenolic content than vigorous genotypes (Moghadam *et al.,* 2007). From the evaluation of 16 vigorous nucellar polyembryonic mango genotypes, it was evident that the vigorous rootstocks had less phenol content in leaves (Srivastav *et al.*, 2009).

Marie (2001) conducted an investigation on dwarfing potential of indigenous mango varieties of Kerala. She categorised the different varieties of rootstocks into low growth potential (LGP) and high growth potential (HGP) group. The stomatal density was the highest in Muvandan (HGP) whereas the lowest stomatal density was in Vellaikolamban (LGP). The highest total phenol content was found in cultivars Kalapady and Vellaikolamban (low growth potential group/ low vigour group) while, the lowest phenol content was found in cultivars Chandrakaran and Muvandan (high growth potential group).

The significantly higher phenolic contents in leaves and buds were obtained from less vigorous polyembryonic mango rootstock Starch. The most vigorous rootstock Nekkare, Bappakai and Kerala 5 recorded the least phenolic

content. The less vigorous monoembryonic genotypes, Amrapali, Chausa and Langra had more phenolic content in their leaves as well as buds, whereas vigorous genotypes Bombay Green, PusaArunima, and Dashehari had lower phenolic contents (Abirami *et al.*, 2011b). Among 11 apple rootstocks, the highest phenolic compound was recorded in super dwarf rootstocks P 22 and P 61 (Kviklys *et al.*, 2014).

Majumdar *et al.* (1972) classified the mango rootstocks based on the vigour through the assessment of bark percentage. They classified Kurukkan, Goa, Chausa and wild mango as vigorous as they had lowest percentage of bark while, Olour and Totapuri Red Small were grouped under less vigorous with high bark percentage.

Singh *et al,* (1986) found a negative correlation between bark percentage and plant height. The highest bark/ wood ratio was reported in dwarf species of guava but it was not a potential tool for predicting the vigour of plant at early stage (Saroj*et al.*, 1997). Srivastav *et al.* (2009) conducted an experiment to study the relationship between various physiological parameters associated with vigour of mango seedlings. Total of 16 nucellar polyembryonic genotypes were selected for study. They found a negative correlation between stem bark percentage and plant vigour.

According to Mendel (1951), the reduced transpiration rate is associated with low vigour in citrus.

In stem anatomy, Mukherjee and Das (1980) observed thick bark in Vellaikolamban followed by Ambalavi, Olour and Mylepelian whereas, the bark was narrow in Dashehari. The xylem vessel number and vessel size per unit area in vascular bundles and metaxylem area were the least in Vellaikolamban, indicating the dwarfing potential.

Shenoy (2016) found a weak and non-significant correlation between stomatal density and plant height in mango. The highest stomatal density was recorded in vigorous cultivars Kurukkan and Rumani whereas, the cultivars Bappakai was grouped under dwarf. Based on stomatal density, the dwarf cv. Vellaikolamban was classified under medium tall. The highest phenol content was

recorded in cv. Kalapady (58.20 mg/g) followed by Vellaikolamban (53.33 mg/g) and the lowest value was recorded for cv. Muvandan (37.60 mg/g). A weak, non-significant negative correlation found between plant height and total phenol content. A weak positive and non-significant correlation existed between bark percentage and plant height. The lowest bark percentage was noted in cv. Muvandan (9.90 %) while highest was in cv. Creeping (16.64 %). The dwarf cv. Vellaikolamban recorded the highest phloem – xylem ratio (0.78) and found a negative correlation between phloem – xylem ratio and plant height.

The proportion of xylem and bark could be a useful tool to classify the mango seedlings based on various vigour classes in the early stages of growth. The vigour of mango seedlings could be attributed to high percentage of xylem and negative correlation was observed between the bark percentage and plant vigour (Majumdar *et al.*, 1972).

According to Wang and Faust (1987), the dwarfness in 10 year old seedlings of hybrid apple cultivar (Gold Spur Delicious x Red Spur Delicious) exhibited the highest phloem xylem ratio. Kurian and Iyer (1992) conducted an investigation on 24 mango cultivars of different vigour groups. The results of the study indicated that the higher primary phloem to xylem ratio of young shoots were associated with low vigour. The width of cortex had no relationship with plant vigour. There was a negative correlation between phloem to xylem ratio and tree vigour. This character could be utilized for screening of genotypes for tree vigour at early growth stage. Hence based on phloem to xylem ratio, they categorized the mango rootstocks into least vigorous (ratio >1), moderately vigorous (0.6-1) and most vigorous (<0.6).

According to Santamaria *et al.* (2002), the dwarf cultivar Colin V-33 possessed lower vulnerability index than vigorous cultivar Fuerte and Hass in avocado. Trifilo *et al.* (2007) found that the shoots of Leccino Dwarf had narrower conduits than the vigorous rootstocks in olive. More than 90 per cent of the conduits were <25 µm.

Goncalves *et al.* (2007) detected a positive correlation between xylem conduit and plant vigour in sweet cherry tree. The trees grafted on vigorous rootstocks possessed higher xylem conduit than trees on the dwarfing rootstocks. According to Raimondo *et al.* (2009), the dwarf olive rootstock 'LD' had higher number of conduits and narrower xylem conduits than the vigorous rootstock 'LM'.

Saeed *et al.* (2010) conducted an experiment to study the anatomical features of stem, leaves and roots of citrus rootstocks belonging to different vigour groups. Troyer citrange, rough lemon, swinglecitrumelo, sweet lime, carrizocitrange, sour orange and flying dragon were examined to evaluate the relationships between their anatomical features and vigour. They concluded that lower proportion of phloem both in stems and roots, larger xylem vessel elements and low bark /wood ratio in the stem were found in vigorous rootstock rough lemon than the less vigorous rootstock Flying Dragon. Also they revealed that the number of xylem vessel elements had a negative correlation with plant height.

The characteristic difference in xylem vessels could be an important factor that determine the dwarfing nature of graft-compatible peach rootstocks. Through theoretical means, the estimation of dwarfing potential of specific genotypes can be assessed by calculating the xylem hydraulic conductance based on the number of vessels and dimensions per unit area of xylem. At the mean time by practical means, the anatomical measurements of xylem may be useful indicators for predicting the vigour of rootstocks during early growth stages (Tombesi *et al.*, 2010).

Tombesi *et al.* (2011) examined the xylem tissues which were taken from shoots, trunk and roots of rootstocks derived from the genetic cross between 'Harrow Blood' and 'Okinawa' peaches and the tissues were compared with the vigorous 'Nemaguard' rootstock (control). They found that the dwarfing rootstocks were characterized with fewer large xylem vessels and more number of smaller vessels than the vigorous rootstocks. More vigorous rootstocks had higher

weighted mean vessel diameter and noted more hydraulic conductance than the dwarfing peach rootstocks.

Hegazi *et al.* (2013) discussed a possible way to screen the dwarfing potential of different olive cultivars through stem anatomy. According to them, good indication of dwarfing potential was xylem and phloem percentage as well as xylem vessels percentage with different size classes. Based on the evaluation, high dwarfing potential was found in cultivar Cairo7. Based on stem anatomy, Rashedy *et al.* (2014) reported low phloem percentage, high xylem percentage and high number of large vessels in vigorous mango varieties.

2.4 Vegetative propagation in mango

Mango can be propagated easily by various methods. Seed propagation is mainly aimed for the production of rootstocks especially meant for improved cultivars. Seedlings may require 6 -10 years or even more to bear the fruits and quality may not be up to the mark. Such fruits may be smaller in size, characterized with fibrous flesh, resinous flavour and poor colour development or uneven distribution compared to the true to types. Hence desired cultivars can be propagated either by grafting, budding or by other vegetative means. Those grafted or budded mango plants will usually start bearing fruits at the age of 3-5 years of propagation (Pinto *et al.*, 2017). As these retain the characteristics of the mother plants, earlier fruiting and flowering can be obtained, and these plants remain relatively smaller at initial growth phase which in turn helps to accommodate more number of plants per unit area and gives the yield quite earlier with much higher net returns/ unit area. Hence various methods of grafting *viz.*, veneer grafting, softwood grafting, epicotyl grafting, side grafting etc. are being adopted with varying degrees of success rates (Hartmann *et al.*, 1997). The success of grafting techniques rely on the selection of desirable variety, method of grafting, time of grafting, age of scion and stock, growing conditions of grafts, nature of scion, scion defoliation period, nodes on scion, leaf retention on rootstocks, length of scion, etc. (Akter *et al.*, 2016).

2.4.1 Effect of propagation methods

Singh and Srivastava (1980) reported that softwood grafting done on August month recorded the highest graft success (90.00 %) in mango followed by July (64.85 %). Dhungana (1984) revealed that the epicotyl grafting done using four month old scion during August month recorded highest final graft survival in mango. No significant difference in survival percentage was observed in the case of veneer grafting during August, September and October. Ratan (1985) standardized the method of epicotyl grafting in mango and found that when 3 to 4 months old matured scion of 8 cm long, which were pre-cured for ten days and grafted on five to ten days old mango rootstock at 6 to 8 cm height resulted in the highest percentage of sprouting and survival of mango grafts during July-August.

In mango, 95 per cent graft success was obtained when the seedlings were propagated by stone grafting (4-6 days old rootstock) and softwood grafting (1 year old rootstock) and 84 to 97 per cent success was obtained from veneer grafting (Srivastava, 1989). Singh *et al.* (2014) conducted an experiment to standardize the method of grafting in mango cv. Amrapali. Stone grafting done on 10th August resulted in higher graft success.

Differential response of mango varieties to epicotyl grafting was studied by Radha and Aravindakshan (2000). The highest survival percentage was recorded for cv. Kalapady (84.50 %) and lowest for Mulgoa (39.60 %) at six months after grafting. The cultivars Bangalora (71.50 %), Neelum (70.80 %) and Mundappa (69.00 %) also recorded relatively high survival percentage. In order to compare the growth rate of the grafts, the height of the plants were recorded at an interval of six and twelve months after grafting. The significantly higher graft height was recorded in Bangalora (36.50 cm), while lowest was in Chandrakaran (26.30 cm). The highest graft height was recorded in cv. Bangalora (78.50 cm) and the least was in cv. Chandrakaran (62.00 cm) at twelve months after grafting.

Jacob *et al.* (2001) evaluated the success of softwood grafting in some commercial mango hybrids. They observed that less number of days for initiation of sprout was recorded during the month of July, August, September and October. The graft success in different hybrids showed significant variation in different

seasons. Nair *et al.* (2002) studied the effect of different methods of epicotyl grafting *viz.*, wedge, whip, slice and veneer on graft success in mango and obtained earliest scion sprouting in wedge method of grafting.

According to Islam *et al.* (2004), the highest growth of rootstock (2.74 cm) and scion (15.20 cm), mean number of new shoots (2.13), number of leaves (21.55) and final survival of grafts (68.76 %) were recorded in modified cleft grafting followed by the cleft grafting. Sabeky (2005) reported that the highest graft success percentage in mango was recorded in side grafting (65.80 %) followed by softwood grafting (63.70 %) and the least success (47.50 %) was noted in shield budding (47.50 %).

Epicotyl grafting with 8 to 16 days old rootstocks was the best in mango. More percentage of graft success was found in scion those were defoliated 4-15 days prior to grafting. In *in-situ* method of grafting, the young rootstocks with brown leaf colour stage was the best to obtain the highest graft success (Patil *et al.*, 2006).

Earliest bud sprouting (13.00 days), highest graft-take (96.66 %) and graft survival (90.00 %) were recorded in veneer grafting performed on 15thJuly (Singh *et al.*, 2012). Singh *et al.* (2014) studied the growth and survival of stone grafts as influenced by age of rootstock in mango. Stone grafting was performed by cleft method using Amrapali as scion stick in the month of July-August on seedling rootstock of four age groups i.e. 5, 10, 15 and 20 days. The graft height (24.40 cm), sprouting percentage (83.00 %) of scion and girth of rootstock (6.30 mm) were found significantly higher on grafts made on 10 days old rootstock and the least values were recorded for 20 day old rootstocks. The leaf length (17.12 cm) and width (4.80 cm) were the highest on 10 days old rootstocks. The highest percentage of graft survival was recorded in grafts made on 5 days and 10 days old rootstocks.

Among different grafting methods, the higher success in stone grafting might be attributed to the complete and much stronger graft union before bud sprouting. The preservation of higher amount of stored food material in cotyledons as well as the active growing stage of rootstock ultimately resulted in

greater graft success in case of stone/ epicotyl grafting. The optimum and equal rate of metabolic activities both in stock and scion enables the proper union of grafts and higher graft success in mango (Singh *et al.,* 2014). The highest initial as well as final success of epicotyl grafts were found in grafts made out of 15 days old mango seedlings. With regard to the duration of pre-defoliation, 10 days prior defoliation recorded best results (Upadhya *et al.*, 2014).

Kumar *et al.* (2015) studied the effect of different propagation techniques on survivability of mango. Among different methods, veneer grafting done in the month of July recorded the highest survival percentage (82.00 %), whereas the highest graft survival percentage (47.66 %) was in epicotyl grafting. The least number of days for sprouting was recorded in epicotyl grafting. The earliest sprouting, higher survivability of grafts and saleable plants, least percentage of mortality as well as best vegetative growth performance were obtained in veneer method of grafting done in the month of August in which the scion sticks were defoliated 9 days prior to grafting operation in mango (Majeed *et al.*, 2015).

The highest graft success (91.59 %), survivability (88.75 %), earliest bud breaking (11.10 days) and first leaf opening (14.22 days) were found in cleft grafting with the defoliation of scion 9 days prior to the grafting operation in mango cv. Amrapali (Akter *et al.,* 2016). The highest survival (66.75 %) and graft success (62.00 %) of mango grafts were obtained from stone grafting whereas the lowest survival (57.39 %) as well as success (53.50 %) were found in softwood grafting. The best results obtained from stone grafting might be due to the high relative humidity (88.30 %), congenial temperature (28.50 ^{0}C) and fairly well distributed amount of shower (51.5 mm) that prevailed during the month of August under Bangalore conditions (Sampath *et al.,* 2017).

The better and early cambial union of rootstock and scion as well as the firmness between the scion stick and stock held with each other without any hindrance from the plant tissue resulted in early formation of callus which ultimately resulted in early sprouting of grafts in veneer method of grafting compared to the softwood and epicotyl grafting. Such grafts also made better results in terms of vegetative growth as well as graft performance in mango cv.

Dashehari (Sami-Ullah *et al.*, 2017). Highest graft success (80.00 %), survival percentage (71.11 %) and growth of grafts in terms of highest shoot length (14.99 cm), plant height, more number of leaves (14.85), highest scion girth (8.81 mm) and stock girth (9.25 mm) were obtained by softwood grafting done on 15th September in mango (Karna *et al.*, 2018).

2.4.2 Effect of Modified environments

Desai and Patil (1984) revealed that the stone grafted mango plants maintained in glasshouse recorded the highest graft success (70.00 %) than the grafts maintained in open condition (40.00 %). Jinturkar and Narwadkar (1989) reported that the success of epicotyl grafting under glass house (51.00 %) was found better than under tree shade (42.00 %) or in grafts maintained under greenhouse (24.00 %) in mango. The highest success percentage of epicotyl grafting was obtained when the plants were maintained under specialized structures *viz.*, glass house, mist chamber or thatched house where the relative humidity, temperature and light are kept optimum (Reddy and Kohli, 1988).

Nair *et al.* (2002) explicated the effects of different colours of polyhouse (white, blue and red), open conditions and various methods of epicotyl grafting (veneer, wedge, whip and side grafting) on graft success and vigour of grafts in mango. They revealed that wedge grafts maintained under red polyhouse recorded earliest bud sprouting of scions (12.00 days), percentage scion take (97.78%), graft success (86.70 %), graft height (15.43 cm), leaf length (11.01 cm) and leaf width (2.46 cm). Pandey and Singh (2002) reported significantly more graft success and survival percentage due to the incorporation of polytube as an anti-transpirant compared to the open condition in mango epicotyl grafts. Savani (2006) recorded highest sprouting percentage of grafts in mango cv. Kesar under polyhouse than the open filed condition.

Among different structural conditions *viz.*, shade net (50 % and 75 % shade), ventilated poly house, open condition and partial shade under coconut trees, the highest sprout length (6.00 cm), total number of sprouted grafts (71.27 %), highest graft height (17.92 cm), number of leaves per graft (17.37), the least number of days required for sprouting (12.11 days) and the highest graft survival

(67.18 %) were recorded in the grafted mango plant maintained under naturally ventilated polyhouse 90 days after grafting (veneer). The highest survival percentage of grafts under natural ventilated polyhouse might be attributed to the climatic factors prevailing during the month of July (early part of monsoon season) *viz.*, 32.69 -90.70 % RH, temperature range of 22-43 ^{0}C and 36.62 K lux light intensity, which ultimately resulted in the more cambial activity both in scion and the rootstock. At the same time, the selected scions seemed to be in a physiologically very active condition which enables better sap flow (Sivudu *et al.*, 2013).

2.4.3 Effect of scion varieties

Maiti and Biswas (1980) conducted an experiment to study the effect of different scion varieties and types of scion shoot (defoliated or not defoliated) on graft success of mango. They found that defoliated scion shoot of cv. Fazli recorded the highest graft success (96.00 %) followed by the cultivars RaneePasand (94.00 %) and Kohinoor (90.00 %). Pandey and Singh (2001) elucidated the effect of scion varieties and time of epicotyl grafting on graft success and survivability of mango. They revealed that pre-activated shoots of four-to-five months old Amrapali grafted on 16th August recorded the highest graft sprouting, success and survivability followed by cultivars Mallika and Dashehari. They opined that the variation in success of grafting among different genotypes might be attributed to the genetic make-up which influences the histological and physiological development within the particular grafts.

Radhamony *et al.* (1989) elucidated the effect of different scion cultivars on mango stone grafting for commercial propagation under Kerala conditions. The scions of different lengths *viz.*, 6, 8 and 10 cm of six cultivars were selected for stone grafting onto an unnamed rootstock. The scion of cvs. Prior and Banganappally (8 cm long) recorded the highest percentage of scion growth (84.00 % in both cultivars). The lowest percentage of survival (12-22 %) was for the cultivar Mulgoa with the scion length of 6 cm.

Geetha (1993) studied the influence of various polyembryonic mango rootstocks on grafting and establishment of grafts. She revealed that Neelum and

Banganapalli scions grafted on rootstock Puliyan resulted in the highest survival percentage of grafts. But the initial success was found better when Muvandan and Chandarakaran rootstocks were grafted with Neelum during June. The poor graft success was noticed for the combination of Chandrakaran - Banganapally and Bangalora-Neelum. With regard to the growth parmeters, highest girth of stock (1.92 cm) and scion (1.66 cm) and the sprout length (12.91 cm) were obtained from the monoembryonic rootstock, Bangalora, grafted with Neelum either in the month of July or August, whereas the polyembryonic rootstocks grafted with Banganapally were less vigorous with respect to these parameters.

The highest graft intake was recorded in mango cultivars Mallika and Khader during the September month, whereas the least graft intake was in cv. Neeleshan. From the study, it was concluded that Mallika and Khader were the most promising varieties for mango softwood grafting (Reddy *et al.*, 1996). The success of veneer and cleft method of grafting at different graft heights were evaluated by Kumar *et al.* (2000). More than 85.00 per cent of graft success, both in veneer and cleft method was obtained when the scions of Dashehari variety were grafted at grafting heights of 75 and 100 cm on seedling rootstock and both the methods were found to be equally successful.

At Kerala Agricultural University, Radha and Aravindakshan (2000) conducted an experiment to study the response of different scion varieties to epicotyl grafting of mango on commercial scale. The cultivars selected for the study include Banglora, Alphonso, Bennet Alphonso, Mundappa, Kalapady, Nadasala, Mulgoa, Prior, Banganapally, Chandrakaran, Neelum, Imampasand, Jehangir, and Suvarnarekha. The highest survival percentage after 6 months of grafting was obtained from Kalapady (84.00 %) followed by Banglora (71.50 %), Neelum (70.80 %) and Mundappa (69.00 %) used as scion while lowest was recorded in Mulgoa (39.60 %) showing greatest variation among different cultivars. The growth rate of grafts in terms of graft height concerned, the highest graft height after 12 months of grafting was recorded in Banglora (78.50 cm) and the lowest was in Chandrakaran (62.00 cm).

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The success of softwood grafting in some commercial hybrids of mango were evaluated by Jacob *et al.* (2001). The scion cultivars Arka Puneet, Arka Aruna, Amrapali, Ratna, Mallika, Prabha Shankar and Sindhu were utilized for the study. The least number of days for sprout initiation was recorded in Mallika (11.74 days) and more number of days for Sindhu (19.57 days). The least number of days for sprout initiation was recorded when the grafting operation was done in the month of July, August, September and October. The grafting success in different mango hybrid grafts varied significantly according to different seasons due to the direct effect of environmental factors. But seasonal effect had much influence on graft success than the varietal difference.

Three different types of propagation methods *viz.,* splice grafting, cleft grafting and budding were practiced in three commercial mango varieties such as Palmer, Haden and Tommy Atkins during the winter period by Pereira *et al.* (2004). The rootstock used for the experiment was cv. Espada. Cent per cent success and establishment of grafts were found in splice method of grafting with Haden and Palmer as scion. In cv. Tommy Atkins, 100 per cent establishment was observed in cleft method of grafting followed by splice grafting (90.00 %). Gurudutta *et al.* (2004) studied the response of four mango cultivars *viz.*, Amrapali, Dashehari, Langra and Mallika towards mango stone grafting. The cv. Dashehari used as scion showed vigorous growth and recorded the highest scion length and height of new graft, whereas cv. Mallika recorded the highest scion girth six month after grafting (MAG) compared to all other varieties.

Prasanth *et al.* (2006) reported the least number of days for graft sprouting (27.30 days) and highest graft survival (46.50 %) in cv. Khader, used as scion in epicotyl grafting of mango. Alam *et al.* (2006) elucidated the effect of different varieties of scion on graft success in mango stone grafting. They concluded that the cv. Langra grafted onto 15 days old rootstock recorded the greatest graft success (66.67 %) followed by Langra grafted onto 20 days old rootstock (53.33 %). The lowest graft success (10.00 %) was obtained from cv. BARI Aam-3 grafted onto 5 and 30 days old rootstock. The highest graft height (25.07 and

24.73 cm) was obtained from cv. Langra grafted on 15 and 20 days old rootstock respectively.

Jana (2007) conducted an experiment to study the response of mango cultivars to top-veneer grafting technique. Mallika, Amrapali, Dashehari, Langra, Zardalu, Tommy Atkins, Malda and Bombay Green were selected for the study. The pooled data analysis during the year 2000 and 2001 indicated that the cv. Tommy Atkins accounted for highest graft success (93.34 %), sprout length (30.60 cm) and more number of leaves (22.95) at 90 days after grafting. The largest number of sprouts were recorded in cv. Amrapalli (2.36). The highest N-S and E-W spread was observed in Langra. With respect to the girth and height, all the cultivars equally responded well. Ram *et al.* (2012) reported that the highest graft success and overall performance of stone grafted mango plants were obtained by using Amrapali as scion cultivar whereas the lowest success percentage was recorded in cv. Lucknow Safeda.

Ajal and Kizito (2015) studied the effect of different scions on graft success (splice grafting) and wound healing of mangoes under nursery conditions. They found significant difference among scion varieties in scion length, time required for bud sprouting and final plant survival under Uganda conditions. The scion varieties Bire, Suu, and Kate were grafted onto rootstock Kagogwa. They found that the variety Kate was most compatible with rootstock Kagogwa followed by Suu. The increase in length of scion had direct effect on graft success because of the significant difference among the scion varieties. The Kate variety recorded highest final survival (70.00 %).

Mahesh *et al.* (2017) studied the effect of different scions and grafting time on graft survivability in softwood grafting of mango. Results revealed that among different scion varieties, Baneshan recorded the highest graft survivability (86.00 %), while Khader recorded the lowest percentage (48.60 %) of graft survivability at 90 days after grafting. The softwood grafting done on 15th August was the best in terms of graft success and survivability whereas the least graft success was obtained from the grafting done on 15th September. The variation in graft success might be due to the difference in phenolic contents and differential

capability of stocks in callus formation. The scion maturity is also an important factor which decides the success of grafting.

The higher graft success in mango with different scion cultivars were in the order of Ngwangwa (100.00 %), Zizi (60.00 %), and Sindano (52.50 %), grafted onto rootstock Ngwangwa under Tanzania conditions. There was a significant effect on stock-scion interaction with plant height, leaf area and root collar diameter. Scion cv. Dodo grafted onto Ngwangwa recorded highest plant height at four months after grafting. The scion cv. Ngowe grafted on rootstock Zizi had significantly higher root collar diameter. The highest number of leaves were obtained from the scion Alphonso grafted on Ngwangwa followed by cvs. Ngowe, Tommy and Apple when grafted on rootstock Sindano (Minja *et al.*, 2017).

Sampath *et al.* (2017) conducted an experiment to study the response of selections of Kari Ishada mango cultivar to different grafting methods *viz.*, epicotyl and softwood grafting methods. 10 Kari Ishada selections were utilized for the study. Kari Ishada selection responded well to epicotyl grafting and the selection 'KIS-15' showed highest graft success (75.00 %) whereas the lowest percentage of success was recorded in 'KIS-7' (40.00 %). The higher graft survival was obtained in epicotyl grafting (6.75 %) than the softwood grafting (57.39 %). The highest percentage of graft survival was obtained in 'KIS-15' (69.15 %) and the lowest was recorded in 'KIS-7' (50.42 %). The variation in graft success might be due to the difference in concentration of endogenous phenolic component observed in different selections. Among the interactions, epicotyl grafting done on 'KIS-15' recorded the highest graft success (85.00 %) while softwood grafting done on 'KIS-7' recorded the least success (35.00 %). The compatibility between the grafting methods and different scion varieties might be the probable cause of higher graft success.

Bobade *et al.* (2018) elucidated the effect of different scion varieties on growth and biomass production of stone grafts in mango and they found significant variation among the varieties. The highest graft diameter (0.81 cm), fresh weight (16.29 g) and dry weight of shoot (8.75 g) were found in Kesar. The

greatest sprout height (6.02 cm), shoot length (27.97 cm), length of tap root (35.30 cm), length of secondary roots (23.73 cm), fresh weight of root (8.41 g) and dry weight of root (4.94 g) were observed in Mallika, closely followed by Kesar. However the variety Dashehari recorded the highest leaf area (423.84 cm^2) and it was on par with Kesar (418.66 cm^2) and Amrapali (404.34 cm^2). The variety Pairi recorded the highest stionic ratio (0.93) which was on par with Amrapali (0.91) and lowest ratio was in Dashehari (0.79).

Materials and Methods

3. MATERIALS AND METHODS

An investigation entitled "Evaluation of propagation techniques and rootstock studies of mango (*Mangifera indica* L.)" was carried out at the Department of Pomology and Floriculture, College of Agriculture, Vellayani during the year 2016-19. The investigation was taken up utilizing twenty indigenous mango varieties/ collections, collected from the homesteads of different parts of Thiruvananthapuram district of Kerala. The present investigation comprised of four experiments aimed to screen local mango varieties/ collections for polyembryony, to study the pre-sowing treatments, sowing position and age of stone after extraction from fruit on germination of mango stones, to screen local mango varieties for use as dwarfing rootstocks and to study the effect of two propagation methods in three modified environments on three varieties of scions. In this chapter, the materials used and methods adopted for undertaking different experiments are described under the following headings and sub-headings.

3.1 Screening of local mango varieties/ collections for polyembryony

The twenty local mango varieties/ collections from different parts of Thiruvananthapuram district were screened for polyembryony. These trees were geo-referenced. Microsatellite analysis of all the plantlets from two varieties which exhibited the highest per cent of polyembryony were done using twenty SSR primers and their banding patterns were compared with those of their respective mother plants.

3.1.1 Geo-referencing of mango trees

The geo-referencing of each tree was done by using Maps Me application that was installed in android smart phone. The longitude and latitude of respective trees were recorded as listed in table 1.

3.1.2 Source of seed material

The seed materials were collected from the twenty geo referenced trees in different parts of Thiruvananthapuram district of Kerala (Table 2) and these varieties were screened for polyembryony.

Table 1. Geo-referencing of mango trees utilized for the study

Sl. No.	Genotypes	Longitude (^{0} N)	latitude (^{0} E)
1	Kotookonam Varikka	08^{0}26'13.69"N	76^{0}59'08.44"E
2	Thali Manga	08^{0}26'35.41"N	76^{0}59'00.9"E
3	Vellari Manga	08^{0}25'44.19"N	76^{0}59'09"E
4	Kochu Kilichundan	08^{0}25'44.19"N	76^{0}59'09"E
5	Unda Varikka	08^{0}35'50.11"N	76^{0}51'54.65"E
6	Paiveli Local	08^{0}35'49.7"N	76^{0}51'53.24"E
7	Vazhapazhiti	08^{0}25'30.01"N	76^{0}59'19.85"E
8	Pandi Manga	08^{0}25'57.57"N	76^{0}59'09.41"E
9	Champa Varikka	08^{0}26'13.69"N	76^{0}59'08.44"E
10	Kili Manga	08^{0}43'10.63"N	76^{0}54'30.41"E
11	Peraykka Manga	08^{0}26'52.33"N	76^{0}59'57.43"E
12	Sreekaryom Local	08^{0}32'51.71"N	76^{0}56'42.11"E
13	Mylapoo	08^{0}35'50.11"N	76^{0}51'54.65"E
14	Kasthuri	08^{0}26'13.69"N	76^{0}59'08.44"E
15	Attanari	08^{0}25'57.59"N	76^{0}59'09.4"E
16	Pakalkkuri Local	08^{0}32'34.33"N	76^{0}53'13.77"E
17	Kuttara Local	08^{0}43'10.63"N	76^{0}54'30.41"E
18	Vellari Varikka	08^{0}25'30.01"N	76^{0}59'19.85"E
19	Kappa Manga	08^{0}25'57.57"N	76^{0}59'09.41"E
20	Nattumavu	08^{0}32' 34.33"N	76^{0}53'13.77"E

Kotookonam Varikka

Thali Manga

Vellari Manga

Kochu Kilichundan

Plate 1. Varieties screened for polyembryony

Kuttara Local

Vellari Varikka

Kappa Manga

Nattumavu

Plate 1. continued

Mylapoo

Kasthuri

Attanari

Pakalkkuri Local

Plate 1.continued

Champa Varikka

Kili Manga

Peraykka Manga

Sreekaryom Local

Plate 1.continued

Unda Varikka

Paiveli Local

Vazhapazhiti

Pandi Manga

Plate 1.continued

3.1.3 Location

The experiment was conducted at Instructional Farm, College of Agriculture, Vellayani during 2016-18. The geographical co-ordinates of the location of Vellayani are 8^0 5'N latitude and 76^0 9'E longitude with an altitude of 29 m above Mean Sea Level (MSL).

3.1.4 Experimental details

The experiment was laid out in completely randomized design (CRD) with 20 treatments replicated thrice. Fifteen stones were taken per replication from different genotypes for the present study.

3.1.5 Methodology

The ripe mango fruits were collected from trees located in different parts of Thiruvananthapuram district of Kerala. The stones were extracted from the fruit and washed thoroughly to remove the extraneous materials adhering to it. After cleaning, the stones were immersed in water and allowed to sink to bottom of the container. Those stones which floated on the surface were discarded and those which settled at the bottom were utilized for the study. Then the selected stones were dried under shade for one day. Twenty five stones of each variety were sown in stalk end up position in large pro trays which were properly filled with a mixture of red soil, sand, FYM (2:1:1) and labelled. These were placed in green house at 15 cm apart. The stones were irrigated immediately after sowing and subsequently as and when required.

Table 2. List of mango varieties screened for polyembryony

Sl. No.	Treatments	Varieties
1	T_1	Kotookonam Varikka
2	T_2	Thali Manga
3	T_3	Vellari Manga
4	T_4	Kochu Kilichundan
5	T_5	Unda Varikka
6	T_6	Paiveli Local
7	T_7	Vazhapazhiti
8	T_8	Pandi Manga
9	T_9	Champa Varikka
10	T_{10}	Kili Manga
11	T_{11}	Peraykka Manga
12	T_{12}	Sreekaryom Local
13	T_{13}	Mylapoo
14	T_{14}	Kasthuri
15	T_{15}	Attanari
16	T_{16}	Pakalkkuri Local
17	T_{17}	Kuttara Local
18	T_{18}	Vellari Varikka
19	T_{19}	Kappa Manga
20	T_{20}	Nattumavu

Plate 2. Mango genotypes at germination stage

3.1.6 Microsatellite analysis

3.1.6.1 Plant sample

The mango varieties 'Kotookonam Varikka' and 'Kochu Kilichundan' exhibited highest per cent of polyembryony. Hence young, tender and fully expanded leaves from the mother tree as well as the plantlets from stones of 'Kotookonam Varikka' and 'Kochu Kilichundan' were collected.

3.1.6.2 Genomic DNA isolation

Microsatellite analysis of the two parents and all the plantlets which developed from their stones of these two varieties were done using twenty SSR primers and compared. For the isolation of genomic DNA, young, tender and fully expanded leaves from the mother tree and plantlets arised from each variety were collected, labelled and wrapped in aluminum foil and put in a liquid nitrogen box for inactivation of enzymes. The leaves were washed and the midribs and thick veins were removed.

The genomic DNA from the selected varieties were extracted using the method described by Dellaporta *et al.* (1983). Leaf bits of 0.5-1 g were transferred into pre-chilled mortar, quick frozen in liquid nitrogen and ground to a fine powder. The fine powder of the respective samples were transferred to 20 ml centrifuge tubes and mixed with 15 ml of extraction buffer containing 20 µl of ß-mercapto ethanol and 50 mg of PVP (Polyvinyl pyrollidine) and kept at 4 ^{0}C. 1 ml of 20 % SDS was added in each tube and incubated at 65 ^{0}C for 1 hour in a water bath (Beston) with occasional shaking. 5 ml of 5 M potassium acetate was added to it and then kept on ice (0 ^{0}C) for 20 minutes. Centrifugation (Centrifuge 5430 R Eppendorf) was performed at 12,000 rpm for 20 minutes. The clear aqueous phase was transferred to a fresh sterile tube. Then added equal volume of ice cold isopropanol and mixed gently by inversion and kept in -20 ^{0}C freezer until DNA was precipitated out. Centrifugation was performed at 12,000 rpm for 10 minutes. Then the DNA pellet obtained was dissolved in 500 µl sterile double distilled water. Added 3µl of RNase to this DNA solution and incubated at 37 ^{0}C for 1 hour. 500 µl of chloroform: isoamyl alcohol mixture was added to the mixture and

mixed well for 15 minutes. Then the mixture was centrifuged at 12,000 rpm for 15 minutes. The aqueous phase was transferred to another micro centrifuge tube without disturbing the inter phase to remove the insoluble debris. Then added two volumes of ice cold absolute alcohol and 1/10 volume of sodium acetate to the aqueous phase and kept for overnight incubation at -20 ^{0}C. The mixture was then centrifuged at 12,000 rpm for 5 minutes and the supernatant was discarded. DNA pellet was washed with 500 µl of 70 % ethanol and air-dried thoroughly. Then the DNA pellet was dissolved at 100 µl of TE buffer and stored at -20 ^{0}C for further use.

3.1.6.3 Quantification and quality assessment of DNA samples

The quantity of DNA present in each sample was determined by reading the absorbance at 260 nm and 280 nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer). The optical density (OD) of the DNA samples dissolved in the buffer was recorded at 260 nm and 280 nm. The ratio obtained from the readings at 260 and 280 nm (OD 260/OD 280) indicated the estimate of the purity of DNA samples. A ratio between 1.7 and 1.8 indicated good quality DNA (Sambrook and Russell, 2001). Quality was assessed by using gel electrophoresis with 5µl of crude DNA sample on agarose gel (0.8%) and stained with ethidium bromide (ETBR). Since an OD of 1.0 at 260 nm represents 50 ng/ml of DNA, the quantity of DNA in the sample was estimated by employing the following formula:

Amount of DNA (ng/ml) = A_{260} x 50 x dilution factor, where A_{260} is absorbance at 260 nm

3.1.6.4 Dilution of DNA samples

For PCR analysis, the stock DNA samples after quantification were diluted to 50 ng/µl of working solutions. The DNA dilutions were prepared by using the formula as given below.

$$M_1V_1 = M_2V_2$$

Where M_1 is the stock DNA concentration, V_1 is the volume of stock to be diluted, M_2 is the concentration of working solution and V_2 is the volume of

working solution to be prepared. Then the required volume from the stock was transferred to 0.5 ml micro centrifuge tube, and the volume was made to 100 µl using TE buffer. The DNA working solutions were kept at -20 °C for future use.

3.1.6.6 PCR amplification using SSR primers (PCR analysis)

PCR reactions were carried out in a 25 µl reaction mixture which consisted of

i)	Genomic DNA (~ 25ng/µl)	- 2.0 µl
ii)	PCR Taq Mixture	- 12.5 µl
iii)	Forward primer (1 µM)	- 2.5 µl
iv)	Reverse primer (1 µM)	-2.5 µl
v)	Autoclaved distilled water	-5.5 µl
	Total volume	25 µl

PCR reaction was carried out using Master Cycler gradient 5331-Eppendorf version 2.30. 31-09, Germany. The thermal cycling was carried out with the following programme.

i.	Initial denaturation	- 94 ^{0}C for 3 minutes	
ii.	Denaturation	- 94 ^{0}C for 1 minute	35 cycles
iii.	Primer annealing	- 53 ^{0}C to 55 ^{0}C for 1 minute	
iv.	Primer extension	- 72 ^{0}C for 1 minute	
v.	Final extension	- 72 ^{0}C for 5 minutes	
vi.	Incubation	- 4 ^{0}C for infinity to hold the sample	

3.1.6.7 Detection of polymorphism between the plantlets obtained from two polyembryonic mango varieties with their mother plant using SSR primers

Twenty primer combinations were screened by PCR and their sequences are enlisted in table 3. The amplified products were run along with marker (100 bp ladder) on 2 % agarose gel using 1X TBE buffer and stained with ethidium bromide. The DNA profile was visualized under UV (312 nm) trans-illuminator and documented in gel documentation system (Syngene G box documentation system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern among the plantlets with their respective mother plant.

Table 3. List of SSR primers and their base sequences used for the study

Primer name	Sequence (5'-3')	Anne-aling temp. (^{0}C)	Melti-ng temp. (^{0}C)	Allele size range (bp)
SSR- 16	F: GCTTTATCCACATCAATATCC R: TCCTACAATAACTTGCC	54	54	160-170
SSR-19	F: AATTATCCTATCCCTCGTATC R: AGAAACATGATGTGAACC	54	54	135-145
SSR-20	F: CGCTCTGTGAGAATCAAATGGT R:GGACTCTTATTAGCCAATGGGATG	58	58	295-310
SSR-24	F: GATGAAACCAAAGAAGTCA R: CCAATAAGAACTCCAACC	53	53	310-346
SSR-26	F: GCCCTTGCATAAGTTG R: TAAGTGATGCTGCTGGT	52	52	170-182
SSR-52	F: AAAAACCTTACATAAGTGAATC R: CAGTTAACCTGTTACCTTTTT	52	52	207-248
SSR-84	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	54	58	200-260
SSR-85	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	52	58	250-310
SSR-89	F: CGCCGAGCCTATAACCTCTA R: ATCATGCCCTAAACGACGAC	54	55	110-140
MNGSSR-14	F: TCATTAAGCTGTGGCAACCA R: CATTGCATAGATGTGGTCATT	55	59	110-140

Table 3. List of SSR primers and their base sequences used for the study (continued)

Primer name	Sequence (5'-3')	Annealing temp. (^{0}C)	Melting temp. (^{0}C)	Allele size range (bp)
MilIHR 10	F: CGATTCAAGACGGAAAGGAA R:TTCAAGCACAGACGACCAAC	55	53	161-184
MilIHR 11	F: CAGTGAAACCACCAGGTCAA R: TGGCCAGCTGATACCTTCTT	55	63.7	203-213
MilIHR 12	F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	55	53	153-187
MilIHR 13	F: CCCAGTTCCAACATCATCAG R: TTCCTCTGGAAGAGGGAAGA	55	50	169-193
MilIHR 15	F: CTAACCATTCGGCATCCTCT R:TCTGTGATAGAATGGCAAAAGAA	55	54	135-194
MilIHR 21	F: TTTGGCTGGGTGATTTTAGC R: TTAATTGCAGGACTGGAGCA	55	53	230-262
MilIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	55	52	127-148
MilIHR 24	F: GCTCAACGAACCCAACTGAT R: TCCAGCATTCAATGAAGAAGTT	55	52	237-260
MilIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	55	52	210-229
MilIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	55	53	222-244

3.1.7 Germination characters

3.1.7.1 Germination (%)

The germination percentage was calculated once the germination of all the stones has been completed. It was calculated by counting number of mango stones germinated out of total stones sown in the polybag (Patel, 2015).

$$\text{Germination percentage} = \frac{\text{Number of stones germinated}}{\text{Total number of stones sown}} \times 100$$

3.1.7.2 Number of plantlets produced per stone

The germinated stones of selected varieties were closely examined for polyembryony. After the completion of germination, the number of plantlets produced per stone of each variety was recorded and the average was calculated (Geetha, 1993).

3.1.7.3 Percentage polyembryony (%)

Percent polyembryony was calculated by dividing the number of stones having multiple seedlings (more than one seedling) by total number of germinated stones and multiplying with 100 (Kumar, 2015).

$$\text{Percent polyembryony} = \frac{\text{Stones having multiple seedlings}}{\text{Total number of germinated stones}} \times 100$$

3.1.7.4 Mean germination time (MGT)

Mean germination time is an accurate measure of the time taken for a batch of stones to germinate and is expressed in days (Czabator, 1962).

Mean germination time = $\Sigma f.x/\Sigma f$, where f is the number of stones germinated on day x

3.1.7.5 Germination index (GI)

Germination index was calculated 60 days after sowing by dividing the germination percentage by time taken for 50 per cent germination (Kendrick and Frankland, 1969).

$$\text{Germination index} = \frac{\text{Germination percentage}}{\text{Time taken for 50 per cent germination}}$$

3.1.7.6 Seedling vigour index – I (growth basis)

Seedling Vigour Index- I was calculated 120 DAS based on the following formula:

Seedling Vigour Index

= germination percentage (%) x [shoot length (cm) + root length (cm)]

(Rao *et al.*, 2006)

Statistical Analysis

The means of all the treatments were calculated and the ANOVA for all the characters were performed by 'F' variance test at 5 per cent level of significance.

3.1 Effect of pre-sowing treatments, sowing position and age of stone after extraction from the fruit on germination of mango stones.

3.2.1 Experimental details

The mango stones of Kotookonam Varikka were utilized for the experiment. The experiment was laid out in Factorial Completely Randomized Block Design with forty two treatments replicated thrice. The treatments comprised of combinations of sowing positions, age of stone after extraction from fruit and pre sowing treatments.

3.2.2 Treatment details

Factor A: Sowing positions

S_1: Flat

S_2: Stalk end up

Factor B: Age of stone after extraction from fruit

A_1: Freshly extracted stone

A_2: 10 days after extraction

A_3: 20 days after extraction

Factor C: Pre sowing treatments

T_1: GA_3 -100 ppm

T_2: GA_3 -200 ppm

T_3: KNO_3 -1 ppm

T_4: KNO_3 - 2 ppm

T_5: Cow dung slurry

T_6: Water

T_7: Control (No treatment)

3.2.3 Methodology

The treatments comprised of different combinations of two sowing positions, three different age group of stones after extraction from fruit, seven pre-sowing treatments and their combinations. Fruits of 'Kotookonam Varikka' variety of mango were selected for the study. After extraction, the stones were washed thoroughly to remove extraneous material adhering to it. Then these stones were immersed in water and allowed to sink to the bottom of the container. Stones floating on the surface of water were discarded and those which settled at the bottom were used for experimentation. The mango stones were soaked in the above solutions for 24 hours prior to sowing. The seed beds were prepared in field and FYM was added. The treated mango stones of different age groups were sown at proper spacing in stalk end up and flat positions in the seed bed. Twenty five stones of each treatment were replicated thrice. The stones were irrigated immediately after sowing. Subsequently, the beds were watered as and when required.

3.2.4 Observations

3.2.4.1 Days taken for initiation of germination (days)

The number of days taken for the emergence of radicles in randomly selected polybags in each replication were recorded and expressed as average number of days taken for the initiation of germination as suggested by Aatla, 2011.

3.2.4.2 Days taken for 50% of germination (days)

The number of days taken for 50 per cent sprouting in each replication was recorded and expressed as average number of days taken for 50 per cent germination (Aatla, 2011).

3.2.4.3 Germination (%)

The germination percentage was calculated once the germination of all the stones has been completed. It was calculated by counting number of mango stone germinated out of total stones sown in the polybag (Patel, 2015).

$$\text{Germination percentage} = \frac{\text{Number of stones germinated}}{\text{Total number of stones sown}} \times 100$$

3.2.4.4 Rate of germination

The rate of germination was determined by dividing the germination percentage by the number of days taken for germination (Bewley and Black, 1982).

$$\text{Rate of germination} = \frac{\text{Germination percentage}}{\text{Number of days taken for germination}}$$

3.2.4.5 Seedling length (cm)

The seedling length was measured from randomly selected plants of each replication by a metric scale from shoot base to the shoot tip of the seedlings. The mean value was calculated 120 days after sowing (Mukundbhai, 2014).

3.2.4.6 Dry weight of seedling (g)

The seedlings were dried moisture free in a hot air oven at 80 °C for 48 hours till constant weight was attained. Then the dry weight of samples were recorded by using an electronic balance and expressed in grams. The mean values were recorded at 120 DAS (Mukundbhai, 2014).

3.2.4.7 Vigour index - I (growth basis)

Vigour Index- I was calculated 120 DAS based on the following formula:

Vigour Index –I

= germination percentage (%) x [shoot length (cm) + root length (cm)

(Rao *et al.*, 2006)

3.2.4.8 Vigour index - II (weight basis)

Vigour Index- II was calculated 120 DAS based on the following formula:

Vigour Index – II

= germination percentage (%) x dry weight of seedling (g)

(Kumar *et al.*, 2007)

Statistical Analysis

The means of all the treatments were calculated and the ANOVA for all the characters were performed by 'F' variance test at 5 per cent level of significance.

3.3 Screening of mango varieties for use as dwarfing rootstock

The germination, vegetative and growth features and physiological and anatomical features were carefully observed and recorded for each variety utilized in this experiment to identify its dwarfing potential.

3.3.1 Source of seed material

Out of twenty varieties utilized in experiment I, ten were screened at seedling stage for use as rootstocks in order to impart dwarfness (Table 4).

Table 4. List of mango varieties used in screening as dwarfing rootstock

Sl. No.	Treatments	Varieties
1	T_1	Kotookonam Varikka
2	T_2	Kasthuri
3	T_3	Thali Manga
4	T_4	Kochu Kilichundan
5	T_5	Vellari Varikka
6	T_6	Pallikkal Local
7	T_7	Kili Manga
8	T_8	Kappa Manga
9	T_9	Paiveli Local
10	T_{10}	Unda Vaarikka

3.3.2 Experimental details

The experiment was laid out in completely randomized design (CRD) with ten treatments replicated thrice.

3.3.3 Methodology

Ten indigenous mango varieties of Thiruvananthapuram district were selected for the experiment. The stones were extracted from the ripe fruits and washed thoroughly to remove the extraneous materials adhering to it. After cleaning, the stones were immersed in water and allowed to sink to bottom of the container. Those stones which floated on the surface were discarded and those which settled at the bottom were utilized for the study. Then the selected stones were dried under shade for one to two days. Twenty five stones of each variety were sown in polythene bags which were properly filled with a mixture of soil, sand and FYM, labelled with tags and placed in green house at proper spacing. The stones were irrigated immediately after sowing. Subsequently, the bags were irrigated as and when required. The observations were recorded from five randomly selected seedlings in each treatment replicated thrice.

3.3.4 Anatomical studies

Anatomical studies of stem and root of each variety were done at six month after sowing. Pieces of the plant materials of 2-3 cm length were taken for free hand section. The top portion of the material was dipped in distilled water. Then transverse sections were made using sharp razor blade as fast as possible and placed in a watch glass containing water. With the help of a delicate brush, the thinnest section of the sample materials were selected. Oblique and incomplete sections were discarded. Then transferred the thin sections into another watch glass containing water. Few drops of saffranin stain were added into it and left for 3-5 minutes without any disturbances. Then the stain was drained off. Thin sections were washed if necessary. The selected sections were placed at the centre of slide and a drop of glycerine added over the material. The slide was then covered with a cover slip. The root and stem sections were observed under a compound microscope after staining and mounting. For permanent slide preparation, thinnest sections were left in saffranin for 3-5 minutes, washed in clean water until excess stain was removed and stained with fast green for 3 minutes. Later, the excess stain was washed off. Dehydrated the sections in ascending alcohol series 30 %, 50 %, 70 %, 90 % and 100 % alcohol for 4 minutes each. Clearing was done twice in xylene for 5 minutes. Then the sections were mounted on clean glass slide without air bubbles using DPX (Distyrene Plasticizer Xylene) mountant and a clean cover slip was put on it. The required images were taken in Leica microscope (DM 1000 using Leica software). The photographs were made from each cross-section at 4 X, 10 X and 40 X magnification to measure various anatomical features.

3.3.5 Observations

i) Germination characters

The germination characters were recorded four months after sowing.

3.3.5.1 Germination (%)

The germination percentage was calculated once the germination of all the stones has been completed. It was calculated by counting number of mango stone germinated out of total stones sown in the polybag (Patel, 2015).

$$\text{Germination percentage} = \frac{\text{Number of stones germinated}}{\text{Total number of stones sown}} \times 100$$

3.3.5.2 Seedling length (cm)

The seedling length was measured from randomly selected plants of each replication by a metric scale from shoot base to the shoot tip of the seedlings. The mean value was calculated 120 days after sowing (Mukundbhai, 2014).

3.3.5.3 Dry weight of seedling (g)

The randomly selected seedlings from each replication were uprooted and dried under shade for 3 days. Then the samples were oven dried at 60 °C till the constant weight was attained. The mean dry weight was calculated at 120 days after sowing (Mukundbhai, 2014).

3.3.5.4 Vigour index - I (growth basis)

Vigour Index- I was calculated 4 months after sowing based on the following formula:

Vigour Index –I

= germination percentage (%) x [Shoot length (cm) + Root length (cm)]

(Rao *et al.*, 2006)

3.3.5.5 Vigour index - II (weight basis)

Vigour Index- II was calculated 120 DAS based on the following formula:

Vigour Index – II = germination percentage (%) x dry weight of seedling (g)

(Kumar *et al.*, 2007)

ii) Vegetative and growth characters

The vegetative and growth characters of each variety was recorded at six month after sowing.

3.3.5.6 Plant height (cm)

The seedling height was measured from the collar region to the apex of the main stem of randomly selected seedlings and the mean computed (Aatla, 2011).

3.3.5.7 Number of leaves

The number of leaves per seedling were counted from randomly selected seedlings in each replication. The mean values were calculated (Shenoy, 2016).

3.3.5.8 Leaf length (cm)

The leaf length was measured from the tip of the lamina to the base of leaf petiole. The average length of leaf was recorded from randomly selected plants in each replication and expressed in centimetres (Aatla, 2011).

3.3.5.9 Leaf width (cm)

The width of leaf blade was measured at the point where width was maximum on randomly selected plants in each replication using meter scale and the mean value was computed. Leaf width is expressed in centimetres (Aatla, 2011).

3.3.5.10 Average leaf area (cm^2)

The average leaf area was calculated by dividing the area of all the leaves on a flush by the total number of leaves on it (Babubhai, 2009).

3.3.5.11 Total leaf area (cm^2)

The leaf area was calculated using the leaf area constant derived by using leaf area meter and the leaf length and width. Then the total leaf area was calculated by multiplying the leaf area and total number of leaves on it (Babubhai, 2009).

3.3.5.12 Internodal length (cm)

The second internodal region from the base was measured for internodal length. The average internodal length was recorded and expressed in centimetres (Shenoy, 2016).

3.3.5.13 Number of roots

The number of primary roots were counted and the mean computed (Shenoy, 2016).

3.3.5.14 Root length (cm)

The length of longest root was measured by metric scale and expressed in centimetres (Shenoy, 2016).

3.3.5.15 Dry matter of shoot and root (g)

The seedlings were dried moisture free in a hot air oven at 80°C for 48 hours till constant weight was attained. Then the dry weight of samples were recorded by using an electronic balance and expressed in grams. The mean value was recorded at 120 DAS (Mukundbhai, 2014).

iii) Physiological characters

The physiological parameters of each variety was recorded at six months after sowing.

3.3.5.16 Stomatal density (stomata/mm^2)

Measurement of stomatal density was done as per the procedure described by Bajracharya (1999). Three leaf samples of same age group from each variety

was collected and washed gently by running water in order to remove the adhering dirt and debris and they were allowed to dry properly. Then one or two drops of Xylene thermo cole mix was applied on the dorsal surface of leaf in a uniform manner as to form a very thin film over the leaf sample. Then it was allowed to dry completely. After few minutes, the peel was gently peeled off with a sharp needle. It was placed on the slide and one or two drops of water/ glycerol was added for proper spreading and it was covered it with cover slip and kept on a 40 X compound microscope. The total number of stomata from three microscopic fields were counted and average was calculated. Stomatal density was calculated by total number of stomata per unit area. (Unit area of microscopic field was of 0.089 cm^2 size).

3.3.5.17 Membrane stability index (% leakage)

Measurement of membrane stability index was done as per the procedure described by Leopold *et al.* (1981). The collected leaves of each variety was incubated in distilled water for 45 minutes to gain the turgidity. Then the turgid weight of each sample was recorded and samples were placed under shade in order to wilt. When the leaves lost 60 % of its fresh weight, the leaf punches of 1 cm diameter were taken. In order to leach out the solutes from the cut ends, the leaf punch samples were washed for 1 to 2 minutes in clear water and blotted on clean filter paper. A total of 10 leaf punches were taken and incubated for 3 hours in a beaker containing 20 ml distilled water. The initial leakage of solutes in the bathing medium was estimated by recording the absorbance at 273 nm. Then the beakers were incubated for 15 minutes in hot water bath (100 ^{0}c). The absorbance (final) was again read at 273 nm after the suitable dilution because of the leakage of total solutes contained in the leaf tissue. Then the percent leakage was calculated by the following formula:

$$\text{Percentage leakage} = \frac{\text{Initial absorbance of bathing medium}}{\text{Final absorbance of bathing medium}} \times 100$$

3.3.5.18 Relative Water Content (RWC)

The relative leaf water content was estimated as per the method described by Turner (1981). Fully expanded leaves of each variety were collected and the fresh weight recorded. Then these samples were immersed in a petri dish containing distilled water for three hours. The samples were removed and surface water was blotted off. Then the turgid weight was recorded. To obtain the dry weight, these turgid samples were kept in an oven at 70°C for three days. The relative leaf water content was expressed in per cent and calculated using the following formula;

RWC (%) = [(FW - DW) / (TW–DW)] x 100; where FW is the fresh weight; DW is the dry weight; and TW is the turgid weight.

3.3.5.19 Starch content of leaf

The estimation of starch was done by Anthrone method (Mc Crready *et al.*, 1950). A known quantity (0.1 – 0.5 g) of plant sample was homogenized in hot 80 % ethanol to remove sugars. Then the homogenate was centrifuged and the residue retained. The residue was washed repeatedly with hot 80 % ethanol till the washing turned out to be colourless with anthrone reagent. The residue was dried well over a water bath. 5 ml water and 6.5 ml 52 % perchloric acid were added to the residue and was extracted at 0 ^{0}c for 20 min. Then centrifugation was done and the supernatant saved. The extraction was repeated using fresh perchloric acid and centrifugation done accordingly. Then the supernatant was pooled and made up to a volume of 100 ml. 0.1 ml of the supernatant was pipetted out and made up to 1 ml using distilled water. The standard was prepared by taking 0.2, 0.4, 0.6 0.8 and 1 ml of the working standard and volume made up to 1 ml in each tube with distilled water. Four millilitre of Anthrone reagent was added to each test tubes. These test tubes were heated in a boiling water bath for eight minutes and cooled rapidly. The intensity of colour change from green to dark green was measured at 630 nm. The glucose content in each sample was calculated by using the standard graph. Then the value was multiplied by a factor of 0.9 to derive the starch content.

3.3.5.20 Transpiration rate (m moles m^{-2} s^{-1})

Transpiration rate was measured during the morning hours between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and expressed in m moles m^{-2} s^{-1}.

3.3.5.21 Total phenol content of apical bud and leaves of rootstock (mg phenols/ 100 gm)

Phenol was estimated by using Folin Ciocalteau reagent as per the procedure suggested by Sadasivam and Manickam (2009). A quantity of 0.5-1g of the samples (apical bud and leaves) of each variety was ground with a pestle and mortar in ten time volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected. The residue obtained was re-extracted with five time volume of 80 per cent ethanol. Then the supernatant was collected and pooled. Then the supernatant was dried by evaporating at 50 ^{0}C. The residue was dissolved in 5 ml of water for dilution. Then 0.2-2 ml different aliquots were pipetted out into test tubes and made up the volume to 3 ml in each test tube with distilled water. Added 0.5ml Folin Ciocalteau reagent to the respective tubes. Then 2 ml of 20 per cent of Na_2Co_3 was added after three minutes. The content of test tubes were mixed thoroughly and kept in boiling water for one minute. The absorbance was measured at 650 nm against a reagent blank immediately after cooling. Then the standard curve was drawn using different concentrations of catechol. The phenol content of the samples were estimated from the standard curve and expressed in mg phenols/ 100 g material.

3.3.5.22 Leaf temperature (^{0} C)

Leaf temperature was measured during the morning hours between 9 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and expressed in ^{0}C.

iv) Anatomical analysis of rootstock

From the stem and root cross section of respective varieties, the width of half pith (from the centre of section to the inner periphery of vascular bundles), primary xylem (from inner edge of vascular bundles to cambial ring), primary phloem (from cambial ring to outer periphery of vascular bundles) and bark (from cambial ring to outer periphery of periderm) were measured along with the radial lines from center of pith to periphery of cross section using a calibrated ocular micrometer. These observations were recorded for each replication and mean values were worked out for xylem and phloem area and percentage, total conduit area (mm^2), bark percentage of stem and root, etc. These anatomical studies were done as per the standard procedures detailed by Johanson (1940).

Statistical Analysis

The means of all the treatments were calculated and the ANOVA for all the characters were performed by 'F' variance test at 5 per cent level of significance.

3.4 Effect of propagation methods and modified environments on different varieties of scion.

3.4.1 Experimental details

The experiment was laid out in Factorial Completely Randomized Block Design with eighteen treatments replicated thrice. The treatments comprised of combinations of propagation methods, modified environments and different varieties of scion.

3.4.2 Treatment details

Factor A: Propagation methods

1. Epicotyl grafting
2. Softwood grafting (Veneer)

Factor B: Modified environments

1. Climate controlled (fan and pad)
   (80 % RH and 32 ^{o} C temperature)
2. Humid chamber (Closed tunnel made of poly film with 80-90% RH, 32-35 ^{o} C temperature)
3. Natural shade (75% shade)

Factor C: Varieties of scion

1. Kalapady
2. Neelum
3. Kotookonam Varikka

3.4.3 Methodology of softwood grafting

3.4.3.1 Raising of rootstock plants

After extraction from the fruits, the stones were washed thoroughly to remove extraneous material adhering to it. After extraction, the stones were washed thoroughly to remove extraneous material adhering to it. Then these stones were immersed in water and allowed to sink to the bottom of the container. Stones floating on the surface of water were discarded and those which settled at the bottom were used for experimentation. The mango stones were soaked in GA_3 100 ppm for 24 hours prior to sowing. Sowing was during the first week of June. The germinated mango stones (about 18-23 days after sowing) were lifted carefully and transferred into the polythene bags of 25cm x 20cm size and 300 gauge thickness. The polythene bags were properly filled with the potting mixture in the ratio 2:1:1 (red earth, sand and FYM) and then the germinated stones were carefully placed inside the centre of polythene bag. Then the bags were labelled with tags and placed in green house at proper spacing. The stones were irrigated immediately after sowing. Subsequent irrigation was given based on the moisture level of soil.

3.4.3.2 Selection of rootstock

For the purpose of grafting, vigorously growing healthy rootstocks with normal growth and required age (6 months old) were selected. Then plants were ready for grafting operation once it attained the pencil thickness.

3.4.3.3 Selection and preparation of scion

The scions of cultivars Kalapady, Neelum and Kotookonam Varikka were collected from the mango orchard maintained at the Instructional Farm. The terminal, non-flowering shoots of current seasons growth having dark green coloured leaves, about 12-15 cm long, straight, healthy and free from pests and diseases were selected. Usually scions having three to four months maturity and having same thickness as that of rootstock were preferred for grafting. The scion defoliation was done with the help of sharp secateurs by clipping off the leaf blades leaving one –fourth of the petiole (1 cm length) intact seven days prior to detaching. The defoliated scion shoots were separated from the mother tree during the morning hours on the same day of grafting and kept in shady place to avoid the desiccation. The scion sticks were dipped in 0.2 per cent carbendazim solution for 5 min prior to grafting.

3.4.3.4. Procedure of softwood grafting

The main stem of rootstock having a diameter of 4-10 cm is cut off horizontally. A longitudinal cut (vertical split) of 4.5-8 cm was made at the centre portion of the cut stem of stock. A wedge shaped cut (5 cm each) was made on lower part of scion stick (preferably 3 budded shoot). The graft was secured gently by using 1.5 cm wide, 200 gauge polythene stripe. The grafted plants are kept under shade. In about 15-20 days, the scions developed new flushes and the graft union completed. Once the wound completely healed (45-50 days after grafting), the polythene strip was removed. Care was taken to avoid injuries while removing the strip.

3.4.3.5 Aftercare of the grafts

Grafts were watered immediately after planting in the polythene bags. Irrigation was done at 3 to 4 days interval. During summer, irrigation was done at

an interval of 1-2 days. The sprouts arising from leaf axils of rootstock just below the graft union were frequently removed. The sprouts were removed carefully without disturbing the new growth of scion. Hand weeding was done in order to keep the polythene bags free from weeds and off types. It was done at an interval of 12-15 days. For better growth and development of proper graft union, the polythene strips were removed 90 days after grafting in order to avoid the girdling at the region of graft union.

3.4.4 Methodology of epicotyl /stone grafting

3.4.4.1 Raising of rootstock plants

After extraction from the fruits, the stones were washed thoroughly to remove extraneous material adhering to it. After extraction, the stones were washed thoroughly to remove extraneous material adhering to it. Then these stones were immersed in water and allowed to sink to the bottom of the container. Stones floating on the surface of water were discarded and those which settled at the bottom were used for experimentation. The mango stones were soaked in GA_3 100 ppm for 24 hours prior to sowing. Sowing was during the first week of June. The germinated mango stones (about 18-23 days after sowing) were lifted carefully and transferred into the polythene bags of 25 cm x 20 cm size and 300 gauge thickness. The polythene bags were properly filled with the potting mixture in the ratio 2:1:1 (red earth, sand and FYM) and then the germinated stones were carefully placed inside the centre of polythene bag. Then the bags were labelled with tags and placed in green house at proper spacing. The stones were irrigated immediately after sowing. Subsequent irrigations were given based on requirement.

3.4.4.2 Selection of rootstock

Healthy and vigorous seedlings of 8- 12 days age with straight and stout epicotyl and coppery-red leaves were lifted carefully along with stones without causing any injury to roots. The lifted seedlings were washed immediately with water. Then treated with 0.1 % Bavistin for five minutes and were kept ready for grafting operation.

3.4.4.3 Selection and preparation of scion material

The scions of cultivars Kalapady, Neelum and Kotookonam Varikka were collected from the mango orchard maintained at the Instructional Farm. The terminal, non-flowering shoots of current season growth having dark green coloured leaves, about 12-15 cm long, straight, healthy and free from pests and diseases were selected. Usually scions having three to four months maturity and having same thickness as that of rootstock were preferred for grafting. The scion defoliation was done with the help of sharp secateurs by clipping off the leaf blades leaving 1/4 th of the petiole (1 cm length) intact seven days prior to detaching. The defoliated scion shoots were separated from the mother tree during the morning hours on the same day of grafting and kept in a shady place to avoid desiccation. The scion sticks were dipped in 0.2 per cent carbendazim solution for 5 min prior to grafting.

3.4.4.4 Procedure of epicotyl/ stone grafting

In epicotyl grafting, grafting was performed by cleft method. The top of the seedlings should be removed by giving a complete horizontal cut at 6-8 cm height from the stone. Then a vertical cross cut of about 3 cm was given at the centre of stock plants. The scion sticks of equal or slightly smaller in diameter were utilized for grafting. A similar slanting cut was made to both sides of scion, just below the terminal bud thus forming a wedge. Then it was inserted in the vertical cut of the stock plant in such a way that both sides of scion-wedge contacted the sides of vertical cut. The grafted portion was wrapped with 1.5 cm wide, 200 gauge transparent white polythene strip so as to prevent the water entering into the grafted part.

3.4.4.5 Aftercare of the grafts

Grafts were watered immediately after planting in the polythene bags. Irrigation was done at 3-4 days interval. During summer, irrigation was done at an interval of 1-2 days. The sprouts developing from leaf axils of rootstock just below the graft union were frequently removed. The sprouts were removed carefully without disturbing the new growth of scion. Hand weeding was done in

order to keep the polythene bags free from weeds and off types. It was done at an interval of 12-15 days. For better growth and development of proper graft union, the polythene strips were removed 90 days after grafting in order to avoid the girdling at the region of graft union.

3.4.4 Observations

3.4.4.1 Girth of rootstock (mm)

Girth of rootstock was measured 90 DAG with the help of vernier callipers just 1 cm below the graft union and expressed in millimetre. (Agrawal, 2007).

3.4.4.2Girth of scion (mm)

Girth of scion was measured 90 DAG with the help of vernier callipers just 1 cm above the graft union and is expressed in millimetre (Agrawal, 2007).

3.4.4.3 Length of scion (cm)

The length of scions was measured 90 DAG from the middle of the graft union to the apex of the newly developed shoot by using a measuring scale and expressed in centimetre (Usare, 2016).

3.4.4.4 Graft height (cm)

The height was measured 90 DAG from the point just above the collar region to the terminal end of the grafts and expressed in cm (Usare, 2016).

3.4.4.5 Length of sprout (cm)

The length of sprout was measured from the base of scion bud to the terminal end of graft at 90 DAG and expressed in cm (Agrawal, 2007).

3.4.4.6 Spread of plant (cm)

The spread of plant was recorded 90 DAG with meter scale in two directions (East - West and North –South) and expressed in cm (Bhagat, 1998).

3.4.4.7 Days taken for first sprouting (days)

The number of days required for initiation of sprouting (bud breaking in scion) was recorded as days taken for first sprouting (Agrawal, 2007).

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3.4.4.8 Days taken for last sprouting (days)

The number of days required for completion of sprouting was recorded as number of days taken for last sprouting (Agrawal, 2007).

3.4.4.9 Number of grafts sprouted at weekly intervals (%)

The number of grafts which sprouted at weekly intervals out of the total number of grafts prepared were counted for each treatment and expressed in percentage (Savani, 2009).

3.4.4.10 Initial success percentage (%)

The initial success percentage of the grafts were measured at 30 DAG as the percentage of grafts that put forth the first vegetative flush. Initial success percentage was calculated by the formula (Bhagat, 1998) as follows:

$$\text{Initial success percentage} = \frac{\text{No. of grafts that put forth the first vegetative flush}}{\text{Total number of grafts prepared}}$$

3.4.4.11 Percentage of graft establishment (%)

The percentage of graft established was measured as the percentage of grafts that put forth the second vegetative flush with fully opened leaves at 60 DAG (Agarwal, 2007).

3.4.4.12 Days taken for leaf opening (days)

The number of days required for opening of first leaf from the sprouted scion was counted (Usare, 2016).

3.4.4.13 Number of leaves per graft

Total number of fully opened leaves per graft was counted from randomly selected plants from each treatment at 90 DAG and the average was calculated (Savani, 2009).

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3.4.4.14 Leaf length (cm)

The leaf length was measured 90 DAG from the tip of the lamina to the base of leaf petiole. The average length of leaf was recorded from randomly selected five plants in each replication and expressed in centimetres (Aatla, 2011).

3.4.4.15 Leaf width (cm)

The width of leaf blade was measured at the point where width was maximum on randomly selected plants in each replication using meter scale and the mean value was computed. Leaf width is expressed in centimetres (Aatla, 2011).

3.4.4.16 Leaf area (cm^2)

The leaf area was calculated using the leaf area constant derived using leaf area meter and the leaf length and width. The leaf area was expressed in centimetre square (Abilasha, 2012).

3.4.4.17 Number of nodes on scion

The total number of nodes on scion was counted and recorded at 90 DAG (Sivudu, 2013).

3.4.4.18 Internodal length (cm)

The second internodal region from the base was measured for internodal length. The average internodal length was recorded and expressed in centimetres (Shenoy, 2016).

3.4.4.19 Root length (cm)

The length of longest root was measured by metric scale at 180 DAG and expressed in centimetres (Shenoy, 2016).

3.4.4.20 Number of growth flushes per graft

After grafting, the number of new growth flushes in each treatment was counted at the time of their appearance and the mean value computed (Nair, 2000).

3.4.4.21 Number of days taken between grafting and first vegetative flush

The number of days required for complete opening of the entire leaves in the vegetative flush was counted.

3.4.4.22 Number of days taken between grafting and second vegetative flush

The number of days required for complete opening of the leaves in the second vegetative flush was counted.

3.4.4.23 Survival percentage of grafts (%)

After six months of graft union and sprouting of scion shoots, the data pertaining to number of plants survived were recorded and expressed in percentage using the following formula (Agrawal, 2007):

$$\text{Survival percentage of grafts (\%)} = \frac{\text{Total no. of grafts survived}}{\text{Total no. of grafts prepared}}$$

Statistical Analysis

The means of all the treatments were calculated and the ANOVA for all the characters were performed by 'F' variance test at 5 per cent level of significance.

Results

4. RESULTS

The results of the investigations carried out for evaluation of propagation techniques and rootstock studies of mango were analysed and are presented in this chapter.

4.1: Screening of local mango varieties/ collections for polyembryony

4.1.1 Germination characters of mango genotypes

The germination characters such as germination percentage, number of plantlets produced per stone, percentage polyembryony, mean germination time, germination index, seedling vigour index -I were recorded and the analysed data is urnished below.

4.1.1.1 Germination (%)

The germination percentage of different mango genotypes are presented in table 5(a). The germination percentage differed significantly among the genotypes. The highest germination percentage (73.33 %) was recorded in var. Kappa Manga (T_{19}) and Vellari Varikka-T_{18} (68.89 %), Nattumavu-T_{20} (66.67 %), Kuttara Local- T_{17} (62.22 %), Unda Varikka- T_5 (60.00 %), Pandi Manga- T_8 (60.00 %) and ChampaVaikka- T_9 (60.00 %) were on par. The least germination percentage (31.11 %) was recorded in var. Kotookonam Varikka (T_1).

4.1.1.2 Number of plantlets produced per stone

The data on number of plantlets produced per stone is presented in table 5(a).The number of plantlets produced per stone significantly differed among the varieties under study.Out of seventeen polyembryonic varieties, Kotookonam Varikka (T_1) produced significantly more number of plantlets/ stone (5.00) followed by Kochu Kilichundan-T_4 (4.13) and Mylapoo -T_{13} (3.80), whereas the var. Pandi Manga (T_8) recorded the minimum number of plantlets/ stone (1.67).

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Table 5(a). Germination characters of mango genotypes

Treatments	Germination (%)	Number of plantlets produced per stone	Percentage polyembryony (%)
T_1	31.11	5.00	65.13
T_2	46.67	2.27	29.57
T_3	44.44	3.67	46.90
T_4	33.33	4.13	63.62
T_5	60.00	2.27	23.12
T_6	37.78	3.60	52.11
T_7	55.55	2.20	30.97
T_8	60.00	1.67	20.97
T_9	60.00	2.27	28.06
T_{10}	44.44	3.33	44.30
T_{11}	46.67	3.27	41.79
T_{12}	48.89	3.60	57.68
T_{13}	40.00	3.80	56.30
T_{14}	51.11	2.33	33.88
T_{15}	51.11	2.27	33.80
T_{16}	51.11	2.33	41.75
T_{17}	62.22	1.93	26.35
T_{18}	68.89	----	----
T_{19}	73.33	----	----
T_{20}	66.67	----	----
SE m(±)	5.42	0.17	2.74
CD	15.55	0.49	7.90

--- The monoembryonic varieties (T_{18}, T_{19} and T_{20}) were not included for statistical analysis

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Kotookonam Varikka

Thali Manga

Vellari Manga

Kochu Kilichundan

Plate 3. Extent of polyembryony among the mango genotypes

Unda Varikka

Paiveli Local

Vazhapazhiti

Pandi Manga

Plate 3. Continued

Champa Varikka

Kili Manga

Peraykka Manga

Sreekaryom Local

Plate 3. continued

Mylapoo

Kasthuri

Attanari

Pakalkkuri Local

Plate 3. Continued

Kuttara Local

Vellari Varikka

Kappa Manga

Nattumavu

Plate 3. Continued

4.1.1.3 Percentage polyembryony

The data with regard to percentage polyembryony is presented in table 5(a) exhibited significant differences. The highest percentage polyembryony (65.13 %) was recorded in var. Kotookonam Varikka (T_1). The varieties Kochu Kilichundan (T_4) (63.62 %) and Sreekaryom Local (T_{12}) (57.68 %) were on par and the least percentage of polyembryony (20.97 %) was noted in Pandi Manga (T_8).

4.1.1.4 Mean germination time

The data presented in table 5(b) showed significant difference among the varieties with regard to mean germination time. The mean germination time was the least (17.50 days) in var. Vellari Varikka (T_{18}). The varieties Nattumavu (T_{20}) (18.90 days), Kappa Manga (T_{19}) (20.67 days), Kuttara Local (T_{17}) (22.53 days) and Pandi Manga (T_8) (23.07 days) were on par with Vellari Varikka. The highest mean germination time (33.40 days) was recorded in var. Kotookonam Varikka (T_1).

4.1.1.5 Germination index

The data on germination index of different mango genotypes are presented in table 5(b) and that exhibited significant differences. The highest germination index (2.41) was in var. Kappa Manga (T_{19}). The varieties such as Nattumavu (T_{20}) (2.27) and Vellari Varikka (T_{18}) (2.15) were on par with Kappa Manga and the lowest germination index (0.70) was recorded in var. Kotookonam Varikka (T_1).

4.1.1.6 Seedling vigour index -I

The data on seedling vigour index -I on growth basis 120 DAS is presented in table 5(b). There was significant difference among the treatments under study. The highest vigour index was noted in var. Kappa Manga (T_{19}) (2795.20) and the varieties Vellari Varikka (T_{18}) (2508.53), Nattumavu (T_{20}) (2127.68) and Pandi Manga (T_8) (2233.78) were on par. The least vigour index-I (910.18) was recorded in Kochu Kilichundan (T_4).

Table 5(b).Germination characters of mango genotypes

Treatments	Mean germination time (Days)	Germination index	Seedling vigour index-I (Growth basis)
T_1	33.40	0.70	1029.01
T_2	29.63	1.27	1528.58
T_3	29.27	0.99	1283.70
T_4	30.17	0.76	910.18
T_5	28.57	1.66	2001.02
T_6	32.00	0.91	1174.07
T_7	27.23	1.52	1658.73
T_8	23.07	1.72	2233.78
T_9	28.40	1.53	1950.00
T_{10}	28.73	1.03	1365.16
T_{11}	30.57	1.12	1454.66
T_{12}	29.83	1.18	1643.55
T_{13}	32.73	0.96	932.57
T_{14}	32.80	1.21	1447.38
T_{15}	33.00	1.40	1799.25
T_{16}	28.50	1.35	1833.04
T_{17}	22.53	1.65	1580.91
T_{18}	17.50	2.15	2508.53
T_{19}	20.67	2.41	2795.20
T_{20}	18.90	2.27	2127.68
SE m(±)	2.15	0.22	263.79
CD	6.17	0.62	756.74

4.1.2 Molecular characterization of zygotic and nucellar seedlings

Out of seventeen polyembryonic mango varieties screened, molecular characterization of all the plantlets from two varieties that exhibited highest per cent of polyembryony *viz.,* Kotookonam Varikka and Kochu Kilichundan were done using SSR primers in comparison with their mother plants (Table 6).

The selected polyembryonic mango varieties were screened using 20 SSR primers, which were reported in earlier works on mango (Begum *et al.* 2012; Sane *et al.* 2015). The SSR primers *viz.*, SSR- 16, SSR- 19, SSR- 20, SSR- 24, SSR-26, SSR- 52, SSR- 84, SSR- 85, 8SSR- 9, MNGSSR-14, MiIIHR 11, MiIIHR 10, MiIIHR 15, MiIIHR 21, MiIIHR 23, MiIIHR 24, MiIIHR 12, MiIIHR13, MiIIHR 31 and MiIIHR 34 primers uniformly amplified the DNA with an allele size range of 160-170 bp, 135-145 bp, 295-310 bp, 310-346 bp, 170-182 bp, 207-248 bp, 200-260 bp, 250-310 bp, 110-140 bp, 110-140 bp, 203-213 bp, 161-184 bp, 135-194 bp, 230-262 bp, 127-148 bp, 237-260 bp, 153-187 bp, 169-193 bp, 210-229 bp and 222-244 bp respectively (Table 3).

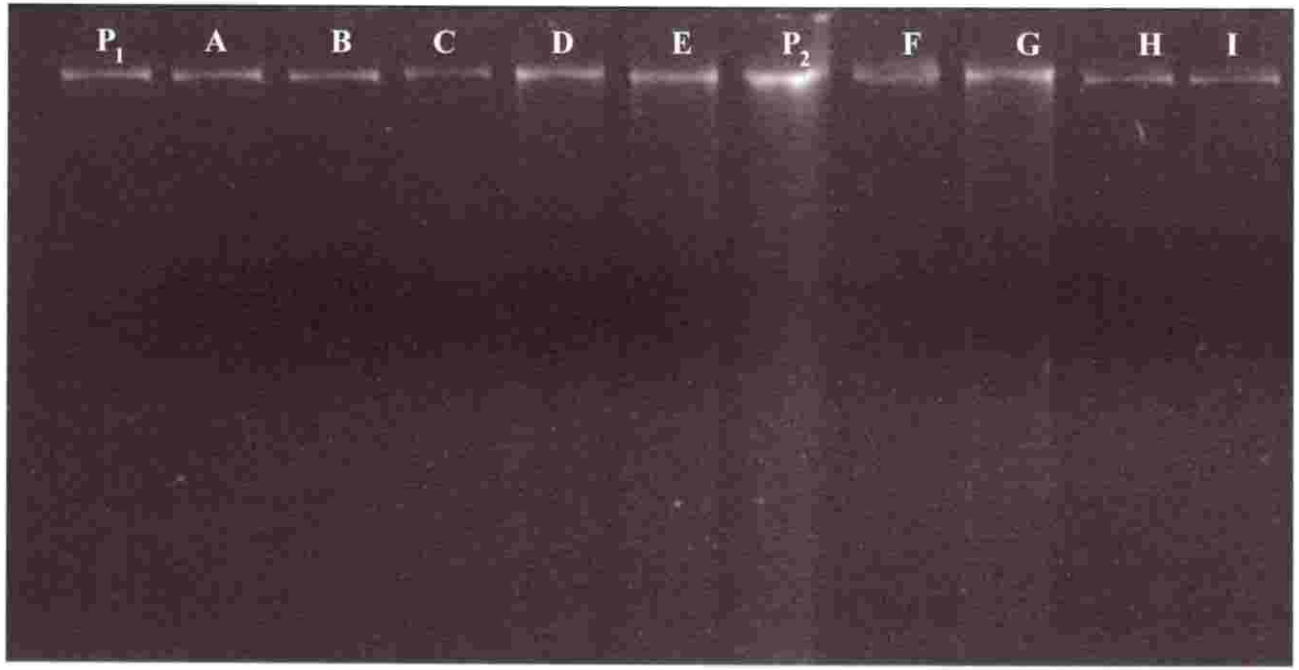

Plate 4.Quality of DNA isolated from parents and progenies
(DNA bands on 0.8 % agarose gel)

P_1: Kotookonam Varikka (Mother plant); A – E: Plantlets from P_1; P_2: Muvandan (Mother plant); F - I: plantlets from P_2

Table 6. Concentration and purity of isolated DNA identified by spectrophotometric method

Sl. No.	Samples	A_{260} value (nm)	A_{280} value (nm)	A_{260}/A_{280} (Ratio)	Quantity of DNA (ng/µl)
1	P_1	0.052	0.030	1.73	1438
2	A	0.098	0.056	1.75	1876
3	B	0.041	0.022	1.86	2182
4	C	0.096	0.054	1.78	1972
5	D	0.102	0.055	1.85	1726
6	E	0.067	0.038	1.76	2175
7	P_2	0.099	0.054	1.83	1638
8	F	0.116	0.069	1.68	1744
9	G	0.046	0.025	1.84	1369
10	H	0.123	0.069	1.78	2243
11	I	0.067	0.038	1.76	1760

P_1 - mother plant (Kotookonam Varikka), A- E: plantlets obtained from P_1, P_2 - mother plant (Kochu Kilichundan), F-I: plantlets obtained from P_2.

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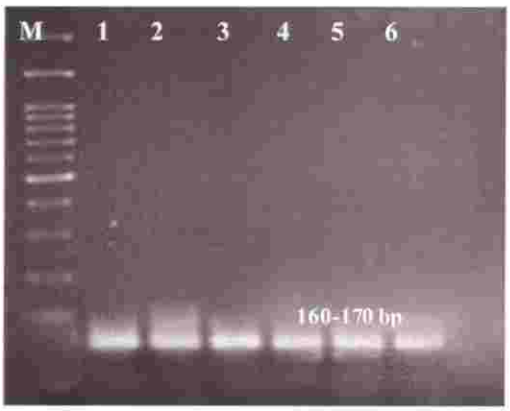

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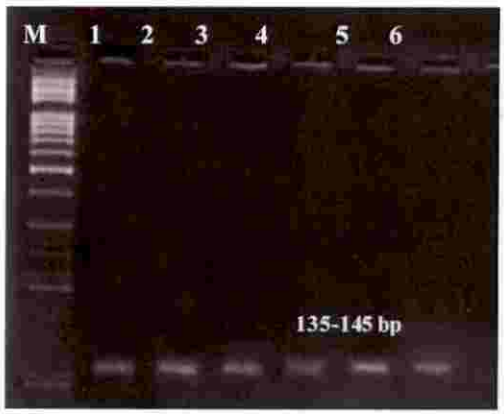

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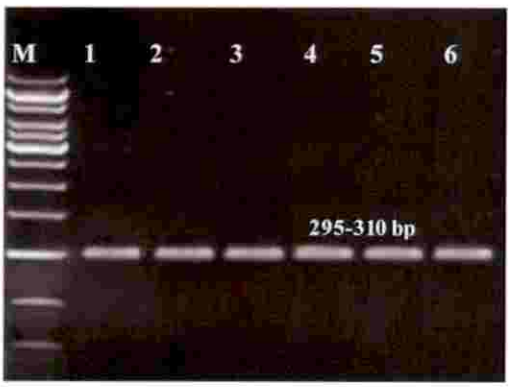
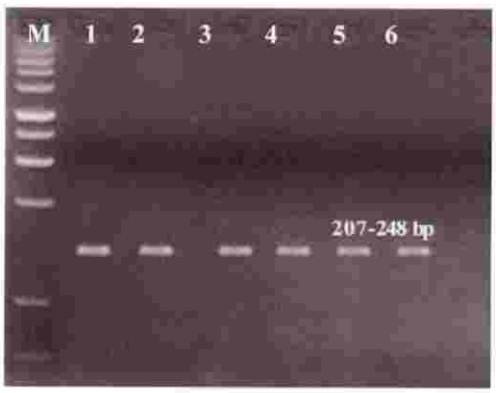


SSR -20

SSR -24

SSR -26

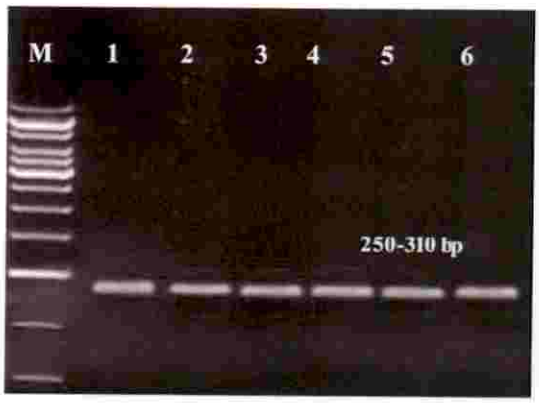


SSR -52

1- Mother plant (Kotookonam Varikka), 2-6: Plantlets M: 100 bp ladder

Plate 5. Amplification profiles of genomic DNA of plantlets obtained from var. Kotookonam Varikka and mother plant using SSR primers

SSR -84



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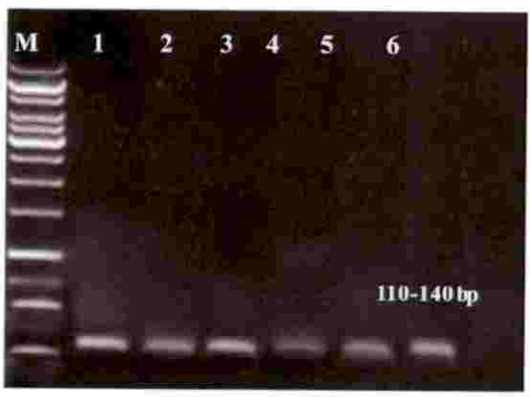
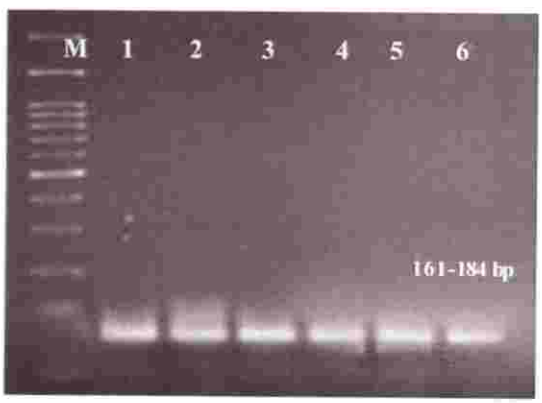


SSR -89

MNGSSR -14



MiIHR 10

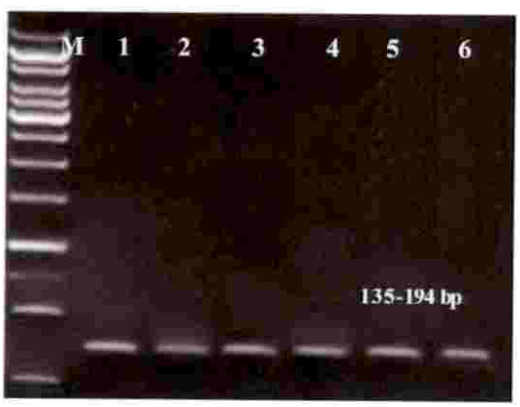
MiIHR 11

1- Mother plant (Kotookonam Varikka), 2-6: Plantlets M: 100 bp ladder

Plate 5. continued

MiIIHR 12

MiIIHR 13



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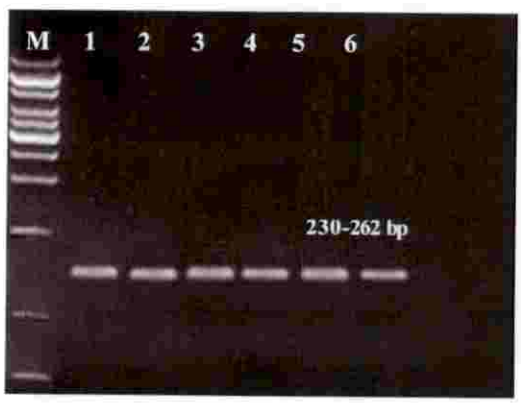
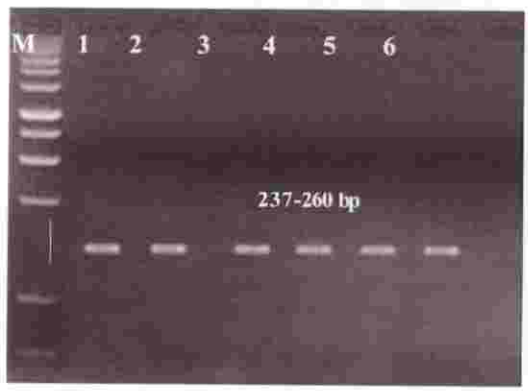


MiIIHR21

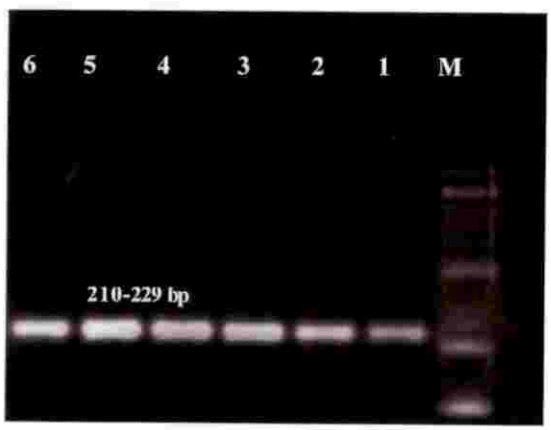
1- Mother plant (Kotookonam Varikka), 2-6: Plantlets M: 100 bp ladder

Plate 5. continued

MiIIHR 23



MiIIHR 24



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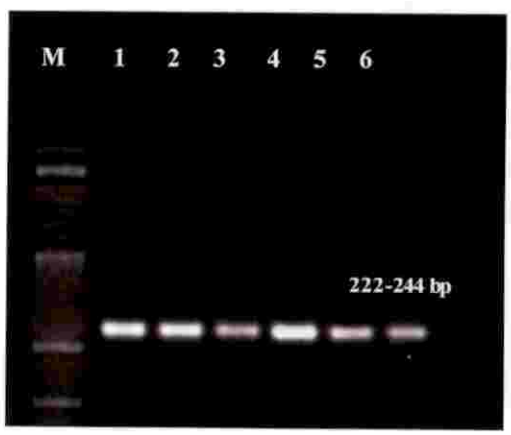

MiIIHR 34

1- Mother plant (Kotookonam Varikka), 2-6: Plantlets M: 100 bp ladder

Plate 5. continued

4.2: Effect of pre-sowing treatments, sowing position and age of stone after extraction from the fruit on germination of mango stones

4.2.1 Days taken for initiation of germination

The data on days taken for initiation of germination as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatmentsis presented in table 7(a).

4.2.1.1 Effect of sowing positions

The days taken for initiation of germination of mango stone was significantly influenced by different sowing positions. Earliness in germination (22.95 days) was recorded in stalk end up sowing method over flat position (29.15 days).

4.2.1.2 Effect of age of stones after extraction from the fruit

It is evident from table 7(a) that age of stones after extraction from the fruit was significantly influenced the days taken for initiation of germination of mango stones. Freshly extracted stones were germinated earlier (18.56 days) than the sowing at 10 days after extraction (24.56 days) and 20 days after extraction from the fruit (35.03 days).

4.2.1.3 Effect of pre sowing treatments

It is evident from table 7(a) that the pre-sowing treatments had a significant effect on number of days taken for initiation of germination of mango stones. The stones treated with 200 ppm GA_3 (T_2) recorded the least number of days for initiation of germination (22.62 days), followed by 100 ppm GA_3 (23.89 days) and 1 ppm KNO_3 (24.49 days). The highest number of days required for initiation of germination (31.01 days) was recorded in control (T_7).

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Table 7(a). Germination characters of mango as influenced by sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Days taken for initiation of germination	Days taken for 50 percent germination	Germination (%)	Rate of germination
Effect of sowing positions				
Flat (S_1)	29.15	40.91	40.95	0.26
Stalk end up (S_2)	22.95	31.75	60.85	0.47
SE(m)	0.05	0.17	0.69	0.001
CD	0.13	0.47	1.94	0.004
Effect of Age of stone after extraction from fruit				
Freshly extracted stone (A_1)	18.56	31.29	59.84	0.47
10 days after extraction (A_2)	24.56	36.50	52.38	0.36
20 days after extraction (A_3)	35.03	41.20	40.48	0.28
SE(m)	0.06	0.20	0.85	0.002
CD	0.16	0.57	2.38	0.005
Pre sowing treatments				
GA_3 - 100 ppm (T_1)	23.89	33.94	55.19	0.43
GA_3 - 200 ppm (T_2)	22.62	31.78	62.59	0.48
KNO_3 - 1 ppm (T_3)	24.49	34.17	52.96	0.42
KNO_3 - 2 ppm (T_4)	25.69	35.56	50.00	0.36
Cow dung slurry (T_5)	25.78	35.78	53.19	0.35
Water (T_6)	28.84	40.11	42.96	0.31
Control [no treatment] (T_7)	31.01	42.94	37.41	0.25
SE(m)	0.09	0.31	1.29	0.003
CD	0.24	0.87	3.63	0.008

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4.2.1.4 Effect of interactions

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant [Table 8(a)] with respect to number of days taken for initiation of germination. Earliness in germination (16.00 days) was recorded in treatment S_2A_1 (stalk end up position + freshly extracted stones) followed by S_2A_2 (stalk end up position + sowing of stones 10 days after extraction from fruit) (21.11 days) while the highest number of days for initiation of germination (38.32 days) was recorded for S_1A_3 (flat position + sowing of stones 20 days after extraction from fruit).

The interaction effect between sowing positions and pre sowing treatments was significant for days taken for initiation of germination of mango stones [Table 9(a)]. The least number of days for initiation of germination (19.51 days) was noted in the treatment S_2T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) followed by S_2T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (20.47 days). The highest number of days required for initiation of germination (34.06 days) was recorded in S_1T_7 (Flat position of sowing + without treatment).

The interaction effect betweenage of stones after extraction from the fruitand pre sowing treatments was significant for days taken for initiation of germination of mango stones [Table 10(a)]. The least number of days for initiation of germination (15.37 days) was noted in the treatment A_1T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) followed by A_1T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (16.33 days). Whereas, the highest number of days required for initiation of germination (41.20 days) was recorded in A_3T_7 (sowing of stones 20 days after extraction from fruit + without treatment).

The interaction between sowing positions, age of stones after extraction from the fruit and pre sowing treatments found significant for days taken for initiation of germination of mango stones [Table 11(a)]. The least number of days for initiation of germination of mango stone (13.00 days) was recorded in $S_2A_1T_2$ (stalk end up position of sowing + freshly extracted stones + soaking of mango

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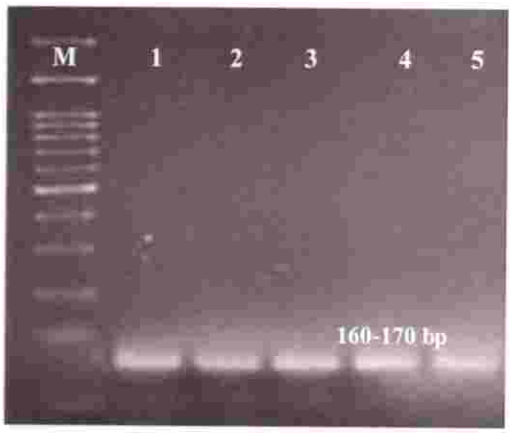

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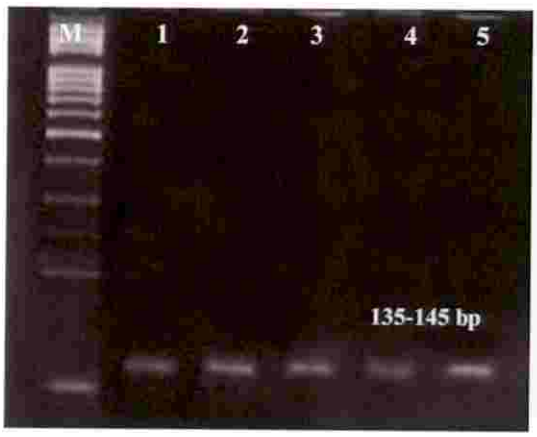

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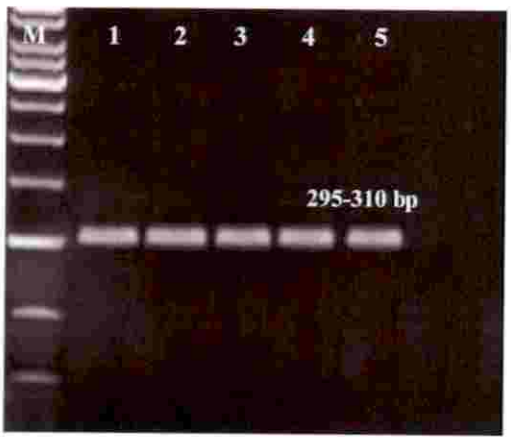

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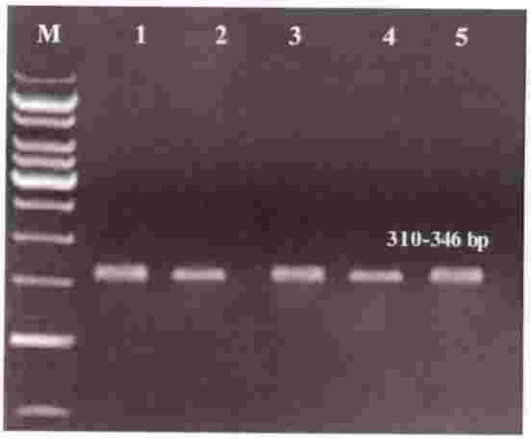

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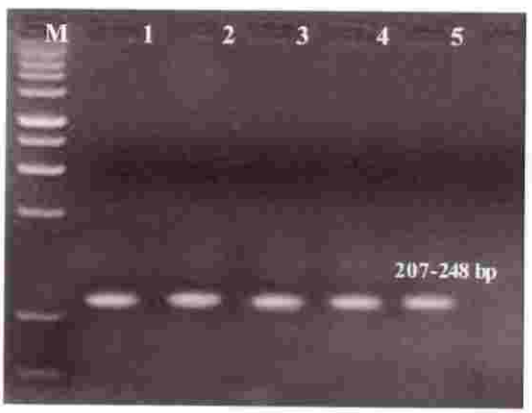
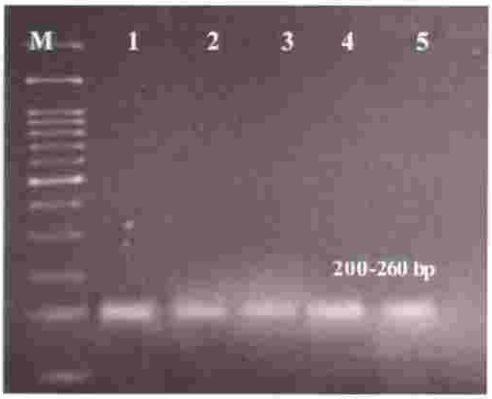
SSR -26



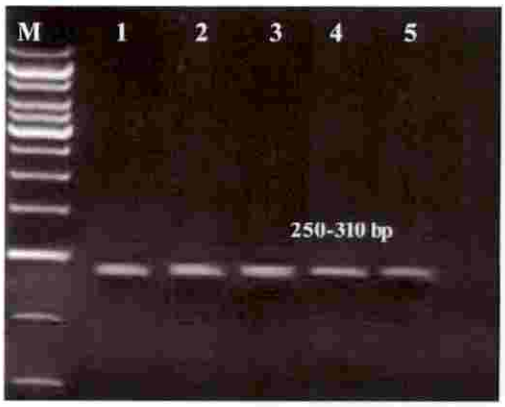
SSR -52

1- Mother plant (Kochu Kilichundan), 2-5: Plantlets M: 100 bp ladder

Plate 6. Amplification profiles of genomic DNA of plantlets obtained from var. Kochu Kilichundan and mother plant using SSR primers

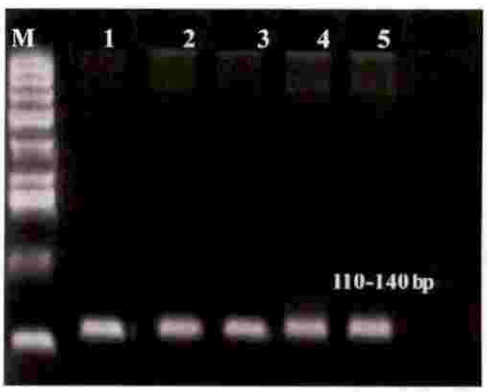


SSR -84

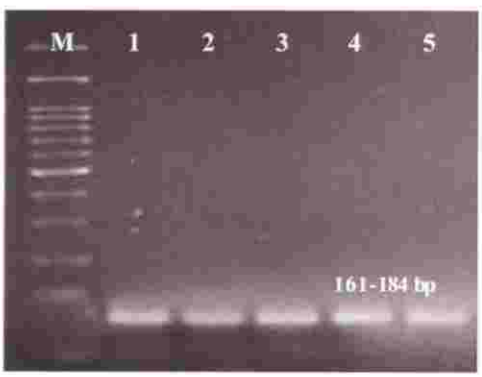


SSR -85

SSR -89



MNGSSR -14



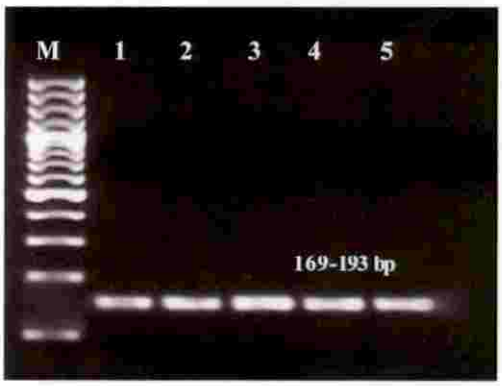
MiIHR 10

MiIHR 11

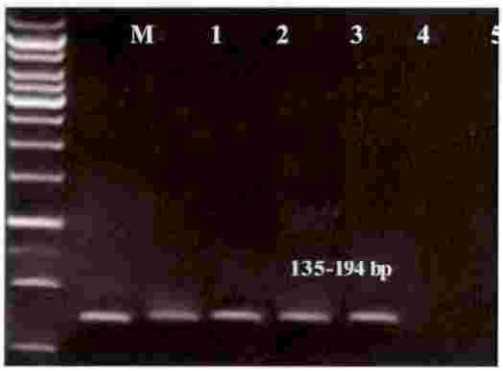
1- Mother plant (Kochu Kilichundan), 2-5: Plantlets M: 100 bp ladder

Plate 6.continued

MiIIHR 12



MiIIHR 13



MiIIHR 15

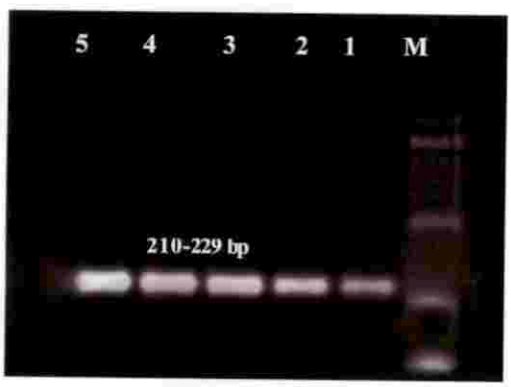
MiIIHR 21

1- Mother plant (Kochu Kilichundan), 2-5: Plantlets M: 100 bp ladder

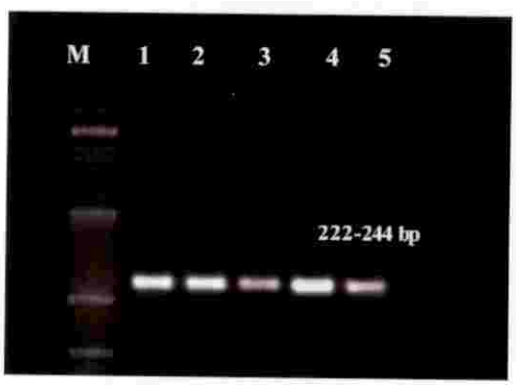
Plate 6.continued

MiIIHR 23

MiIIHR 24



MiIIHR 31



MiIIHR 34

1- Mother plant (Kochu Kilichundan), 2-5: Plantlets M: 100 bp ladder

Plate 6.continued

stones at 200 ppm GA_3), which was on par with treatment $S_2A_1T_1$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (13.53 days), while the highest number of days required for initiation of germination (43.20 days) was recorded in $S_1A_3T_7$ (flat position of sowing + sowing of stones 20 days after extraction from fruit + without treatment).

4.2.2 Days taken for 50 per cent of germination

The data on days taken for 50 per cent germination as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatments are presented in table 7(a).

4.2.2.1 Effect of sowing positions

The perusal of data presented in table 7(a) clearly indicated that the days taken for 50 per cent germination of mango stone was significantly influenced by different sowing positions. The least number of days taken for 50 per cent germination (31.75 days) was noted in stalk end up position compared to flat position (40.91 days).

4.2.2.2 Effect of age of stones after extraction from the fruit

It is evident from table 7(a) that age of stones after extraction from the fruit was significantly influenced the days taken for 50 per cent germination of mango stones. The least number of days taken for 50 per cent germination (31.29 days) was recorded for freshly extracted stones compared to stones sown at 10 days after extraction from fruit and 20 days after extraction from the fruit. Sowing of stones at 20 days after extraction from fruit recorded the highest number of days for 50 per cent germination (41.20 days).

Table 7(b). Germination characters of mango as influenced by sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index- II (Weight basis)
Effect of sowing positions				
Flat (S_1)	28.12	7.23	1192.08	305.72
Stalk end up (S_2)	34.63	9.77	2176.50	603.27
SE(m)	0.10	0.04	26.48	12.49
CD	0.28	0.12	74.48	35.13
Effect of Age of stone after extraction from fruit				
Freshly extracted stone (A_1)	36.60	9.85	2241.88	594.08
10 days after extraction (A_2)	31.57	8.32	1714.50	458.53
20 days after extraction (A_3)	25.95	7.33	1096.50	310.87
SE(m)	0.12	0.05	32.43	15.30
CD	0.34	0.15	91.22	43.03
Pre sowing treatments				
GA_3 - 100 ppm (T_1)	35.70	10.39	1984.48	546.23
GA_3 - 200 ppm (T_2)	34.70	10.01	2310.02	657.09
KNO_3 - 1 ppm (T_3)	33.26	9.22	1834.42	513.80
KNO_3 - 2 ppm (T_4)	32.27	8.59	1694.86	454.53
Cow dung slurry (T_5)	30.05	7.77	1740.60	444.77
Water (T_6)	27.82	7.12	1248.63	316.76
Control [no treatment] (T_7)	25.83	6.39	977.03	248.33
SE(m)	0.19	0.08	49.54	23.37
CD	0.53	0.22	139.34	65.73

4.2.2.3 Effect of pre sowing treatments

The pre-sowing treatments had a significant effect on number of days taken for 50 per cent germination of mango stones [Table 7(a)]. The stones treated with 200 ppm GA_3 (T_2) recorded the least number of days for 50 per cent germination (31.78 days), whereas the highest number of days for 50 per cent germination (42.94 days) was noted in control (T_7).

4.2.2.4 Effect of interactions

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant [Table 8(a)] with respect to number of days taken for 50 per cent germination. The least number of days taken for 50 per cent germination (28.14 days) was recorded in S_2A_1 (stalk end up position + freshly extracted stones) followed by S_2A_2 (stalk end up position + sowing of stones 10 days after extraction from fruit) (31.71 days), while the highest number of days for 50 per cent germination (47.00 days) was recorded for S_1A_3 (flat position + sowing of stones 20 days after extraction from fruit).

The interaction effect between sowing positions and pre sowing treatments was significant for days taken for 50 per cent germination of mango stones [Table 9(a)]. The least number of days taken for 50 per cent germination (27.67 days) was recorded in S_2T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3), followed by S_2T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (29.22 days). The highest number of days for 50 per cent germination (48.56 days) was recorded in S_1T_7 (flat position of sowing + without treatment).

The interaction effect of age of stones after extraction from the fruit and pre sowing treatments did not show significant results with respect to number of days for 50 per cent germination [Table 10(a)].

The interaction effect between sowing positions, age of stones after extraction from the fruit and pre sowing treatments with respect to number of days taken for 50 per cent germination was non-significant [Table 11(a)].

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Plate 7. Effect of pre sowing treatments on seedling length of freshly harvested mango stones sown in stalk end up method

Table 8(a). Interaction effect of sowing positions and age of stones after extraction from the fruit on germination of mango stones

Treatments	Days taken for initiation of germination (Days)	Days taken for 50 per cent germination (Days)	Germination (%)	Rate of germination
S_1A_1	21.12	34.43	48.89	0.41
S_1A_2	28.00	41.29	39.68	0.23
S_1A_3	38.32	47.00	34.29	0.14
S_2A_1	16.00	28.14	70.79	0.52
S_2A_2	21.11	31.71	65.08	0.50
S_2A_3	31.74	35.38	46.67	0.40
SE m(±)	0.08	0.29	1.20	0.003
CD (0.05)	0.23	0.81	3.36	0.007

Table 8(b). Interaction effect of sowing positions and age of stones after extraction from the fruit on germination of mango stones

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index- II (Weight basis)
S_1A_1	33.04	8.20	1639.86	287.36
S_1A_2	28.65	7.15	1148.50	407.36
S_1A_3	22.66	6.35	787.89	222.45
S_2A_1	40.15	11.49	2843.90	780.80
S_2A_2	34.49	9.49	2280.50	629.71
S_2A_3	29.25	8.32	1405.11	399.31
SE m(±)	0.18	0.07	45.86	21.63
CD (0.05)	0.49	0.21	129.00	60.85

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4.2.3 Germination (%)

The mean data on germination percentage as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatmentsis presented intable 7(a).

4.2.3.1 Effect of sowing positions

With regard to sowing positions, the results obtained were significant for germination percentage [Table 7(a)]. Higher germination percentage (60.85 %) was noted in stalk end up position compared to flat position (40.95 %).

4.2.3.2 Effect of age of stones after extraction from the fruit

The data presented in table 7(a) clearly indicated that there was significant effect due to age of stones after extraction from the fruit on germination percentage at 60 days after sowing. The highest germination percentage (59.84 %) was recorded in freshly extracted stones. Sowing of stones at 20 days after extraction from fruit recorded the lowest germination percentage (40.48 %).

4.2.3.3 Effect of pre sowing treatments

It is apparent from the data presented in table 7(a) that there was a significant difference with respect to germination percentage due to different pre-sowing treatments. The highest germination percentage (62.59 %) was recorded when mango stones treated with T_2 (200 ppm GA_3) followed by 100 ppm GA_3 (55.19 %), whereas the lowest germination percentage (37.41 %) was observed in treatment T_7 (control).

4.2.3.4 Effect of interactions

On statistical analysis, the interaction of sowing positions and age of stones after extraction from the fruit was significant for germination percentage 60 DAS [Table 8(a)]. The germination percentage was higher (70.79 %) in stalk end up method of sowing of freshly extracted stones (S_2A_1), followed by stalk end up method of sowing of stones at 10 days after extraction from fruit (S_2A_2). The least percentage of germination (34.29 %) was noted in flat method of sowing of mango stones at 20 days after extraction from fruit (S_1A_3).

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The interaction effect of sowing positions and pre sowing treatments with respect to germination percentage was non-significant [Table 9(a)].

The interaction effect betweenage of stones after extraction from the fruit and pre sowing treatments with respect to germination percentage was non-significant [Table 10(a)].

The interaction effect between sowing positions, age of stones after extraction from the fruit and pre sowing treatments with respect to germination percentage was non-significant [Table 11(a)].

4.2.4 Rate of germination

The data regarding rate of germination as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatments is presented in table 7(a).

4.2.4.1 Effect of sowing positions

There was significant difference in rate of germination as influenced by various sowing positions [Table 7(a]. The stones sown in stalk end up position (S_2) recorded higher germination rate (0.47) as compared to flat position (0.26).

4.2.4.2 Effect of age of stones after extraction from the fruit

The data presented in table 7(a) indicated that different age of stones after extraction from the fruit significantly influenced the rate of germination. Freshly extracted stones recorded the higher rate of germination (0.47) compared to stones sown at 10 days after extraction from fruit (0.36) and 20 days after extraction from fruit (0.28).

4.2.4.3 Effect of pre sowing treatments

It is evidentfrom the data presented in table 7(a) that the pre sowing treatments significantly influenced the rate of germination. The mango stones treated with 200 ppm GA_3 (T_2) recorded highest rate of germination (0.48), whereas the lowest germination rate (0.25) was observed in treatment T_7 (control).

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Table 9(a). Interaction effect of sowing positions and pre-sowing treatments on germination of mango stones

Treatments	Days taken for initiation of germination (Days)	Days taken for 50 per cent germination (Days)	Germination (%)	Rate of germination
S_1T_1	27.31	37.79	52.59	0.30
S_1T_2	25.73	35.89	44.44	0.35
S_1T_3	27.42	39.11	42.22	0.30
S_1T_4	28.87	40.22	37.78	0.27
S_1T_5	28.76	39.89	45.19	0.27
S_1T_6	31.87	44.49	34.07	0.19
S_1T_7	34.06	48.56	30.37	0.16
S_2T_1	20.47	29.22	65.93	0.55
S_2T_2	19.51	27.67	72.59	0.60
S_2T_3	21.56	30.11	63.70	0.53
S_2T_4	22.51	30.89	62.22	0.44
S_2T_5	22.82	31.67	65.18	0.42
S_2T_6	25.82	35.33	51.85	0.42
S_2T_7	27.96	37.33	44.44	0.34
SE m(±)	0.12	0.44	N.S	0.004
CD (0.05)	0.35	1.23	N.S	0.011

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4.2.4.4 Effect of interactions

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant [Table 8(a)] with respect to rate of germination. The highest germination rate (0.52) was recorded in S_2A_1 (stalk end up position + freshly extracted stones) followed by S_2A_2 (stalk end up position + sowing of stones 10 days after extraction from fruit) (0.50) while lowest germination rate (0.14) was recorded in S_1A_3 (flat position + sowing of stones 20 days after extraction from fruit).

The interaction effect between sowing positions and pre sowing treatments was significant for rate of germination [Table 9(a)]. The highest rate of germination (0.60) was noticed in S_2T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) followed by S_2T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (0.55). The lowest germination rate (0.16) was recorded in S_1T_7 (Flat position of sowing + without treatment).

The interaction effect between age of stones after extraction from the fruit and pre sowing treatments was significant for rate of germination of mango stones [Table 10(a)]. The highest rate of germination (0.66) was noted in treatment A_1T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3), followed by A_1T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (0.59). The least germination rate (0.23) was recorded in A_3T_7 (sowing of stones 20 days after extraction from fruit + without treatment).

The interaction effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments was significant for rate of germination of mango stones [Table 11(a)]. The highest rate of germination (0.74) was obtained from the treatment $S_2A_1T_2$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones at 200 ppm GA_3), followed by $S_2A_1T_1$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones at 100 ppm GA_3) (0.66). The least germination rate (0.11 days) was recorded in $S_1A_3T_7$ (flat position of sowing + sowing of stones 20 days after extraction from fruit + without treatment).

4.2.5 Seedling length (cm)

The data on seedling length 120 DAS as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatments is presented in table 7(b).

4.2.5.1 Effect of sowing positions

There was significant difference in seedling length 120 DAS as influenced by various sowing positions [Table 7(b)]. The stones sown in stalk end up position produced significantly higher seedling length (34.63 cm) compared to flat position (28.12 cm).

4.2.5.2 Effect of age of stones after extraction from the fruit

It is apparent from the data given in table 7(b) that there was a significant effect due to age of stones after extraction from the fruit on seedling length 120 days after sowing. The highest seedling length (36.60 cm) was recorded in freshly extracted stones. The stones sown 20 days after extraction from fruit recorded the least seedling length (25.95 cm).

4.2.5.3 Effect of pre sowing treatments

From table 7(b) it is evident that pre-sowing treatments had significant effect on seedling length 120 days after sowing. The highest seedling length (35.70 cm) was noted in GA_3 100 ppm (T_2), followed by 200 ppm GA_3 (34.70 cm). The least seedling length (25.83 cm) was recorded in control (without treatment).

4.2.5.4 Effect of interactions

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant [Table 8(b)] with respect to seedling length 120 DAS. The highest seedling length (40.15 cm) was recorded in treatment S_2A_1 (stalk end up position + freshly extracted stones), followed by S_2A_2 (stalk end up position + sowing of stones 10 days after extraction from fruit) (34.49 cm). The lowest seedling length (22.66 cm) was recorded in S_1A_3 (flat position + sowing of stones 20 days after extraction from fruit).

Table 9(b). Interaction effect of sowing positions and pre-sowing treatments on germination of mango stones

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index - II (Weight basis)
S_1T_1	31.41	8.11	1392.44	381.30
S_1T_2	30.67	8.46	1688.94	432.56
S_1T_3	29.76	7.67	1285.62	329.14
S_1T_4	29.29	7.33	1135.30	282.25
S_1T_5	27.21	6.95	1251.86	317.51
S_1T_6	25.31	6.44	869.93	222.20
S_1T_7	23.17	5.65	720.48	175.10
S_2T_1	39.98	12.32	2576.52	711.15
S_2T_2	38.72	11.91	2931.10	881.62
S_2T_3	36.75	10.76	2383.21	698.45
S_2T_4	35.24	9.85	2254.43	626.80
S_2T_5	32.89	8.59	2229.34	572.02
S_2T_6	30.34	7.80	1627.32	411.31
S_2T_7	28.49	7.13	1233.57	321.55
SE m(±)	0.27	0.11	70.06	33.05
CD (0.05)	0.75	0.32	197.06	92.95

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The interaction effect between sowing positions and pre sowing treatments was significant with respect to seedling length 120 DAS [Table 9(b)]. The highest seedling length (39.98 cm) was obtained from treatment S_2T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3), followed by S_2T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) (38.72 cm). The lowest seedling length (23.17 cm) was recorded in treatment S_1T_7 (Flat position of sowing + without treatment).

The interaction effect between age of stones after extraction from the fruit and pre sowing treatments was significant for seedling length 120 DAS [Table 10(b)]. The highest seedling length (40.84 cm) was noticed in treatment A_1T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3) followed by A_1T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) (39.80 cm). The least seedling length (21.02 cm) was recorded in A_3T_7 (sowing of stones 20 days after extraction from fruit + without treatment).

The interaction between sowing positions, age of stones after extraction from the fruit and pre sowing treatments found significant for seedling length 120 DAS [Table 11(b)]. The highest seedling length (44.43 cm) was noted in treatment $S_2A_1T_1$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones in 100 ppm GA_3) which was on par with $S_2A_1T_2$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones in 200 ppm GA_3) (43.58 cm). The least seedling length (18.81 cm) was noted in $S_1A_3T_7$ (flat position of sowing + sowing of stones 20 days after extraction from fruit + without treatment).

4.2.6 Dry weight of seedling (g)

The data regarding dry weight of seedling 120 DAS as influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatmentsis presented in table 7(b).

4.2.6.1 Effect of sowing positions

With regard to sowing positions, the results obtained was significant for dry weight of seedling120 DAS [Table 7(b)]. The stones sown in stalk end up

position recorded the highest seedling dry weight (9.77 g) over flat position (7.23 g).

4.2.6.2 Effect of age of stones after extraction from the fruit

A close perusal of data [Table 7(b)] revealed that age of stones after extraction from the fruit was influenced the dry weight of seedling. The highest seedling dry weight (9.85 g) was recorded for freshly extracted stones compared to sowing of stones at 10 days after extraction from the fruit (8.32 g) and 20 days after extraction from the fruit (7.33 g).

4.2.6.3 Effect of pre sowing treatments

The pre-sowing treatments had a significant effect on dry weight of seedling [Table 7(b)]. Significantly higher dry weight (10.39 g) was recorded in 100 ppm GA_3 pre-treated stones, followed by 200 ppm GA_3 (10.01 g) and 1 ppm KNO_3 (9.22 g). The sowing of stones without any pre-sowing treatments (control) recorded the least seedling dry weight (6.39 g).

4.2.6.4 Effect of interactions

On statistical analysis, it was inferred that the interaction between sowing positions and age of stone after extraction from the fruit was significant for seedling dry weight 120 DAS [Table 8(b)]. The highest dry weight of seedling (11.49 g) was recorded in stalk end up sowing method of freshly extracted stones (S_2A_1), followed by stalk end up sowing of stones 10 days after extraction from fruit (S_2A_2) (9.49 g). The least seedling dry weight (6.35 g) was noticed in flat method of sowing of mango stones sown 20 days after extraction from fruit (S_1A_3).

The interaction effect of sowing positions and pre sowing treatments on seedling dry weight 120 DAS [Table 9(b)] was significant. The highest dry weight of seedling (12.32 g) was obtained from stalk end up sowing of 100 ppm GA_3 pre-treated stones (S_2T_1), followed by stalk end up method of sowing of 200 ppm GA_3 pre-treated stones (S_2T_2) (11.91g). The least dry weight of seedling (5.65 g) was recorded in flat position sowing of stones without any pre sowing treatments (S_1T_7).

Table 10 (a). Interaction effect of age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Days taken for initiation of germination (Days)	Days taken for 50 per cent germination (Days)	Germination (%)	Rate of germination
A_1T_1	16.33	28.83	63.33	0.59
A_1T_2	15.37	26.67	72.22	0.66
A_1T_3	16.50	28.00	62.22	0.56
A_1T_4	18.70	30.50	57.78	0.41
A_1T_5	19.13	31.00	64.45	0.42
A_1T_6	21.03	35.17	52.22	0.35
A_1T_7	22.83	38.83	46.67	0.27
A_2T_1	22.77	33.67	63.33	0.40
A_2T_2	21.26	32.33	54.45	0.43
A_2T_3	23.70	35.67	54.45	0.40
A_2T_4	24.47	36.00	54.44	0.38
A_2T_5	23.63	35.33	57.78	0.36
A_2T_6	27.03	40.17	44.44	0.31
A_2T_7	29.00	42.33	37.78	0.24
A_3T_1	32.57	39.33	52.22	0.28
A_3T_2	31.23	36.33	47.78	0.33
A_3T_3	33.27	38.83	42.22	0.29
A_3T_4	33.90	40.17	37.78	0.27
A_3T_5	34.60	41.00	43.33	0.27
A_3T_6	38.47	45.00	32.22	0.26
A_3T_7	41.20	47.67	27.78	0.23
SE m(±)	0.15	N.S	N.S	0.005
CD (0.05)	0.42	N.S	N.S	0.013

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The interaction effect of age of stones after extraction from the fruit and pre sowing treatments were significant for seedling dry weight 120 DAS [Table 10(b)]. The highest seedling dry weight (12.16 g) was obtained from treatment A_1T_1 (freshly extracted stones + soaking of mango stones at 100 ppm GA_3), followed by A_1T_2 (freshly extracted stones + soaking of mango stones at 200 ppm GA_3) (11.71 g). The least seedling dry weight (5.70 g) was recorded in treatment A_3T_7 (sowing of stones 20 days after extraction from fruit + without treatment).

The interaction effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments with respect to seedling dry weight was significant [Table 11(b)]. The highest dry weight of mango seedling (14.72 g) was recorded in $S_2A_1T_1$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones at 100 ppm GA_3) which was on par with $S_2A_1T_2$ (stalk end up position of sowing + freshly extracted stones + soaking of mango stones at 200 ppm GA_3) (14.25 g). The least seedling dry weight (4.71 g) was recorded in $S_1A_3T_7$ (flat position of sowing + sowing of stones 20 days after extraction from fruit + without treatment).

4.2.7 Vigour index- I

The mean data on seedling vigour index- I 120 DASas influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatmentsis presented in table 7(b).

4.2.7.1 Effect of sowing positions

There was significant difference in seedling vigour index- I 120 DAS as influenced by various sowing positions [Table 7(b)]. The stones sown in stalk end up position produced higher vigour index-I (2176.50) over flat position (1192.08).

4.2.7.2 Effect of age of stones after extraction from the fruit

It is apparent from the data given in table 7(b) that age of stones after extraction from the fruit had significant influence over seedling vigour index- I. The highest vigour index- I (2241.88) was recorded for freshly extracted stones (A_1) 120 DAS. The stones sown 20 days after extraction from fruit (A_3) recorded the lowest vigour index- I (1096.50).

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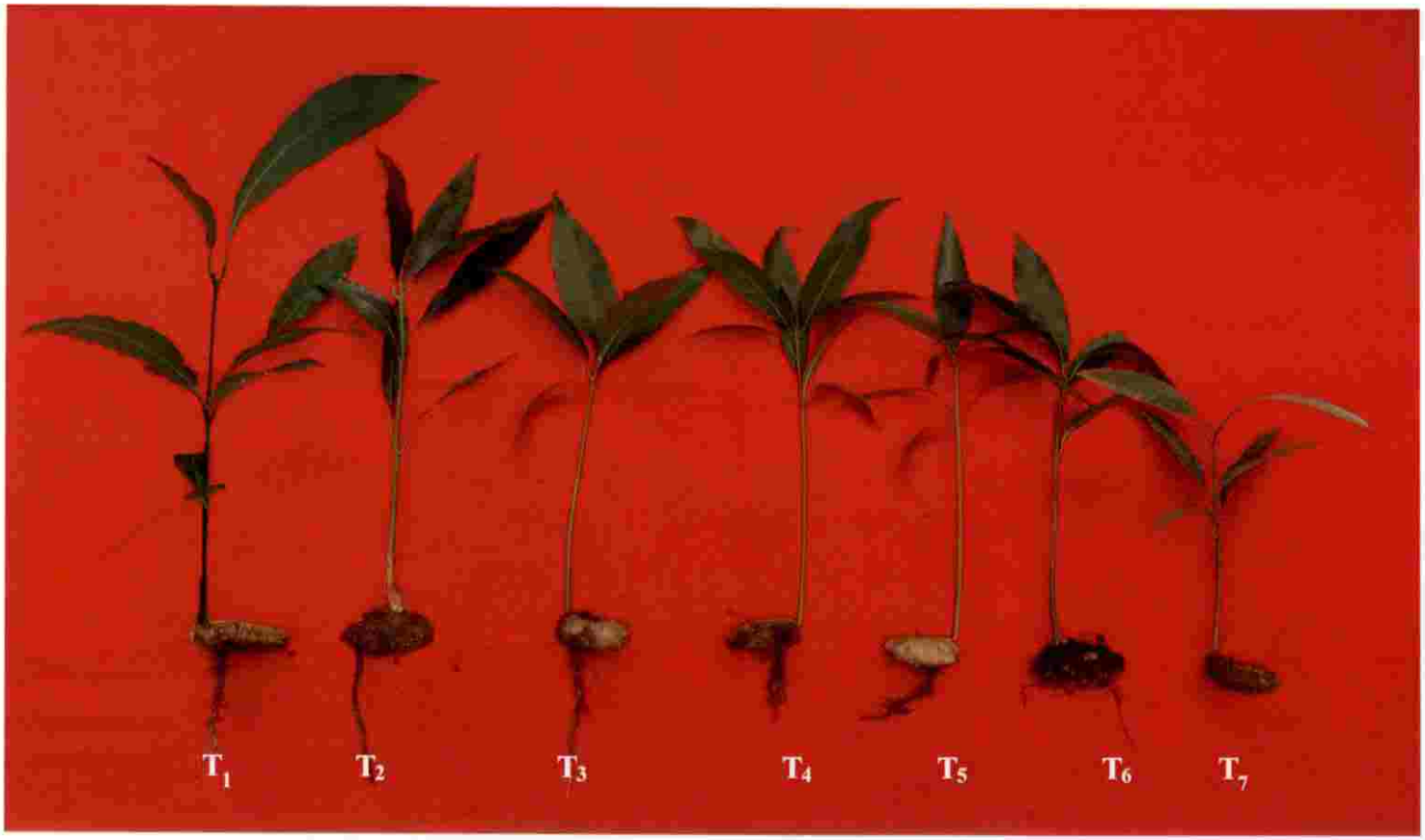

Plate 8. Effect of pre sowing treatments on seedling length of freshly harvested mango stones sown in flat method

Table 10(b). Interaction effect of age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index- II (Weight basis)
A_1T_1	40.84	12.16	2558.96	625.83
A_1T_2	39.80	11.71	2975.53	871.11
A_1T_3	38.43	10.83	2430.32	698.30
A_1T_4	38.13	10.29	2258.79	616.12
A_1T_5	35.23	8.96	2356.03	591.68
A_1T_6	32.85	7.90	1757.16	419.20
A_1T_7	30.88	7.06	1356.35	336.33
A_2T_1	36.43	9.93	2356.85	579.28
A_2T_2	34.88	10.22	1937.46	652.39
A_2T_3	33.61	8.80	1875.62	496.42
A_2T_4	32.70	8.19	1825.96	465.60
A_2T_5	30.06	7.51	1778.00	444.12
A_2T_6	27.72	7.14	1245.15	324.92
A_2T_7	25.59	6.42	982.44	247.04
A_3T_1	29.82	8.39	1597.67	433.57
A_3T_2	27.72	8.79	1457.02	447.77
A_3T_3	29.40	8.03	1197.32	346.68
A_3T_4	25.96	7.29	999.84	281.86
A_3T_5	24.85	6.83	1087.78	298.50
A_3T_6	22.91	6.32	743.57	206.16
A_3T_7	21.02	5.70	592.29	161.61
SE m(±)	0.33	0.14	N.S	N.S
CD (0.05)	0.92	0.39	N.S	N.S

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4.2.7.3 Effect of pre sowing treatments

From table 7(b) it is evident that pre-sowing treatments had significant effect on vigour index- I 120 days after sowing. Significantly higher vigour index- I (2310.02) was observed in 200 ppm GA_3 treated stones (T_2), followed by 100 ppm GA_3 (1984.48). The least vigour index- I (977.03) was recorded in control (without treatment).

4.2.7.4 Effect of interactions

On statistical analysis of the data, the interaction of sowing positions and age of stone after extraction from the fruit was significant for vigour index- I [Table 8(b)]. The largest values for vigour index- I (2843.90) was recorded in stalk end up method of sowing of freshly extracted stones (S_2A_1), followed by stalk end up of sowing of stones at 10 days after extraction from fruit (S_2A_2) (2280.50). The least vigour index- I (787.89) was in flat method sowing of mango stones at 20 days after extraction from fruit (S_1A_3).

Significant effect was observed in interaction between sowing position, pre sowing treatments and vigour index-I 120 DAS [Table 9(b)]. The highest vigour index-I (2931.10) was recorded in stalk end up sown 200 ppm GA_3 pre-treated stones (S_2T_1), followed by stalk end up sown 100 ppm GA_3 pre-treated stones (S_2T_2) (2576.52). The least vigour index-I (720.48) was recorded in flat position sown mango stones without any pre sowing treatments (S_1T_7).

The interaction effect betweenage of stones after extraction from the fruit and pre sowing treatments with respect to vigour index-I was non-significant [Table 10(b)].

The interaction effect between sowing positions, age of stones after extraction from the fruit and pre sowing treatments with respect to vigour index- I was non-significant [Table 11(b)].

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Table 11(a). Interaction effect of sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Days taken for initiation of germination (Days)	Days taken for 50 per cent germination (Days)	Germination (%)	Rate of germination
$S_1A_1T_1$	19.13	31.33	53.33	0.51
$S_1A_1T_2$	17.73	30.33	62.22	0.58
$S_1A_1T_3$	18.33	31.67	51.11	0.51
$S_1A_1T_4$	21.00	33.67	46.67	0.40
$S_1A_1T_5$	22.20	33.33	53.33	0.41
$S_1A_1T_6$	23.73	38.34	40.00	0.26
$S_1A_1T_7$	25.73	42.33	35.55	0.21
$S_1A_2T_1$	26.00	37.34	51.11	0.24
$S_1A_2T_2$	24.00	36.33	42.22	0.25
$S_1A_2T_3$	26.53	41.33	40.00	0.24
$S_1A_2T_4$	27.67	41.67	35.55	0.26
$S_1A_2T_5$	26.87	39.33	44.45	0.26
$S_1A_2T_6$	31.60	45.34	33.33	0.21
$S_1A_2T_7$	33.27	47.66	31.11	0.15
$S_1A_3T_1$	36.80	44.67	37.38	0.14
$S_1A_3T_2$	35.47	41.00	44.45	0.21
$S_1A_3T_3$	37.40	44.33	35.55	0.16
$S_1A_3T_4$	37.94	45.34	31.11	0.14
$S_1A_3T_5$	37.20	47.00	37.78	0.14
$S_1A_3T_6$	40.27	51.00	28.89	0.12
$S_1A_3T_7$	43.20	55.67	24.45	0.11

Table 11(a).Interaction effect of sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones (continued)

Treatments	Days taken for initiation of germination (Days)	Days taken for 50 per cent germination (Days)	Germination (%)	Rate of germination
$S_2A_1T_1$	13.53	26.34	73.33	0.66
$S_2A_1T_2$	13.00	23.00	82.22	0.74
$S_2A_1T_3$	14.67	24.33	73.33	0.60
$S_2A_1T_4$	16.40	27.34	68.89	0.42
$S_2A_1T_5$	16.07	28.66	75.56	0.42
$S_2A_1T_6$	18.33	32.00	64.45	0.45
$S_2A_1T_7$	19.93	35.33	57.78	0.33
$S_2A_2T_1$	19.53	30.00	66.67	0.56
$S_2A_2T_2$	18.53	28.33	75.55	0.60
$S_2A_2T_3$	20.87	30.00	68.89	0.58
$S_2A_2T_4$	21.27	30.33	73.33	0.50
$S_2A_2T_5$	20.40	31.34	71.11	0.45
$S_2A_2T_6$	22.47	35.00	55.55	0.41
$S_2A_2T_7$	24.73	37.00	44.45	0.33
$S_2A_3T_1$	28.33	34.00	57.78	0.42
$S_2A_3T_2$	27.00	31.67	60.00	0.44
$S_2A_3T_3$	29.13	33.33	48.89	0.42
$S_2A_3T_4$	29.87	35.00	44.45	0.41
$S_2A_3T_5$	32.00	35.00	48.89	0.41
$S_2A_3T_6$	36.67	39.00	35.55	0.40
$S_2A_3T_7$	39.20	39.67	31.11	0.34
SE m(±)	0.21	N.S	N.S	0.007
CD (0.05)	0.60	N.S	N.S	0.019

174745

4.2.8 Vigour index II (cm)

The data regarding vigour index- II 120 DASas influenced by different sowing positions, age of stones after extraction from the fruit and pre sowing treatmentsis presented intable 7(b).

4.2.8.1 Effect of sowing positions

There was significant difference in vigour index- II 120 DAS as influenced by various sowing positions [Table 7(b)]. The stones sown in stalk end up position produced significantly higher vigour index- II (603.27) over flat position (305.72).

4.2.8.2 Effect of age of stones after extraction from the fruit

It is evident from the data given in table 7(b) that there was a significant effect for age of stones after extraction from the fruit on vigour index- II 120 days after sowing. The highest vigour index- II (594.08) was recorded for freshly extracted stones (A_1). The stone sown 20 days after extraction from fruit (A_3) recorded the lowest vigour index- II (310.87).

4.2.8.3 Effect of pre sowing treatments

From table 7(b) it is evident that pre-sowing treatments had significant effect on vigour index- II 120 days after sowing. The highest vigour index- II (657.09) was observed in 200 ppm GA_3 treated mango stones (T_2), followed by 100 ppm GA_3 (546.23). The lowest vigour index- II (248.33) was recorded for control.

4.2.8.4 Effect of interactions

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant for vigour index- II [Table 8(b)]. The highest vigour index- II (780.80) was recorded for stalk end up sowing of freshly extracted stones (S_2A_1), followed by stalk end up sowing of stones at 10 days after extraction from fruit (S_2A_2) (629.71). The least vigour index- II (222.45) was noted in flat position sowing of mango stones at 20 days after extraction from fruit (S_1A_3).

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Table 11(b). Interaction effect of sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index- II (Weight basis)
$S_1A_1T_1$	37.26	9.60	1918.80	511.44
$S_1A_1T_2$	36.02	9.16	2319.70	569.87
$S_1A_1T_3$	34.83	8.65	1781.80	442.24
$S_1A_1T_4$	34.42	8.36	1611.42	390.17
$S_1A_1T_5$	31.55	7.75	1680.84	412.84
$S_1A_1T_6$	29.50	7.35	1180.86	293.35
$S_1A_1T_7$	27.71	6.49	985.58	231.59
$S_1A_2T_1$	32.31	8.00	1348.99	351.10
$S_1A_2T_2$	31.92	8.32	1650.36	408.90
$S_1A_2T_3$	30.68	7.56	1230.04	302.38
$S_1A_2T_4$	30.30	7.23	1074.99	257.56
$S_1A_2T_5$	27.04	6.78	1203.30	300.97
$S_1A_2T_6$	25.31	6.39	816.15	211.73
$S_1A_2T_7$	23.01	5.75	715.64	178.88
$S_1A_3T_1$	24.67	7.16	909.52	281.37
$S_1A_3T_2$	24.08	7.45	1096.77	318.91
$S_1A_3T_3$	23.77	6.81	845.01	242.81
$S_1A_3T_4$	23.14	6.40	719.48	199.01
$S_1A_3T_5$	23.05	6.32	871.44	238.70
$S_1A_3T_6$	21.12	5.58	612.77	161.51
$S_1A_3T_7$	18.81	4.71	460.21	114.83

Table 11(b).Interaction effect of sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones (continued)

Treatments	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (Growth basis)	Vigour index- II (Weight basis)
$S_2A_1T_1$	44.43	14.72	3199.11	740.22
$S_2A_1T_2$	43.58	14.25	3631.36	1172.35
$S_2A_1T_3$	42.85	13.00	3078.84	954.35
$S_2A_1T_4$	42.89	12.21	2906.15	842.05
$S_2A_1T_5$	38.92	10.18	3031.21	770.52
$S_2A_1T_6$	36.20	8.46	2333.45	545.04
$S_2A_1T_7$	34.06	7.63	1727.12	441.08
$S_2A_2T_1$	40.55	12.13	2525.93	807.47
$S_2A_2T_2$	37.85	11.87	3063.35	895.88
$S_2A_2T_3$	36.55	10.04	2521.19	690.45
$S_2A_2T_4$	35.10	9.16	2576.93	673.64
$S_2A_2T_5$	33.09	8.25	2352.69	587.25
$S_2A_2T_6$	30.13	7.90	1674.15	438.09
$S_2A_2T_7$	28.18	7.09	1249.24	315.19
$S_2A_3T_1$	34.96	10.13	2004.51	576.62
$S_2A_3T_2$	34.73	9.61	2098.58	585.76
$S_2A_3T_3$	31.67	9.24	1549.62	450.55
$S_2A_3T_4$	28.78	8.19	1280.20	364.70
$S_2A_3T_5$	26.65	7.34	1304.12	358.29
$S_2A_3T_6$	24.70	7.05	874.36	250.80
$S_2A_3T_7$	23.24	6.68	724.35	208.39
SE m(±)	0.46	0.20	N.S	N.S
CD (0.05)	1.30	0.54	N.S	N.S

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The interaction effect between sowing positions and pre sowing treatments on vigour index-II 120 DAS [Table 9(b)] was significant. The highest vigour index-II (881.62) was obtained from stalk end up method of sowing of 200 ppm GA_3 pre-treated stones (S_2T_2) followed by stalk end up method of sowing of 100 ppm GA_3 pre-treated stones (S_2T_1) (711.15). The lowest vigour index-II (175.10) was noted in flat position sowing of mango stones without any pre sowing treatments (S_1T_7).

The interaction effect of age of stones after extraction from the fruit and pre sowing treatments with respect to vigour index- II was non-significant [Table 10 (b)].

The interaction effect between sowing positions, age of stones after extraction from the fruit and pre sowing treatments with respect to vigour index-II was non-significant [Table 11(b)].

4.3 Screening of mango varieties for use as dwarfing rootstock

An attempt has been made to identify the local mango varieties for using as dwarfing rootstock based on morphological, physiological and anatomical features. The data collected were analysed and results are presented below.

4.3.1 Germination characters

The germination characters of stones of selected indigenous varieties such as germination (%), seedling length, dry weight of seedling, seedling vigour index- I and seedling vigour index- II were recorded and the analyzed data are furnished below.

4.3.1.1 Germination percentage

The average data on germination percentage at 4 months after sowing (MAS) of different mango genotypes were presented in table 12. The germination percentage was differed significantly among the varieties under study. The close perusal of data presented in Table 4.14 clearly indicated that the highest germination percentage (71.11 %) was recorded in var. Kappa Manga and the varieties Vellari Varikka (62.22 %), Thali Manga (62.22 %) and Kasthuri (60.00

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%) were on par with Kappa Manga. The least germination percentage (42.22 %) was recorded in var. Kotookonam Varikka (T_1).

4.3.1.2 Seedling length

The data presented in table 12 showed significant difference among the varieties with regard to seedling length at 4 MAS. The statistical analysis (Table 12) of seedling length revealed that the var.Kochu Kilichundan (T_4) recorded the least seedling length (29.48 cm), followed by UndaVarikka (33.19 cm) and Paiveli Local (37.28 cm). The highest seedling seedling length (56.11cm) was recorded in Kappa Manga (T_8).

4.3.1.3 Dry weight of seedling

The examination of data regarding the seedling dry weight among different varieties under study were presented in table 12 indicated significant differences. The analysis on the dry weight of seedling revealed that the lowest seedling dry weight (9.66 g) was noted in Unda Varikka (T_{10}), followed by Kili Manga (T_7) (11.94 g), Paiveli Local (T_9) (12.43 g) and Kochu Kilichundan (T_4) (13.79 g). The highest seedling dry weight (25.51 g) was recorded in Vellari Varikka (T_5).

4.3.1.4 Vigour index- I

The data on seedling vigour index -I on growth basis of different indigenous mango at 4 MAS is presented in table 12. There was significant difference among the treatments under study. The least vigour index- I (1373.69) was recorded in Kochu Kilichundan (T_4). The varieties Unda Varikka (1476.99), Pallikkal Local (1578.38), Paiveli Local (1906.63) and Kili Manga (2330.34) were on par with Kochu Kilichundan. The highest vigour index- I (3993.19) was noticed in var. Kappa Manga (T_8).

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Table 12. Germination characters of different mango genotypes

Treatments	Germination (%)	Seedling length (cm)	Dry weight of seedling (g)	Vigour index -I (growth basis)	Vigour index-II (weight basis)
T_1	42.22	53.53	20.64	2734.56	1055.35
T_2	60.00	41.27	18.52	2475.38	1110.98
T_3	62.22	45.69	14.18	2844.92	883.30
T_4	46.67	29.48	13.79	1373.69	645.15
T_5	62.22	51.27	25.51	3419.60	1699.78
T_6	51.11	48.69	19.41	1578.38	819.51
T_7	46.67	49.66	11.94	2330.34	557.89
T_8	71.11	56.11	24.08	3993.19	1712.14
T_9	51.11	37.28	12.43	1906.63	635.36
T_{10}	44.44	33.19	9.66	1476.99	429.14
SE m(±)	5.58	0.56	0.14	337.11	88.16
CD	16.57	1.67	0.42	1001.48	261.91

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4.3.1.5 Vigour index- II

The data on seedling vigour index -II on weight basis of different mango varieties at 4 MAS is presented in table 12. There was significant difference among the treatments under study. The least vigour index-II (429.14) was recorded in Unda Varikka (T_{10}). The varieties Kili Manga (557.89), Paiveli Local (635.36) and Kochu Kilichundan (645.15) were on par with Unda Varikka. The highest vigour index- II (1712.14) was recorded in var. Kappa Manga (T_8).

4.3.2 Vegetative and growth characters

Data pertaining to the variation in vegetative and growth features *viz.*,plant height, number of leaves, leaf length, leaf width, average leaf area, total leaf area, internodal length, number of roots, root length, dry matter of shoot and root of selected indigenous varieties were recorded and the analyzed data are presented below.

4.3.2.1 Plant height

The plant height was recorded at 6 months after germination [Table 13(a)]. All the varieties showed significant differences with respect to plant height. The plant height was lowest (38.77 cm) in var. Kochu Kilichundan (T_4) and it was on par with Unda Varikka (40.20 cm). Kappa Manga (T_8) differed significantly from all the varieties with respect to plant height (71.66 cm).

4.3.2.2 Number of leaves

The data on number of leaves of different indigenous mango varieties are presented in table 13(a). There was significant difference among the treatments under study. Six month after germination, the variety Paiveli Local produced least number of leaves (18.27), followed by Kochu Kilichundan (20.27) and Unda Varikka (23.20). The Kappa Manga produced significantly higher number of leaves (34.60).

Table 13(a).Vegetative and growth characters of different mango genotypes

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
T_1	68.19	27.48	16.35	4.30
T_2	56.03	32.73	13.79	4.09
T_3	60.75	29.60	14.49	4.31
T_4	38.77	20.27	9.80	3.23
T_5	65.93	31.20	16.18	4.33
T_6	63.76	29.13	20.78	5.65
T_7	65.30	26.13	14.43	4.29
T_8	71.66	34.60	22.39	5.77
T_9	53.24	18.27	11.95	4.06
T_{10}	40.20	23.20	12.59	4.07
SE m(±)	0.49	0.36	0.23	0.04
CD	1.45	1.06	0.67	0.12

Table 13(b). Vegetative and growth characters of different mango genotypes

Treatments	Average leaf area (cm^2)	Total leaf area (cm^2)	Internodal length (cm)
T_1	40.87	428.98	8.48
T_2	35.51	410.85	4.87
T_3	40.52	534.66	6.89
T_4	18.53	153.60	3.16
T_5	43.20	619.34	7.65
T_6	69.48	552.23	6.05
T_7	32.02	360.35	6.58
T_8	72.90	1,462.99	9.25
T_9	30.49	337.63	5.83
T_{10}	22.57	174.81	3.53
SE m(±)	0.34	10.18	0.10
CD	1.02	30.23	0.29

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4.3.2.3 Leaf length

The data presented in table 13(a) showed significant difference among the varieties with regard to leaf length 6 months after germination. The shortest leaf length (9.80 cm) was noticed in Kochu Kilichundan, followed by Paiveli Local (11.95 cm) and Unda Varikka (12.59 cm). The var. Kappa Manga (T_8) recorded significantly higher leaf length (22.39 cm) than other varieties.

4.3.2.4 Leaf width

The data on leaf width of different mango genotypes 6 month after germination were presented in table 13(a). The leaf width differed significantly among the varieties under study. The perusal of data presented in Table 13. a clearly indicated that the lowest leaf width (3.23 cm) was recorded in var. Kochu Kilichundan, followed by Paiveli Local (4.06 cm) and Unda Varikka (4.07 cm). The var. Kappa Manga (T_8) produced leaves with highest width (5.77 cm).

4.3.2.5 Average leaf area

The data regarding average leaf area of different mango genotypes at 6 month after germination were presented in table 13(b). The average leaf area differed significantly among the varieties under study. At 6 MAS, the var. Kochu Kilichundan (T_4) recorded the least average leaf area (18.53 cm^2), followed by Unda Varikka (22.57 cm^2). The Kappa Manga (T_8) produced leaves with highest average leaf area(72.90 cm^2).

4.3.2.6 Total leaf area

Significantdifference between treatments were observed with respect to total leaf area [Table 13(b)]. At 6 MAS, the var. Kochu Kilichundan (T_4) recorded the least total leaf area (153.60 cm^2) and it was on par with Unda Varikka (174.81 cm^2). The Kappa Manga (T_8) produced leaves with highest total leaf area(1462.99 cm^2) than other varieties.

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Table 13(c).Vegetative and growth characters of different mango genotypes

Treatments	Number of roots	Root length (cm)	Dry matter of shoot (g)	Dry matter of root (g)
T_1	48.97	40.76	8.26	4.44
T_2	32.80	37.68	5.26	2.57
T_3	43.87	32.97	7.28	3.29
T_4	28.53	35.02	3.10	1.46
T_5	39.93	45.51	10.29	6.16
T_6	38.07	42.02	5.40	4.70
T_7	34.86	28.98	1.42	1.26
T_8	47.20	44.98	8.40	5.71
T_9	24.87	34.45	2.17	2.24
T_{10}	20.80	25.88	1.30	1.14
SE m(±)	0.33	0.29	0.07	0.09
CD	0.98	0.88	0.20	0.29

158

4.3.2.7 Internodal length

From the analyzed data on internodal length 6 month after germination, it is evident [Table 13(b)] that all the ten selected indigenous mango varieties under study differed significantly from each other in internodal length. The var. Kochu Kilichundan recorded least internodal length (3.16 cm), followed by Unda Varikka (3.53 cm). The var. Kappa Manga recorded the highest internodal length (9.25 cm).

4.3.2.8 Number of roots

The average data on number of roots of different mango genotypes 6 month after germination are presented in table 13(c). The number of roots differed significantly among the varieties under study. The perusal of data presented in Table 4.17 clearly indicated that the var. Unda Varikka (20.80) recorded the least number of roots, followed by Paiveli Local (24.87) and Unda Varikka (28.53). The var. Kotookonam Varikka produced significantly more roots (48.97).

4.3.2.9 Root length

The root length was recorded 6 month after germination [Table 13(c)]. All the varieties differed significantly from each other with respect to their root length. The variety Unda Varikka recorded lowest root length (25.88 cm), followed by Kili Manga (28.98 cm) and Thali Manga (32.97 cm). The var. Vellari Varikka recorded the highest root length (45.51 cm).

4.3.2.10 Dry matter of shoot

The dry matter of shoot differed significantly among the varieties under study [Table 13(c)]. The var. UndaVarikka (T_{10}) recorded the lowest dry matter content of shoot (1.30 g) and was on par with Kili Manga (1.42 g). The var.Vellari Varikka recorded highest dry matter of shoot (10.29 g).

4.3.2.11 Dry matter of root

The dry matter of root differed significantly among the varieties under study [Table 13(c)]. The var. UndaVarikka (T_{10}) recordedtheleast dry matter

Kotookonam Varikka

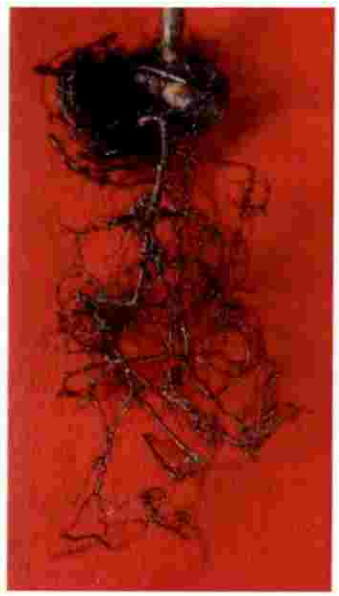
Kasthuri

Thali Manga

Kochu Kilichundan

Plate 9. Root system of mango genotypes at 6 MAS

Vellari Varikka



Pallikkal Local

Kili Manga

Kappa Manga

Plate 9.continued

Paiveli Local

Unda Varikka

Plate 9. continued

content of root (1.14 g) and was on par with Kili Manga (1.26 g). The variety Vellari Varikka recorded significantly the highest dry matter of root (6.16 g).

4.3.3 Physiological and anatomical characters

The physiological characters of selected indigenous mango varieties such as stomatal density, membrane stability index (% leakage), Relative Water Content (RWC), starch content of leaf, transpiration rate, total phenol content of apical bud, total phenol content of leaves of rootstock, bark percentage of root, bark percentage of shoot, leaf temperature and anatomical features were recorded and the analyzed data are furnished below.

4.3.3.1 Stomatal density

It can be intended from table 14(a) that there was a significant difference among the selected indigenous mango varieties with respect to the stomatal density. The lowest stomatal density (39.69) was noticed in Unda Varikka, followed by Kochu Kilichundan (51.68) and Paiveli Local (57.48). The highest stomatal density was observed in Vellari Varikka (101.11).

4.3.3.2 Membrane stability index (% leakage)

The data regarding the membrane stability index of different mango genotypes 6 month after germination are presented in table 14(a). The membrane stability index among the varieties were found non- significant.

4.3.3.3 Relative Water Content (RWC)

The examination of data regarding relative water content of different mango varieties presented in table 14(a) indicated non-significant differences.

4.3.3.4 Starch content of leaf

The starch content of leaf differed significantly among the varieties under study [Table 14(a)].The var. Kasthuri (T_2) recorded the highest starch content (8.53 %), followed by Paiveli Local (8.38 %), Unda Varikka (8.35 %) and Kochu Kilichundan (8.28 %). The var. Kappa Manga (T_8) recorded the lowest (7.26 %) starch content of leaf.

Table 14(a). Physiological characters of different mango genotypes

Treatments	Stomatal density	Membrane stability index (percentage leakage) (%)	Relative Water Content (%)	Starch content of leaf (%)
T_1	89.13	55.93	94.12	7.36
T_2	79.39	57.94	94.15	8.53
T_3	61.41	49.77	94.38	7.70
T_4	51.68	52.60	95.14	8.28
T_5	101.11	54.28	94.92	7.53
T_6	83.07	54.56	94.39	7.40
T_7	87.63	48.96	94.92	7.41
T_8	99.62	50.95	95.58	7.26
T_9	57.48	51.11	95.57	8.38
T_{10}	39.69	52.32	95.52	8.35
SE m(±)	1.97	N.S	N.S	0.04
CD	5.84	N.S	N.S	0.13

Table 14(b). Physiological characters of different mango genotypes

Treatments	Transpiration rate (m moles $m^{-2}s^{-1}$)	Total phenol content of apical bud (mg/g)	Total phenol content of leaves of rootstock (mg/g)
T_1	1.43	41.55	20.62
T_2	1.11	54.60	25.36
T_3	0.77	51.34	23.38
T_4	0.73	60.57	29.03
T_5	1.31	44.01	21.35
T_6	0.81	48.52	21.51
T_7	0.79	47.91	23.14
T_8	1.01	40.75	19.88
T_9	0.78	51.85	24.13
T_{10}	0.75	58.56	25.74
SE m(±)	N.S	0.21	0.24
CD	N.S	0.63	0.73

4.3.3.5 Transpiration rate

The data regarding the transpiration rate of different mango genotypes at 6 month after germination are presented in table 14(b). The transpiration rate among the varieties were non- significant.

4.3.3.6 Total phenol content of apical bud

It can be intended from table 14(b) that there was significant difference among the selected indigenous mango varieties with respect to the total phenol content of apical bud. The var. Kochu Kilichundan (T_4) recorded significantly the highest phenol content (60.57 mg/g), followed by Unda Varikka (58.56 mg/g) and Kasthuri (54.60 mg/g). The var. Kappa Manga (T_8) recorded the lowest phenol content of apical bud (40.75 mg/g).

4.3.3.7 Total phenol content of leaves

The data on total phenol content of leaves of different indigenous mango varieties are presented in table 14(b). There was significant difference among the treatments under study. Six month after germination, var. Kochu Kilichundan (T_4) recorded the highest total phenol content of leaves (29.03 mg/g), followed by Unda Varikka (25.74 mg/g) and Kasthuri (25.36 mg/g) T_1 (32.00). The variety Kappa Manga (19.88 mg/g) recorded the lowest phenol content of leaves.

4.3.3.8 Bark percentage of root

The bark percentage of root differed significantly among the varieties under study [Table 14(c)]. The var. Kochu Kilichundan (T_4) recorded the highest bark percentage of root (23.69 %), followed by Paiveli Local (18.35 %) and Unda Varikka (17.85 %). The least bark percentage of root was noted in Kappa Manga (5.36 %).

Table 14(c). Physiological characters of different mango genotypes

Treatments	Bark percentage of root (%)	Bark percentage of shoot (%)	Leaf temperature (^{0}C)
T_1	7.88	17.39	28.68
T_2	11.51	17.64	28.60
T_3	14.42	18.81	29.04
T_4	23.69	34.02	28.37
T_5	8.95	11.99	28.88
T_6	12.93	17.71	28.82
T_7	11.96	18.87	29.21
T_8	5.36	8.38	29.05
T_9	18.35	24.89	28.70
T_{10}	17.85	20.59	28.74
SE m(±)	0.10	0.22	N.S
CD	0.32	0.67	N.S

4.3.3.9 Bark percentage of shoot

Thebark percentage of shoot of different mango varieties presented in table 14(c) indicated significant differences. The var. Kochu Kilichundan (T_4) recorded significantly the highest bark percentage of root (34.02 %), followed by Paiveli Local (24.89 %) and Unda Varikka (20.59 %). The lowest bark percentage of root was noted in Kappa Manga (8.38 %).

4.3.3.10 Leaf temperature

The data regarding leaf temperature of different mango genotypes 6 month after germination are presented in table 14(c). The leaf temperature was non-significant among the varieties under study.

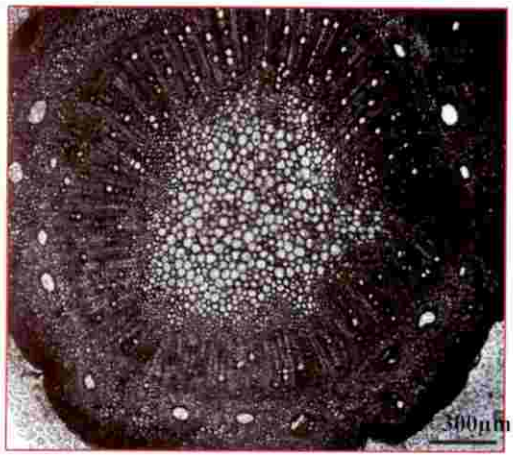
4.3.3.11 Anatomical analysis of root stock

4.3.3.11.1 Xylem area

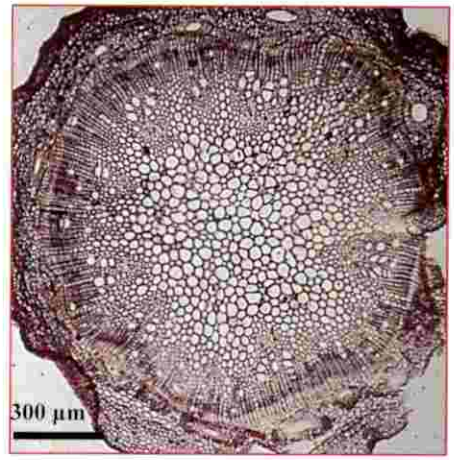
The data regarding the xylem area of stem as well as root of different mango genotypes 6 month after germination are presented in table 15(a) and table 16(a) respectively. The lowest xylem area of stem (1.93 mm^2) was noted in Kochu Kilichundan, followed by Unda Varikka (2.28 mm^2). The highest stem xylem area was noted in Kappa Manga (6.07 mm^2). The var. Unda Varika recorded the lowest root xylem area (1.34 mm^2), followed by Kochu Kilichundan (2.41 mm^2). The var. Kotookonam Varikka recorded the highest xylem area in root (10.86 mm^2).

4.3.3.11.2 Xylem percentage

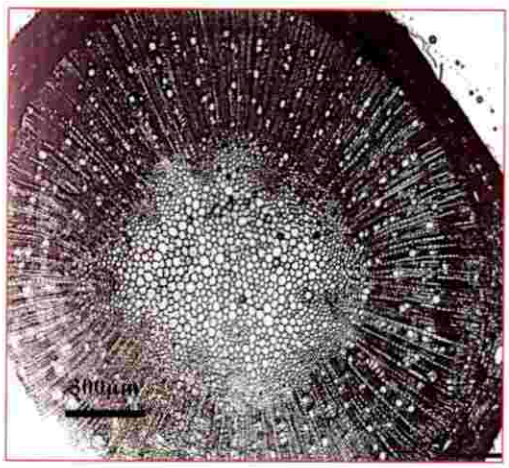
The data regarding the xylem percentage of stem [Table 15(a)]as well as root [Table 16(a)] of different mango genotypes 6 month after germination revealed that the lowest percentage xylem in stem (27.09 %) was noted in Unda Varikka (T_{10}), followed by Kasthuri (T_2) (33.72 %) and Kochu Kilichundan (T_4) (36.19 %). The highest xylem percentage of stem was noted in Vellari Varikka (T_5) (54.46 %). The least percentage of xylem in root (26.84 %) was noted in Unda Varikka (T_{10}), followed by Kochu Kilichundan (T_4) (40.89 %). The highest xylem percentage of root was noted in Kappa Manga (T_8) (78.73 %).



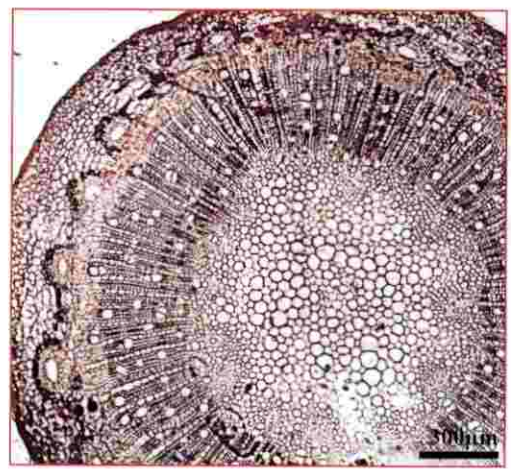
Kotookonam Varikka



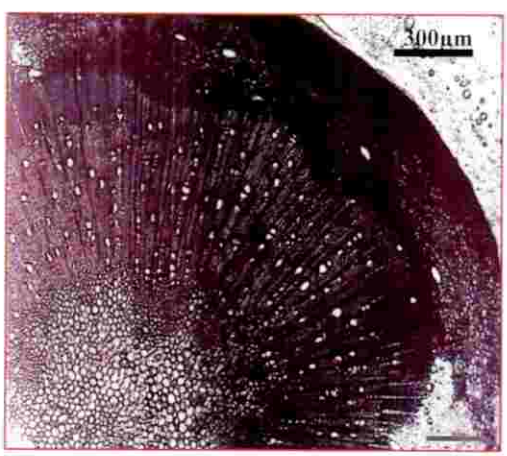
Kasthuri



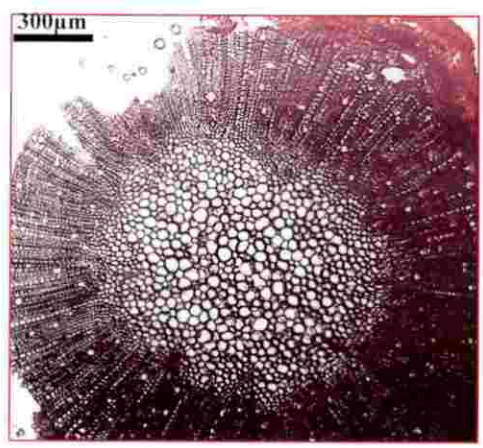
Thali



Kochu Kilichundan

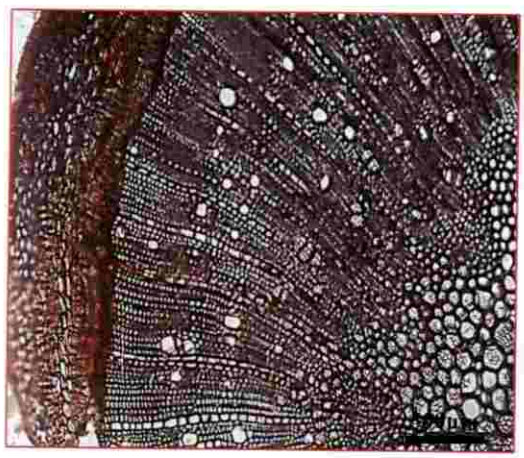


Vellari Varikka



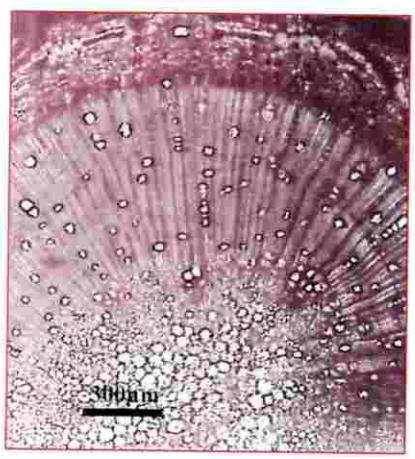
Pallikkal Local

Plate 10. Stem cross section (4 X) of mango genotypes at 6 MAS

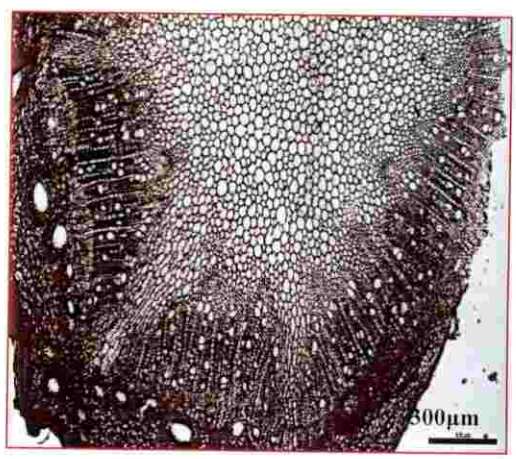


Kili Manga

Kappa Manga



Paiveli Local



Unda Varikka

Plate 10. continued

Table 15(a). Anatomical features (stem cross section) of different mango genotypes

Treatments	Xylem area (mm^2)	Xylem percentage (%)	Phloem area (mm^2)	Phloem percentage (%)
T_1	6.04	46.82	1.53	21.22
T_2	2.68	33.72	2.38	24.65
T_3	2.70	36.63	1.68	24.89
T_4	1.93	36.19	3.48	28.65
T_5	5.71	54.46	1.76	21.24
T_6	3.67	47.77	1.55	22.89
T_7	3.84	48.13	1.75	23.33
T_8	6.07	50.71	1.52	21.71
T_9	2.46	38.76	2.87	27.40
T_{10}	2.28	27.09	3.62	33.53
SE m(±)	0. 08	0.35	0.01	0.13
CD	0.25	1.03	0.05	0.39

Table 15(b). Anatomical features (stem cross section) of different mango genotypes

Treatments	Phloem/ Xylem ratio	Total conduit area (mm^2)
T_1	0.45	7.56
T_2	0.90	5.07
T_3	0.67	4.38
T_4	0.78	5.42
T_5	0.39	7.48
T_6	0.48	5.23
T_7	0.49	5.59
T_8	0.42	7.59
T_9	0.70	5.33
T_{10}	0.99	5.91
SE m(±)	0.008	0.08
CD	0.024	0.24

4.3.3.11.3 Phloem area

The data regarding the phloem area of stem as well as root of different mango genotypes 6 month after germination are presented in table 15(a) and table 16(a) respectively. The highest phloem area of stem (3.62 mm^2) was noted in Unda Varikka (T_{10}), followed by Kochu Kilichundan (T_4) (3.48 mm^2). The lowest stem phloem area was noted in Kappa Manga (T_8) (1.52 mm^2). The var. Kochu Kilichundan (T_4) recorded highest root phloem area (6.60 mm^2), followed by Paiveli Local (T_9) (5.38 mm^2) and Unda Varikka (T_{10}) (5.05 mm^2). The var. Kotookonam Varikka (T_1) recorded the lowest phloem area in root (0.56 mm^2).

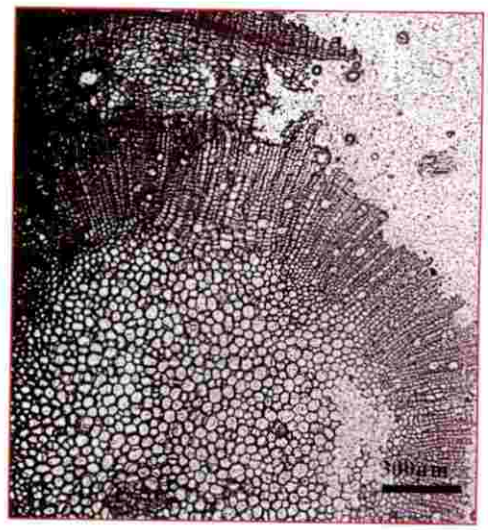
4.3.3.11.4 Phloem percentage

The data regarding the phloem percentage of stem [Table 15(a)] as well as root [Table 16(a)] of different mango genotypes 6 month after germination revealed that the highest percentage of phloem in stem (33.53 %) was noted in Unda Varikka (T_{10}), followed by Kochu Kilichundan (T_4) (28.65 %) and Paiveli Local (T_9) (27.40 %). The lowest phloem percentage of stem was noted in Kotookonam Varikka (T_1) (21.22 %). The highest percentage of phloem in root (36.22 %) was noted in Unda Varikka (T_{10}), followed by Paiveli Local (T_9) (34.17 %) and Kochu Kilichundan (T_4) (33.48 %). The lowest phloem percentage of root was noticed in Kappa Manga (T_8) (9.79 %).

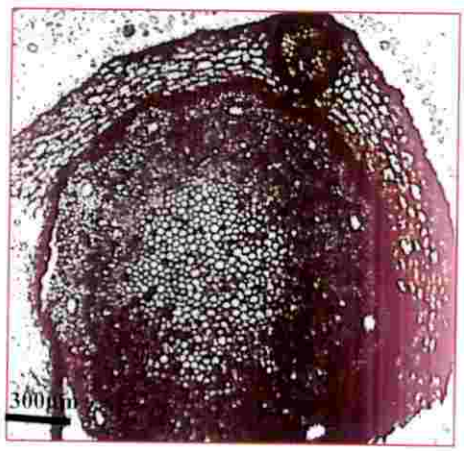
4.3.3.11.4 Phloem/ Xylem ratio

The data regarding the phloem/ xylem ratio of stem [Table 15(b)] as well as root (Table 16. b) of different mango genotypes 6 month after germination revealed that the highest phloem/ xylem ratio of stem (0.99) was noted in Unda Varikka (T_{10}), followed by Kasthuri (T_2) (0.90) and Kochu Kilichundan (T_4) (0.78). The lowest phloem/ xylem ratio was noted in Vellari Varikka (T_5) (0.39). The highest phloem/ xylem ratio of root (1.35) was noted in Unda Varikka (T_{10}), followed by Kochu Kilichundan (T_4) (0.81) and Paiveli Local (T_9) (0.68). The lowest phloem/ xylem ratio of root was noticed in Kappa Manga (T_8) (0.13).

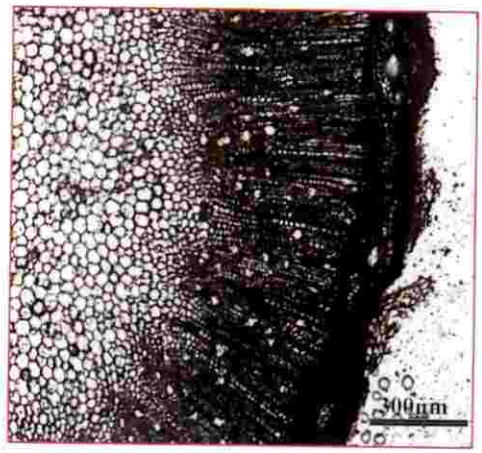
Kotookonam Varikka



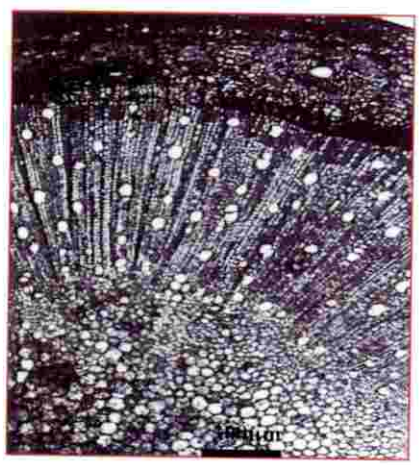
Kasthuri



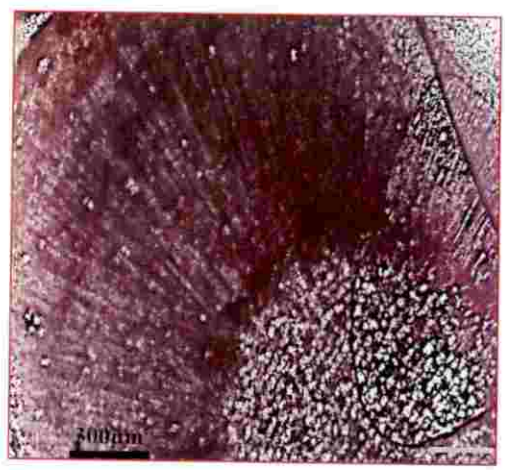
Thali



Kochu Kilichundan

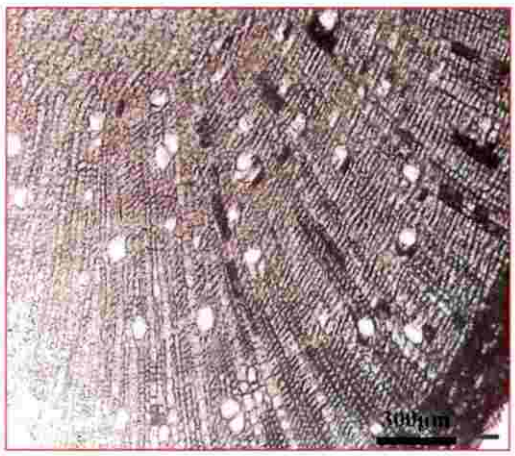


Vellari Varikka

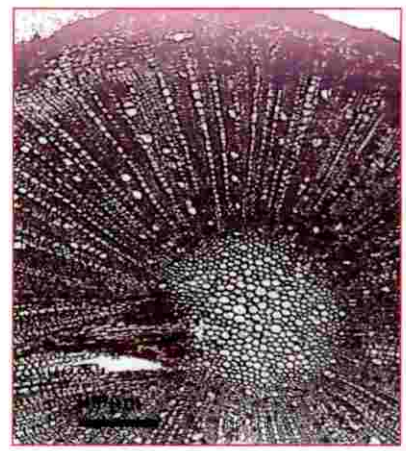


Pallikkal Local

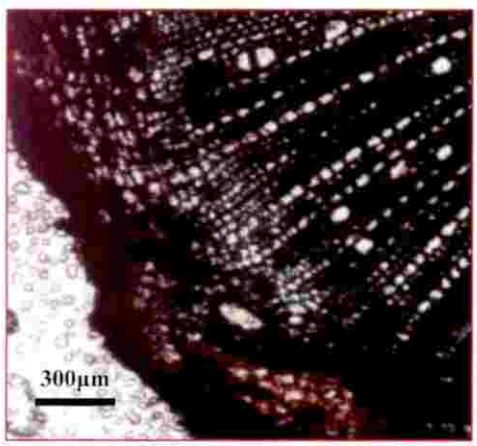
Plate 11. Root cross section (4 X) of mango genotypes at 6 MAS



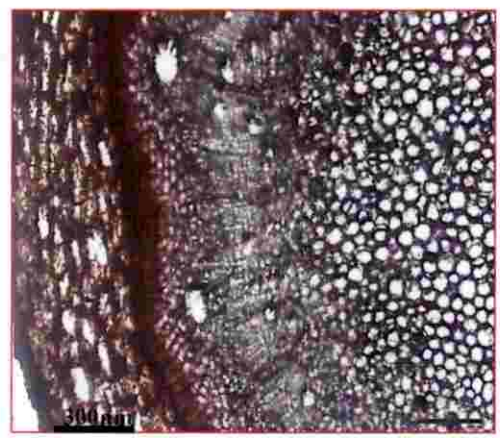
Kili Manga



Kappa Manga



Paiveli Local



Unda Varikka

Plate 11. continued

Table 16(a). Anatomical features (root cross section) of different mango genotypes

Treatments	Xylem area (mm^2)	Xylem percentage (%)	Phloem area (mm^2)	Phloem percentage (%)
T_1	10.86	65.61	0.56	17.48
T_2	3.92	50.87	2.83	27.23
T_3	5.58	52.14	2.08	28.04
T_4	2.41	40.89	6.60	33.48
T_5	6.62	65.68	2.01	22.59
T_6	5.82	53.85	2.46	25.44
T_7	6.85	53.96	1.17	16.49
T_8	9.94	78.73	0.62	9.79
T_9	3.92	50.09	5.38	34.17
T_{10}	1.34	26.84	5.05	36.22
SE m(±)	0.17	0.36	0.02	0.09
CD	0.49	1.06	0.08	0.27

Table 16(b). Anatomical features (root cross section) of different mango genotypes

Treatments	Phloem/ Xylem ratio	Total conduit area (mm^2)
T_1	0.26	11.43
T_2	0.53	6.98
T_3	0.54	7.67
T_4	0.81	9.02
T_5	0.34	8.64
T_6	0.46	8.28
T_7	0.31	8.02
T_8	0.13	10.57
T_9	0.68	9.30
T_{10}	1.35	6.38
SE m(±)	0.010	0.17
CD	0.029	0.52

4.3.3.11.6 Total conduit area

The data regarding total conduit area of stem as well as root of different mango genotypes 6 month after germination are presented in table 15(b) and table 16(b) respectively. The lowest total conduit area of stem (4.38 mm^2) was noted in Thali Manga (T_3), followed by Kasthuri (T_2) (5.07 mm^2) and Pallikkal Local (T_6) (5.23 mm^2). The highest stem total conduit area was noted in Kappa Manga (T_8) (7.59 mm^2). The var. Unda Varika (T_{10}) recorded the lowest root total conduit area (6.38 mm^2), followed by Thali Manga (T_3) (7.67 mm^2) and Kili Manga (T_7) (8.02 mm^2). The var. Kotookonam Varikka (T_1) recorded the highest total conduit area of root (14.43 mm^2).

4.4: Effect of propagation methods and modified environments on different varieties of scion

4.4.1 Girth of rootstock

The data on girth of rootstock 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.1.1 Effect of propagation methods

The methods of grafting showed significant effect on the girth of rootstock 90 DAG [Table 17(a)]. The highest rootstock girth (7.37 mm) was recorded in softwood grafted plants (P_2), whereas the least girth (4.25 mm) was observed in epicotyl grafted plants (P_1).

4.4.1.2 Effect of modified environments

The data recorded on girth of rootstock 90 DAG revealed that there was significant effect due to modified environmental conditions [Table 18(a)]. Among three different modified environmental conditions, fan and pad system produced the grafts with the highest rootstock girth (5.97 mm) followed by humid chamber (5.85 mm). The least rootstock girth (5.61 mm) was noted under natural shade (75 % shade).

4.4.1.3 Effect of varieties of scion

The close perusal of data [Table 19(a)] indicated that, there was significant differences in rootstock girth as influenced by different varieties of scion 90 DAG. The highest rootstock girth (6.05 mm) was observed in Kotookonam Varikka, followed by Neelum (5.78 mm) and the least girth (5.59 mm) was in Kalapady (V_1).

4.4.1.4 Effect of interactions

On statistical analysis, the interaction between propagation methods and modified environments was found non-significant for rootstock girth 90 DAG [Table 20(a)].

The data presented in table 21(a) indicated that there was significant difference due to interaction between propagation methods and varieties of scion in rootstock girth 90 DAG. The highest rootstock girth (7.68 mm) was recorded in P_2V_3 (softwood grafts of kotookonam Varikka) followed by P_2V_2 (softwood grafts of Neelum) (7.29 mm). The lowest rootstock girth (4.03 mm) was recorded in the treatment combination P_1V_1 (epicotyl grafts of Kalapady).

The data presented in table 22(a) indicated that there was non-significant difference due to interaction between modified environments and varieties of scion for rootstock girth 90 DAG.

Interaction effect of different propagation methods, modified environments and varieties of scion was found non-significant [Table 23(a)] with respect to rootstock girth 90 DAG.

4.4.2 Girth of scion (cm)

The data on girth of scion 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.2.1 Effect of propagation methods

The methods of grafting showed significant effect on the girth of scion [Table 17(a)] 90 DAG. The highest scion girth (7.41 mm) was recorded in softwood grafted plants (P_2), whereas the least rootstock girth (5.08 mm) was observed in epicotyl grafted plants (P_1).

4.4.2.2 Effect of modified environments

The data presented in table 18(a) revealed that modified environment conditions had significant effect on scion girth 90 DAG. Fan and pad system was found significantly superior over humid chamber and natural shade (75 % shade) in scion girth of 6.31 mm. The least scion girth (6.18 mm) was observed under natural shade.

4.4.2.3 Effect of varieties of scion

The data regarding scion girth of mango grafts as influenced by different varieties of scion was recorded 90 DAG and presented in table 19(a). The var. Kotookonam Varikka (V_3) produced grafts with highest scion girth (6.44 mm), followed by Neelum (6.27 mm). While the least scion girth (6.03 mm) was in Kalapady (V_1).

4.4.2.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(a)] with respect to scion girth 90 DAG. The maximum scion girth (7.69 mm) was recorded in P_2M_1 (softwood grafting under fan and pad system) followed by P_2M_2 (softwood grafting under humid chamber) (7.43 mm). The minimum scion girth (4.93 mm) was recorded in the treatment combination P_1M_3 (epicotyl grafting under natural shade).

On statistical analysis, the interaction between propagation methods and varieties of scion on scion girth 90 DAG was found significant [Table 21(a)]. The softwood grafts of Kotookonam Varikka recorded significantly the highest scion girth (7.69 mm), followed by softwood grafts of Neelum (7.43 mm), while the least scion girth (4.93 mm) was recorded in epicotyl grafts of variety Kalapady.

Humid chamber

Shade net (75 % shade)

Plate 12. Humid chamber and Shade net (75 % shade)

Table 17(a). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)
	Initial (at the time of grafting)	Final	Girth increment		
Epicotyl grafting (P_1)	3.43	4.25	0.81	5.08	15.80
Softwood grafting (P_2)	6.49	7.37	0.90	7.41	15.28
SE m(±)	0.01	0.01	0.008	0.01	0.02
CD (0.05)	0.04	0.04	0.024	0.03	0.05

Table 17(b). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Graft height (cm)	Length of sprout (cm)	Spread of plant (cm)	
			N-S direction	E-W direction
Epicotyl grafting (P_1)	23.63	5.49	20.09	19.05
Softwood grafting (P_2)	26.03	5.44	20.83	19.53
SE m(±)	0.02	0.01	0.06	0.04
CD (0.05)	0.06	0.03	0.17	0.13

The data presented in table 22(a) indicated that there was significant difference due to interaction between modified environments and varieties of scion for scion girth 90 DAG. The maximum scion girth (6.53 mm) was recorded in M_1V_3 (fan and pad system + kotookonam Varikka), followed by M_2V_3 (humid chamber + Kotookonam Varikka) (6.42 mm). The least scion girth (6.01mm) was recorded in M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(a)] with respect to scion girth 90 DAG.

4.4.3 Length of scion (cm)

The mean data on length of scion at 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.3.1 Effect of propagation methods

Effect of grafting methods on length of scion was found significant 90 DAG [Table 17(a)]. The epicotyl grafted plants recorded significantly higher scion length (15.80 cm) than the softwood grafted plants (15.28 cm).

4.4.3.2 Effect of modified environments

The data presented in table 18(a) showed that there was significant effect of environment conditions on length of scion 90 DAG. The highest scion length (15.86 cm) was observed under fan and pad system (M_1) than in humid chamber (M_2) and natural shade (M_3). The least scion length (15.37 cm) was noted in natural shade (75 % shade).

4.4.3.3 Effect of varieties of scion

Among different varieties of scion, the highest scion length was recorded in var. Kotookonam Varikka (15.90 cm), followed by Neelum (15.50 cm) 90 days after grafting, whereas the least scion length was in Kalapady (15.23 cm) [Table 19 (a)].

4.4.3.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(a)] with respect to scion length 90 DAG. The highest scion length (16.32 cm) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), followed by P_1M_2 (epicotyl grafting under humid chamber) (15.73 cm). The lowest scion length (15.09 mm) was recorded in the treatment combination P_2M_3 (softwood grafting under natural shade).

On statistical analysis, the interaction between propagation methods and varieties of scion for length of scion 90 DAG was significant [Table 21(a)]. The epicotyl grafts of Kotookonam Varikka (P_1V_3) recorded the highest scion length (16.32 cm), followed by epicotyl grafts of Neelum (15.50 cm). While the least scion length (15.09 cm) was recorded in softwood grafts of variety Kalapady (P_2V_1).

The data presented in Table 22(a) indicated that there was significant difference due to interaction between modified environments and varieties of scion in length of scion 90 DAG. The highest scion length (16.19 cm) was recorded in M_1V_3 (fan and pad system + Kotookonam Varikka), which was on par with M_1V_2 (fan and pad system + Neelum) (16.10 cm). The least scion length (15.07 cm) was recorded in the treatment combination M_3V_1 (Natural shade + Kalapady).

The interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(a)] with respect to length of scion 90 DAG. The highest scion length (16.80 cm) was recorded in $P_1M_1V_3$ (epicotyl grafts of Kotookonam Varikka under fan and pad system), followed by $P_1M_1V_2$ (epicotyl grafting of Neelum under fan and pad system) (16.36 cm). The least scion length (14.89 cm) was noted in treatment combination $P_2M_3V_1$ (softwood grafts of Kalapady under natural shade).

Table 17(c). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Days taken for first sprouting (days)	Days taken for last sprouting (days)	Number of grafts sprouted at weekly intervals (%)		
			First week	Second week	Third week
Epicotyl grafting (P_1)	12.19	22.02	36.91	67.16	83.46
Softwood grafting (P_2)	12.33	22.65	32.59	60.86	76.17
SE m(±)	0.02	0.10	0.51	0.59	0.75
CD (0.05)	0.07	0.29	1.46	1.68	2.14

Table 17(d). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Initial success percentage (%)	Percentage of graft establishment (%)	Days taken for leaf opening (days)
Epicotyl grafting (P_1)	83.21	72.22	15.07
Softwood grafting (P_2)	76.30	65.93	15.61
SE m(±)	0.61	0.50	0.056
CD (0.05)	1.74	1.44	0.16

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4.4.4 Graft height (cm)

The data regarding graft height 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.4.1 Effect of propagation methods

Effect of grafting methods on graft height was found significant 90 DAG [Table 17(b)]. The softwood grafted plants recorded the highest graft height (26.03 cm) than epicotyl grafted plants (23.63 cm).

4.4.4.2 Effect of modified environments

The data recorded on graft height 90 DAG revealed that there was significant effect due to modified environmental conditions [Table 18(b)]. Among the three different environmental conditions, fan and pad system resulted the highest graft height (26.17 cm), followed by humid chamber (24.76 cm). The shortest graft height (23.56 cm) were noted under in shade (75 % shade).

4.4.4.3 Effect of varieties of scion

The data [Table 19(a)] revealed significant variation in graft height of different mango varieties 90 DAG. The highest graft height (25.38 cm) was observed in Kotookonam Varikka, followed by Neelum (24.87 cm).While the shortest graft height (24.24 cm) was in Kalapady.

4.4.4.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(a)] with respect to graft height 90 DAG. The maximum graft height (27.75 cm) was recorded in P_2M_1 (softwood grafting under fan and pad system) followed by P_2M_2 (softwood grafting under humid chamber) (25.73 cm). The shortest graft height (22.51 cm) was recorded in the treatment combination P_1M_3 (epicotyl grafting under natural shade).

The interaction effect of propagation methods and varieties of scion was found significant [Table 21(a)] with respect to graft height 90 DAG. The largest

graft height (26.87 cm) was recorded in P_2V_3 (softwood grafts of Kotookonam Varikka), followed by P_2V_2 (softwood grafts ofNeelum) (26.04). The lowest graft height (23.30 cm) was recorded in the treatment combination P_1V_1 (epicotyl grafts of Kalapady).

The data presented in table 22(b) indicated that there was significant difference due to interaction between modified environments and varieties of scion in graft height 90 DAG. The significantly the highest graft height (26.86 cm) was recorded in M_1V_3 (Fan and pad system + Kotookonam Varikka), followed by M_1V_2 (Fan and pad system + Neelum) (26.60 cm). The lowest graft height (22.82 cm) was recorded in the treatment combination M_3V_1 (Natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(b)] with respect to graft height 90 DAG. The largest graft height (28.76cm) was recorded in the treatment combination $P_2M_1V_3$ (softwood grafts of Kotookonam Varikka under fan and pad system) and the shortest graft height (22.17cm) was noted in the treatment combination $P_1M_3V_1$ (epicotyl grafts of Kalapady under natural shade).

4.4.5 Length of sprout (cm)

The data on length of sprout 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.5.1 Effect of propagation methods

The methods of grafting showed significant effect on sprout length [Table 17(b). At 90 DAG, epicotyl grafted plants recorded significantly longer sprout length (5.49 cm) than softwood grafted plants (5.44 cm).

Table 17(e). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Number of leaves per graft	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Epicotyl grafting (P_1)	15.40	15.27	3.36	41.69
Softwood grafting (P_2)	14.33	15.04	2.98	41.28
SE m(±)	0.08	0.01	0.08	0.12
CD (0.05)	0.23	0.04	0.24	0.35

Table 17(f). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
Epicotyl grafting (P_1)	21.63	5.20	20.49	1.76
Softwood grafting (P_2)	20.81	5.14	25.17	1.65
SE m(±)	0.07	0.01	N.S	0.03
CD (0.05)	0.20	0.03	N.S	0.08

Table 17(g). Vegetative and growth characters of mango grafts as influenced by various propagation methods

Propagation methods	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
Epicotyl grafting (P_1)	26.06	44.98	65.19
Softwood grafting (P_2)	27.95	45.80	54.47
SE m(±)	0.10	0.08	0.51
CD (0.05)	0.28	0.22	1.47

Table 18(a). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)
	Initial (at the time of grafting)	Final	Girth increment		
Climate controlled [fan and pad] (M_1)	5.04	5.97	0.94	6.31	15.86
Humid chamber (M_2)	5.00	5.85	0.84	6.25	15.40
Natural shade [75 % shade] (M_3)	4.83	5.61	0.77	6.18	15.37
SE m(±)	0.01	0.01	0.01	0.01	0.02
CD (0.05)	0.05	0.04	0.03	0.03	0.06

Plate 13. Climate controlled (Fan and pad system)

4.4.5.2 Effect of modified environments

The data recorded on sprout length 90 DAG revealed that there was significant effect due to environment conditions [(Table 18(b)]. The highest sprout length (5.83 cm) was recorded in fan and pad system, followed by humid chamber (5.51 cm). The lowest sprout length (5.05 cm) was noted in natural shade (75 % shade).

4.4.5.3 Effect of varieties of scion

The data [Table 19(b)] revealed significant variation in sprout length of different mango varieties 90 DAG. The highest sprout length (5.49 cm) was observed in Neelum (V_2), which was on par with Kotookonam Varikka (V_3) (5.48 cm). While the least sprout length (5.42 cm) was in Kalapady (V_3).

4.4.5.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(b)] with respect to sprout length 90 DAG. The highest sprout length (5.57 cm) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), which was on par with P_1M_2 (epicotyl grafting under humid chamber) (5.54 cm). The least sprout length (5.39 cm) was recorded in P_2M_3 (softwood grafting under natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was significant [Table 21(b)] with respect to sprout length 90 DAG. The highest sprout length (5.57 cm) was observed in P_1V_2 (epicotyl grafts of Neelum), which was on par with P_2V_3 (softwood grafts of Kotookonam Varikka) (5.53 cm). The least sprout length (5.39 cm) was noted in P_2V_1 (softwood grafts of Kalapady).

The data presented in table 22(b) indicated that there was significant difference due to interaction between modified environments and varieties of scion for sprout length 90 DAG. The greatest sprout length (5.97 cm) was recorded in M_1V_3 (fan and pad system + Kotookonam Varikka), followed by M_1V_1 (fan and pad system + Kalapady) (5.88 cm). The lowest sprout length (4.97 cm) was recorded in M_3V_1 (Natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(b) with respect to sprout length 90 DAG. The greatest sprout length (6.05 cm) was recorded in treatment combination $P_1M_1V_3$ (epicotyl grafts of Kotookonam Varikka under fan and pad system), which was on par with $P_1M_1V_2$ (epicotyl grafts of Neelum under fan and pad system) (5.98 cm), whereas the least sprout length (4.90 cm) was noted in $P_2M_3V_1$ (softwood grafts of Kalapady under natural shade).

4.4.6 Spread of plant

The data regardingspread of plant 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.6.1 Effect of propagation methods

It is evident from table 17(b) that the methods of propagation significantly influenced the spread of the plant 90 DAG. The spread of the plant in north south direction (N-S) and east west direction were the highest (20.83 cm and 19.53 cm respectively) in softwood grafted plants. Whereas the lowest spread of the plant in both direction was recorded in epicotyl grafting (20.09 cm and 19.05 cm respectively).

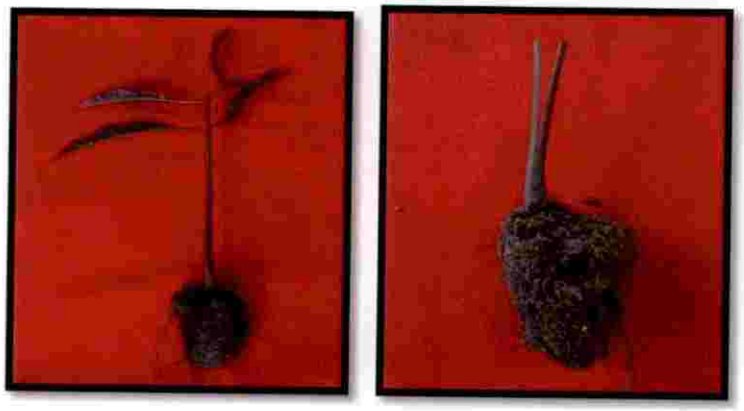
Table 18(b). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Graft height (cm)	Spread of plant (cm)		Length of sprout (cm)
		N-S direction	E-W direction	
Climate controlled [fan and pad] (M_1)	26.17	21.81	20.60	5.83
Humid chamber (M_2)	24.76	20.35	19.15	5.51
Natural shade [75 % shade] (M_3)	23.56	19.22	18.12	5.05
SE m(±)	0.03	0.07	0.05	0.01
CD (0.05)	0.08	0.21	0.16	0.03

Table 18(c). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Days taken for first sprouting (days)	Days taken for last sprouting (days)	Number of grafts sprouted at weekly intervals (%)		
			First week	Second week	Third week
Climate controlled [fan and pad] (M_1)	12.08	22.03	36.30	65.74	81.48
Humid chamber (M_2)	12.11	22.07	34.07	63.52	79.45
Natural shade [75 % shade] (M_3)	12.58	22.90	33.89	62.78	78.50
SE m(±)	0.10	0.16	0.62	0.72	0.90
CD (0.05)	0.29	0.36	1.78	2.06	2.61

Preparation of scion



Preparation of rootstock

Grafting

Plate 14. Epicotyl grafting procedure

4.4.6.2 Effect of modified environments

The data recorded on spread of the plant in North South directions (N-S) and east west direction of mango grafts at 90 days after grafting revealed that there were significant difference due to different environment conditions [Table 18(b)]. The spread of the plant in North South directions (N-S) and East West directions were the highest (21.81 cm and 20.60 cm respectively) under fan and pad system. Whereas the least spread of the plant in both directions was under 75 % shade (19.22 cm and 18.12 cm respectively).

4.4.6.3 Effect of varieties of scion

It is clear from data presented in table 19(b) that, there was significant variation as influenced by different varieties of scion with respect to spread of plant in both directions (N-S and E-W) 90 days after grafting. The spread of the plant in North South (N-S) and East West directions were the highest (22.29 cm and 21.08 cm respectively) in var. KotookonamVarikka whereas, the lowest spread of the plant in both directions were recorded in Kalapady (18.65 cm and 17.56 cm respectively).

4.4.6.4 Effect of interactions

The interaction effect of propagation methods and modified environments was non-significant [Table 20(b)] with respect to spread of plant in North-South direction, whereas it was significant for East-West direction 90 DAG. The highest spread of plant in East- West direction (21.09 cm) was recorded in P_2M_1 (softwood grafting under fan and pad system). The least spread of plant in E-W direction (17.83 cm) was recorded in P_1M_3 (epicotyl grafting under natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was non-significant [Table 21(b)] with respect to plant spread in North-South direction, whereas significant for East-West direction at 90 DAG. The greatest plant spread in East- West direction (21.24 cm) was recorded in P_2V_3 (softwood grafts of Kotookonam Varikka). The least plant spread in E-W direction (17.07 cm) was recorded in P_1V_1 (epicotyl grafts of Kalapady).

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Table 18(d). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Initial success percentage (%)	Percentage of graft establishment (%)	Days taken for leaf opening (days)	Number of leaves per graft
Climate controlled [fan and pad] (M_1)	81.30	71.67	15.11	15.68
Humid chamber (M_2)	79.44	70.00	15.29	14.90
Natural shade [75 % shade] (M_3)	78.52	65.56	15.60	13.98
SE m(±)	0.73	0.61	0.06	0.09
CD (0.05)	2.12	1.76	0.19	0.28

Table 18(e). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Number of nodes on scion
Climate controlled [fan and pad] (M_1)	15.60	3.65	44.98	24.14
Humid chamber (M_2)	15.40	3.33	44.53	23.92
Natural shade [75 % shade] (M_3)	14.42	2.54	34.94	18.32
SE m(±)	0.01	0.10	0.14	0.08
CD (0.05)	0.04	0.28	0.42	0.24

The data presented in table 22(b) indicated that there was non-significant difference due to interaction between modified environments and varieties of scion on plant spread in North – South direction, whereas significant for East-West direction 90 DAG. The greatest plant spread in East- West direction (22.35 cm) was recorded in M_1V_1 (fan and pad system + Kotookonam Varikka), followed by M_2V_3 (humid chamber + kotookonam Varikka) (21.01 cm). The least spread (16.57 cm) was recorded in M_3V_1 (Natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(b)] with respect to spread of plant in North – South and East-West direction 90 DAG.

4.4.7 Days taken for first sprouting

The data regardingdays taken for first sprouting as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.7.1 Effect of propagation methods

The propagation methods had significant impact on the number of days taken for first sprouting [Table 17(c)]. The epicotyl grafted plants resulted in earliness of sprouting (12.19 days) while, the softwood grafted plants recorded comparatively more number of days for first sprouting (12.33 days).

4.4.7.2 Effect of modified environments

The data recorded on number of days taken for first sprouting as influenced by varieties of scion was significant [Table 18(c)] with respect to different modified environments. Earliness in first sprouting (12.08 days) was recorded under fan and pad system (M_1), which was on par with humid chamber (12.11 days). More number of days for first sprouting (12.58 days) was noted under natural shade.

Table 18(f). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
Climate controlled [fan and pad] (M_1)	5.48	23.73	1.91
Humid chamber (M_2)	5.46	23.18	1.71
Natural shade [75 % shade] (M_3)	4.77	21.58	1.52
SE m(±)	0.01	N.S	0.03
CD (0.05)	0.03	N.S	0.09

Table 18(g). Vegetative and growth characters of mango grafts as influenced by modified environments

Modified environments	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
Climate controlled [fan and pad] (M_1)	26.58	44.96	67.26
Humid chamber (M_2)	26.71	45.11	61.11
Natural shade [75 % shade] (M_3)	27.73	46.12	51.11
SE m(±)	0.11	0.09	0.62
CD (0.05)	0.34	0.26	1.80

4.4.7.3 Effect of varieties of scion

Among the three varieties of scion, the least number of days for first sprouting (9.91 days) was recorded in Kalapady (V_1) followed by Kotookonam Varikka (12.12 days). While more number of days (14.74 days) for first sprouting was observed in Neelum [Table 19(c)].

4.4.7.4 Effect of interactions

The interaction effect between propagation methods and modified environments was significant [Table 20(b)] for number of days taken for first sprouting. The earliness in first sprouting (11.58 days) was recorded in P_1M_1 (epicotyl grafting under fan and pad system) which was on par with P_2M_1 (softwood grafting under fan and pad system) (11.80 days). More number of days for first sprouting (12.60 days) was recorded in P_2M_3 (softwood grafting under natural shade).

It is evident from the data that the interaction of propagation methods and varieties of scion was significant [Table 21(b)] with respect to number of days taken for first sprouting. The least number of days taken for first sprouting (9.87 days) was observed in P_1V_1 (epicotyl grafts of Kalapady), which was on par with P_1V_3 (epicotyl grafts of Kotookonam Varikka) (9.96 days) whereas, the largest number of days taken for first sprouting (14.89 days) was noted in P_2V_2 (softwood grafts of Neelum).

The data presented in table 22(c) indicated that there was significant difference due to interaction between modified environments and varieties of scion for number of days taken for first sprouting. The least number of days taken for first sprouting (9.77 days) was recorded in M_1V_1 (fan and pad system + Kalapady), which was on par with M_1V_3 (Fan and pad system+ Kotookonam Varikka) (9.90 days). The largest number of days for first sprouting (15.00 days) was recorded in M_3V_1 (natural shade + Kalapady).

Preparation of scion

Preparation of rootstock

Grafting

Plate 15. Softwood grafting procedure

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(c)] with respect to number of days taken for first sprouting. The earliness in first sprouting (9.53 days) was recorded in $P_1M_1V_1$ (epicotyl grafts of Kalapady under fan and pad system), which was on par with treatments $P_1M_1V_3$ (9.80 days), $P_1M_2V_3$ (10.00 days) and $P_1M_2V_1$ (10.20 days). The largest number of days taken for first sprouting (15.44 days) was recorded in $P_2M_3V_2$ (softwood grafts of Neelum under natural shade).

4.4.8 Days taken for last sprouting

The data regardingdays taken for last sproutingas influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.8.1 Effect of propagation methods

The propagation methods had significant impact on the number of days taken for last sprouting [Table 17(c)] The epicotyl grafted plants required lower number of days for last sprouting (22.02 days) than the softwood grafted plants (22.65 days).

4.4.8.2 Effect of modified environments

The data recorded on number of days taken for last sprouting as influenced by varieties of scion was significant [Table 18(c)] with respect to different modified environmental conditions. The earliness in last sprouting (22.03 days) was recorded under fan and pad system (M_1) which was on par with humid chamber (M_2) (22.07 days). More number of days for the last sprouting (22.90 days) was noted under natural shade (75 % shade).

4.4.8.3 Effect of varieties of scion

Among the three varieties of scion, earliness in last sprouting (20.27 days) was recorded in Kalapady followed by Kotookonam Varikka (22.18 days). More number of days (24.56 days) for last sprouting was observed in Neelum [Table 19(b)].

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Table 19(a). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)	Graft height (cm)
	Initial (at the time of grafting)	Final	Girth increment			
Kalapady (V_1)	4.85	5.59	0.75	6.03	15.23	24.24
Neelum (V_2)	4.91	5.78	0.87	6.27	15.50	24.87
Kotookonam Varikka (V_3)	5.11	6.05	0.95	6.44	15.90	25.38
SE m(±)	0.01	0.01	0.01	0.01	0.02	0.02
CD (0.05)	0.05	0.04	0.03	0.03	0.05	0.08

Table 19(b). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Length of sprout (cm)	Spread of plant (cm)		Days taken for first sprouting (days)	Days taken for last sprouting (days)
		N-S direction	E-W direction		
Kalapady (V_1)	5.42	18.65	17.56	9.91	20.27
Neelum (V_2)	5.49	20.45	19.23	14.74	24.56
Kotookonam Varikka (V_3)	5.48	22.29	21.08	12.12	22.18
SE m(±)	0.02	0.08	0.05	0.11	0.13
CD (0.05)	0.04	0.20	0.15	0.29	0.35

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4.4.8.4 Effect of interactions

With regard to statistical analysis the interaction effect between propagation methods and modified environments was significant [Table 20(b)] for number of days taken for last sprouting. The least number of days taken for last sprouting (21.33 days) was recorded in P_1M_1 (epicotyl grafting under fan and pad system) which was on par with P_1M_2 (21.82 days) and P_2M_1 (21.84 days). The largest number of days taken for last sprouting (23.33 days) was recorded in the treatment combination P_2M_3 (softwood grafting under natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was significant [Table 21(b)] with respect to number of days taken for last sprouting. The least number of days for last sprouting (20.07 days) was observed in P_1V_3 (epicotyl grafting in Kotookonam Varikka) which was on par with P_1V_1 (epicotyl grafting in Kalapady) (20.47 days). The largest number of days taken for first sprouting (24.67 days) was noted in P_2V_2 (softwood grafting in Neelum).

The data presented in table 22(c) indicated that there was no significant difference due to interaction between modified environments and varieties of scion on number of days taken for last sprouting.

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(c)] with respect to number of days taken for last sprouting. The least number of days taken for last sprouting (19.07 days) was recorded in $P_1M_1V_1$ (epicotyl grafting of Kalapady under fan and pad system) which was on par with $P_1M_1V_3$ (epicotyl grafting of Kotookonam Varikka under fan and pad system) (19.27 days) and more number of days taken for first sprouting (25.00 days) was recorded in treatment combination $P_2M_3V_2$ (softwood grafting of Neelum under natural shade).

Table 19(c). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Number of grafts sprouted at weekly intervals (%)			Initial success percentage (%)	Percentage of graft establishment (%)
	First week	Second week	Third week		
Kalapady (V_1)	25.19	52.04	72.60	72.41	61.67
Neelum (V_2)	34.81	64.45	80.56	80.74	70.37
Kotookonam Varikka (V_3)	44.26	75.56	86.30	86.11	75.19
SE m(±)	0.63	0.71	0.91	0.74	0.64
CD (0.05)	1.79	2.05	2.62	2.13	1.77

Table 19(d). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Days taken for leaf opening (days)	Number of leaves per graft	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Kalapady (V_1)	13.97	13.79	14.61	2.93	39.26
Neelum (V_2)	16.81	16.07	15.56	3.26	43.33
Kotookonam Varikka (V_3)	15.23	14.72	15.26	3.64	42.78
SE m(±)	0.07	0.10	0.02	0.10	0.15
CD (0.05)	0.20	0.29	0.05	0.28	0.43

4.4.9 Number of grafts sprouted at weekly intervals

The data on number of grafts sprouted at weekly intervals as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.9.1 Effect of propagation methods

The perusal of data presented in table 17(c) clearly indicated that the number of grafts sprouted at weekly intervals were significantly influenced by different propagation methods. Higher number of sprouted grafts at weekly intervals (first, second and third week after grafting) (36.91 %, 67.16 % and 83.46 % respectively) was obtained from epicotyl grafting.

4.4.9.2 Effect of modified environments

The data presented in table 18(c) revealed that modified environment conditions had significant effect on number of grafts sprouted at weekly intervals. During the first and second week after grafting, fan and pad system (M_1) was significantly superior over humid chamber (M_2) and natural shade (M_3) with respect to the highest number of sprouted grafts (36.30 % and 65.74 % respectively). During third week, more number of sprouted grafts were recorded under fan and pad system (81.48 %), which was on par with those under humid chamber (79.45 %). The least number of sprouted grafts at weekly intervals were observed under natural shade (75 % shade).

4.4.9.3 Effect of varieties of scion

The perusal of data presented in table 19(c) clearly indicated that the number of grafts which sprouted at weekly intervals was significantly influenced by scion varieties. The var. Kotookonam Varikka produced more number of sprouted grafts at weekly intervals (first, second and third week after grafting) (44.26 %, 75.56 % and 86.30 %, respectively) while, the least was in Kalapady.

Plate 16. Mango grafts maintained under fan and pad system

4.4.9.4 Effect of interactions

The interaction between propagation methods and modified environments was significant [Table 20(c)] with respect to the number of grafts sprouted at weekly intervals. The number of sprouted grafts at weekly intervals (first, second and third week after grafting) were the highest (40.00 %, 70.37 % and 87.41 % respectively) in P_1M_1 (epicotyl grafting under fan and pad system). During second week, the treatment P_1M_2 (epicotyl grafting under humid chamber) was on par with P_1M_1. The least number of sprouted grafts at weekly intervals (31.11 %, 58.15 % and 75.55 %) was recorded in P_2M_3 (softwood grafting under natural shade).

It is evident from the data that the interaction of propagation methods and varieties of scion was significant [Table 21(c)] with respect to the number of grafts sprouted at first and second week and it was non-significant at third week after grafting. The number of sprouted grafts at weekly intervals (first and second week after grafting) were the highest (48.52 % and 81.48 % respectively) in treatment P_1V_3 (epicotyl grafts of Kotookonam Varikka), whereas the lowest number of grafts sprouted at weekly intervals (24.81 % and 51.48 %) was observed in P_2V_1 (softwood grafts of Kalapady).

The data presented in Table 22(c) indicated that there was no significant difference due to interaction of modified environments and varieties of scion for number of grafts sprouted at weekly intervals.

Interaction effect of different propagation methods, modified environments and varieties of scion were found non-significant [Table 23(c)] with respect to number of grafts sprouted at weekly intervals.

Table 19(e). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
Kalapady (V_1)	19.64	4.91	21.40	1.34
Neelum (V_2)	22.44	5.23	20.77	1.74
Kotookonam Varikka (V_3)	22.69	5.38	26.32	2.08
SE m(±)	0.09	0.01	N.S	0.03
CD (0.05)	0.27	0.04	N.S	0.10

Table 19(f). Vegetative and growth characters of mango grafts as influenced by varieties of scion

Varieties of scion	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
Kalapady (V_1)	23.73	44.28	60.93
Neelum (V_2)	30.62	49.92	59.45
Kotookonam Varikka (V_3)	26.68	41.98	59.11
SE m(±)	0.12	0.09	N.S
CD (0.05)	0.32	0.28	N.S

4.4.10 Initial success percentage

The data on initial success percentage 30 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.10.1 Effect of propagation methods

With regard to propagation methods, the results obtained was significant for initial success percentage 30 DAG. The highest percentage of initial success (83.21 %) was noted in epicotyl grafted plants while, the lowest (76.30 %) was in softwood grafted plants [Table 17(d)].

4.4.10.2 Effect of modified environments

The data presented in table 18(d) showed that there was significant effect of modified environment condition on initial success percentage of grafts. At 30 DAG, the maximum initial graft success (81.30 %) was obtained under fan and pad system, which was on par with humid chamber (79.44 %). The lowest percentage of initial graft success was recorded under natural shade (78.52 %).

4.4.10.3 Effect of varieties of scion

With regard to different varieties of scion, the results obtained were found significant for initial success percentage at 30 DAG [Table 19(c)]. The variety Kotookonam Varikka recorded the highest initial graft success (86.11 %), followed by Neelum (80.74 %), whereas the least percentage was recorded in Kotookonam Varikka (72.41 %).

Table 20(a). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)	Graft height (cm)
	Initial (at the time of grafting)	Final	Girth increment			
P_1M_1	4.03	4.03	0.71	5.19	16.32	24.58
P_1M_2	4.33	4.28	0.80	5.11	15.73	23.78
P_1M_3	4.38	4.43	0.91	4.93	15.36	22.51
P_2M_1	7.19	7.15	0.83	7.69	15.50	27.75
P_2M_2	7.36	7.29	0.88	7.43	15.26	25.73
P_2M_3	7.57	7.68	0.97	7.12	15.09	24.59
SE m(±)	0.02	0.02	N.S	0.01	0.03	0.11
CD (0.05)	0.07	0.06	N.S	0.04	0.08	0.04

Table 20(b). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Length of sprout (cm)	Spread of plant (cm)		Days taken for first sprouting (days)	Days taken for last sprouting (days)
		N-S direction	E-W direction		
P_1M_1	5.57	21.90	20.11	11.58	21.33
P_1M_2	5.54	20.04	19.19	12.42	21.82
P_1M_3	5.40	18.33	17.83	12.58	22.47
P_2M_1	5.48	22.68	21.09	11.80	21.84
P_2M_2	5.44	20.86	19.11	12.58	22.80
P_2M_3	5.39	18.96	18.40	12.60	23.33
SE m(±)	0.02	N.S	0.08	0.15	0.18
CD (0.05)	0.05	N.S	0.22	0.42	0.51

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4.4.10.4 Effect of interactions

The interaction effect of propagation methods and modified environments were significant [Table 20(c)] for initial success percentage 30 DAG. The highest initial success percentage (87.04 %) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), followed by P_1M_2 (epicotyl grafting under humid chamber) (82.96 %). The least initial success percentage (75.56 %) was recorded in P_2M_3 (softwood grafting under natural shade).

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(c)] with respect to initial success percentage of grafts.

The data presented in table 22(d) indicated that there was no significant difference due to interaction between modified environments and varieties of scion for initial success percentage of grafts.

Interaction effect of different propagation methods, modified environments and varieties of scion were non-significant [Table 23(d)] with respect to initial success percentage 30 DAG.

4.4.11 Percentage of graft establishment

The data regardingpercentage of graft establishment 60 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.11.1 Effect of propagation methods

The method of propagation showed significant effect on percentage of graft establishment 60 DAG [Table 17(d)]. The highest percentage of graft established (72.22 %) was noted in epicotyl method while, the least percentage (65.93 %) was recorded in softwood grafting.

Table 20(c). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Number of grafts sprouted at weekly intervals (%)			Initial success percentage (%)	Percentage of graft establishment (%)
	First week	Second week	Third week		
P_1M_1	40.00	70.37	87.41	87.04	74.81
P_1M_2	36.67	67.46	83.33	82.96	72.55
P_1M_3	34.07	63.70	79.63	79.63	69.63
P_2M_1	34.07	63.33	77.41	75.93	65.19
P_2M_2	32.59	61.11	75.56	77.41	71.11
P_2M_3	31.11	58.15	75.55	75.56	61.49
SE m(±)	0.88	1.01	1.29	1.05	0.87
CD (0.05)	2.53	2.91	3.71	3.01	2.49

Table 20(d). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Days taken for leaf opening (days)	Number of leaves per graft	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
P_1M_1	14.62	15.87	15.90	3.74	45.19
P_1M_2	15.36	15.66	15.46	3.72	43.88
P_1M_3	15.60	14.77	14.47	3.64	36.84
P_2M_1	14.86	15.49	15.34	3.56	45.12
P_2M_2	15.24	14.26	15.30	3.02	44.84
P_2M_3	15.67	13.29	14.37	2.38	34.84
SE m(±)	0.10	0.14	0.02	N.S	0.21
CD (0.05)	0.27	0.40	0.07	N.S	0.61

4.4.11.2 Effect of modified environments

The data presented in table 18(d) showed that there was significant effect of modified environmental conditions on percentage of graft establishment 60 DAG. The highest percentage of graft establishment (71.67 %) was observed under grafts in fan and pad system, which was on par with humid chamber (70.00 %), whereas the lowest percentage of graft establishment (65.56 %) was recorded in those under natural shade (75 % shade).

4.4.11.3 Effect of varieties of scion

The varieties of scion showed significant effect on percentage of graft establishment 60 DAG [Table 19(c)]. The highest percentage of graft established (75.19 %) was noted in Kotookonam Varikka, followed by Neelum (70.37 %) while, the least percentage (61.67 %) was noted in Kalapady.

4.4.11.4 Effect of interactions

The interaction effect between propagation methods and modified environments was found significant [Table 20(c)] with respect to percentage of graft establishment 60 DAG. The highest percentage of graft establishment (74.81 %) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), which was on par with P_1M_2 (epicotyl grafting under humid chamber) (72.55 %). The lowest percentage of graft establishment (61.49 %) was recorded in the treatment combination P_2M_3 (softwood grafting under natural shade).

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(c)] with respect to percentage of graft establishment.

Table 20(e). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
P_1M_1	24.37	5.38	25.58	2.02
P_1M_2	18.61	5.36	17.33	1.62
P_1M_3	18.60	4.84	18.54	1.58
P_2M_1	23.41	5.58	28.92	1.89
P_2M_2	20.98	5.34	25.82	1.80
P_2M_3	18.04	4.70	20.77	1.47
SE m(±)	N.S	0.09	N.S	0.05
CD (0.05)	N.S	0.05	N.S	0.14

Table 20(f). Interaction effect of various propagation methods and modified environments on vegetative and growth characters of mango grafts

Treatments	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
P_1M_1	25.44	43.84	68.89
P_1M_2	26.02	45.20	64.07
P_1M_3	26.73	45.93	62.59
P_2M_1	27.98	45.02	54.82
P_2M_2	27.16	46.07	55.63
P_2M_3	28.73	46.31	52.96
SE m(±)	0.17	0.13	0.89
CD (0.05)	0.48	0.38	2.55

Table 21(a). Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)	Graft height (cm)
	Initial (at the time of grafting)	Final	Girth increment			
P_1V_1	3.35	4.03	0.69	4.93	15.36	23.30
P_1V_2	3.42	4.28	0.84	5.11	15.73	23.70
P_1V_3	3.52	4.43	0.92	5.19	16.32	23.89
P_2V_1	6.35	7.15	0.81	7.12	15.09	25.17
P_2V_2	6.41	7.29	0.90	7.43	15.50	26.04
P_2V_3	6.71	7.68	0.98	7.69	15.26	26.87
SE m(±)	0.02	0.02	0.01	0.01	0.03	0.11
CD (0.05)	0.07	0.06	0.04	0.04	0.08	0.04

The data presented in table 22(d) indicated that there was significant difference due to interaction between modified environments and varieties of scion on percentage of graft establishment 60 DAG. The highest percentage of graft establishment (80.00 %) was recorded in M_1V_3 (fan and pad system + Kotookonam Varikka), followed by M_1V_2 (fan and pad system + Neelum) (73.33 %). The lowest percentage of graft establishment (51.11 %) was recorded in M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(d)] with respect to percentage of graft establishment.

4.4.12 Days taken for leaf opening

The mean data on days taken for leaf opening as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.12.1 Effect of propagation methods

The methods of grafting showed significant effect on number of days taken for leaf opening [Table 17(d)]. The epicotyl grafted plants recorded the least number of days for leaf opening (15.07 days) than the softwood grafted plants (15.61 days).

4.4.12.2 Effect of modified environments

The significant difference due to modified environment conditions were observed for number of days taken for leaf opening [Table 18(c)]. Regarding the effect of modified environmental conditions, fan and pad system (M_1) was better for earliness in leaf opening (15.11 days), which was on par with humid chamber (M_2) (15.29 days). More number of days for leaf opening (15.60 days) was recorded under natural shade condition.

4.4.12.3 Effect of varieties of scion

The perusal of data presented in table 19(d) showed that there was significant effect of varieties of scion on number of days taken for leaf opening. The var. Kalapady recorded the least number of days (13.97 days) for leaf opening, whereas the var. Neelum recorded the largest number of days (16.81 days) for leaf opening.

4.4.12.4 Effect of interactions

The interaction effect between propagation methods and modified environments was significant [Table 20(d)] for the number of days taken for leaf opening. The least number of days for leaf opening (14.62 days) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), which was on par with P_2M_1 (softwood grafting under fan and pad system) (14.86 days). More number of days taken for leaf opening (15.67 days) was recorded in P_2M_3 (softwood grafting under natural shade).

Interaction effect of propagation methods and varieties of scion was significant [Table 21(d)] with respect to number of days taken for leaf opening. The least number of days for leaf opening (14.28 days) was observed in P_1V_1 (epicotyl grafts of Kalapady), followed by P_2V_1 (softwood grafts of Kalapady) (14.93 days). Whereas the highest number of days for leaf opening (15.62 days) was noted in P_2V_2 (softwood grafts of Neelum).

The data presented in table 22(d) indicated that there was significant difference due to interaction between modified environments and varieties of scion on number of days taken for leaf opening. The least number of days taken for leaf opening (13.83 days) was recorded in M_1V_1 (fan and pad system + Kalapady), which was on par with M_2V_1 (humid chamber + Kalapady) (13.93 days). More number of days for leaf opening (17.07 days) was recorded in M_3V_2 (natural shade + Neelum).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(d)] with respect to number of days taken for leaf opening.

Plate 17. Established grafts (epicotyl) under fan and pad system

Table 21(b). Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Length of sprout (cm)	Spread of plant (cm)		Days taken for first sprouting (days)	Days taken for last sprouting (days)
		N-S direction	E-W direction		
P_1V_1	5.40	18.33	17.07	9.87	20.47
P_1V_2	5.57	20.04	19.15	14.60	24.44
P_1V_3	5.48	21.90	20.92	9.96	20.07
P_2V_1	5.39	18.96	18.05	12.42	21.53
P_2V_2	5.44	20.86	19.31	14.89	24.67
P_2V_3	5.53	22.68	21.24	11.82	22.82
SE m(±)	0.02	N.S	0.08	0.15	0.18
CD (0.05)	0.05	N.S	0.22	0.42	0.51

Table 21(c). Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of grafts sprouted at weekly intervals (%)			Initial success percentage (%)	Percentage of graft establishment (%)
	First week	Second week	Third week		
P_1V_1	37.41	68.52	81.84	74.44	63.70
P_1V_2	40.00	69.63	84.81	84.81	74.44
P_1V_3	48.52	81.48	90.74	90.37	78.52
P_2V_1	24.81	51.48	51.48	70.37	59.63
P_2V_2	25.56	52.59	52.59	76.67	66.30
P_2V_3	32.21	60.37	60.37	81.85	71.86
SE m(±)	0.88	1.01	N.S	N.S	N.S
CD (0.05)	2.53	2.91	N.S	N.S	N.S

Table 21(d). Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Days taken for leaf opening (days)	Number of leaves per graft	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
P_1V_1	14.28	14.27	14.93	2.76	38.99
P_1V_2	15.48	16.61	15.57	3.36	43.38
P_1V_3	15.28	15.31	15.62	3.59	41.47
P_2V_1	14.93	13.31	14.28	2.50	39.52
P_2V_2	15.62	14.13	15.24	3.14	42.09
P_2V_3	15.24	15.53	15.48	3.71	43.47
SE m(±)	0.02	N.S	0.02	0.14	N.S
CD (0.05)	0.07	N.S	0.07	0.41	N.S

4.4.13 Number of leaves per graft

The data regarding number of leaves per graft 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.13.1 Effect of propagation methods

The methods of grafting showed significant effect on number of leaves per graft [Table 17(e)]. The epicotyl grafted plants produced significantly more number of leaver per graft (15.40) 90 DAG than softwood grafted plants (14.33).

4.4.13.2 Effect of modified environments

The significant differences due to modified environment conditions were observed for number of leaves per graft 90 DAG [Table 18(d)]. Regarding the effect of environment conditions, fan and pad system (M_1) was found better for producing the highest number of leaves (15.68) 90 DAG, followed by humid chamber (14.90). The lowest number of leaves per graft (13.98) was observed under natural shade (M_3) during the observation period.

4.4.13.3 Effect of varieties of scion

It is clear from the data [Table 19(d)] that the significant variations due to varieties of scion with respect to number of leaves per graft 90 DAG. The highest number of leaves (16.07) were observed in Neelum while the lowest (13.98) was in Kalapady.

4.4.13.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(d)] for number of leaves 90 DAG. The highest number of leaves per graft (15.87) was produced in P_1M_1 (epicotyl grafting under fan and pad system) and on par with P_1M_2 (15.66) and P_2M_1 (15.49). The least number of leaves per graft (13.29) was recorded in P_2M_3 (softwood grafting under natural shade).

Table 21(e). Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
P_1V_1	20.16	4.97	16.76	1.27
P_1V_2	21.73	5.16	16.38	1.71
P_1V_3	22.98	5.46	20.32	2.02
P_2V_1	22.40	4.86	26.04	1.42
P_2V_2	20.88	5.30	25.15	1.73
P_2V_3	22.40	5.42	24.32	2.13
SE m(±)	N.S	0.02	N.S	N.S
CD (0.05)	N.S	0.05	N.S	N.S

Table 21(f).Interaction effect of various propagation methods and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
P_1V_1	25.40	43.82	55.56
P_1V_2	27.96	44.73	66.67
P_1V_3	23.04	41.42	73.33
P_2V_1	29.76	49.73	46.67
P_2V_2	31.89	50.11	55.56
P_2V_3	24.42	43.42	61.18
SE m(±)	0.17	0.13	N.S
CD (0.05)	0.48	0.38	N.S

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(d)] with respect to number of leaves per graft.

The data presented in table 22(d) indicated that there was significant difference due to interaction between modified environments and varieties of scion on number of leaves per graft 90 DAG. The highest number of leaves per graft (16.87) was recorded in M_1V_2 (fan and pad system + Neelum), followed by M_2V_2 (humid chamber + Neelum) (15.73). The least number of leaves per graft (12.67) was recorded in the treatment combination M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(d)] with respect to number leaves per graft 90 DAG.

4.4.14 Leaf length (cm)

The data on leaf length 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.14.1 Effect of propagation methods

The methods of grafting showed significant effect on leaf length 90 DAG [Table 17(e)]. The epicotyl grafted plants recorded higher leaf length (15.27 cm) than softwood grafted plants (15.04 cm).

4.4.14.2 Effect of modified environments

The data recorded on leaf length [Table 18(e)] revealed that there was significant effect due to environmental conditions. Among three modified environmental conditions, fan and pad system (M_1) resulted in grafts with the highest leaf length (15.60 cm), followed by humid chamber (M_2) (15.40 cm). The least leaf length was recorded under natural shade (14.42 cm).

Table 22(a).Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)
	Initial (At the time of grafting)	Final	Girth increment		
M_1V_1	4.90	5.42	0.65	6.05	15.47
M_1V_2	4.99	5.62	0.81	6.33	16.10
M_1V_3	5.28	6.09	0.85	6.53	16.19
M_2V_1	4.86	5.66	0.73	6.04	15.15
M_2V_2	4.94	5.69	0.84	6.31	15.77
M_2V_3	5.13	5.98	0.94	6.42	15.26
M_3V_1	4.76	5.61	0.86	6.01	15.07
M_3V_2	4.80	5.68	0.96	6.16	15.31
M_3V_3	4.93	5.94	1.03	6.37	15.75
SE m(±)	0.03	0.02	N.S	0.02	0.04
CD (0.05)	0.09	0.08	N.S	0.05	0.11

4.4.14.3 Effect of varieties of scion

The data regarding leaf length of different scion varieties of mango is presented in table 19(d). Significant difference between varieties were observed with respect to leaf length. The highest leaf length (15.56 cm) was observed in Neelum, followed by Kotookonam Varikka (15.26 cm) whereas the smallest length (14.61 cm) was observed in Kalapady.

4.4.14.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(d)] with respect to leaf length 90 DAG. The largest leaf length (15.90 cm) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), followed by P_1M_2 (epicotyl grafting under humid chamber) (15.46 cm). The lowest leaf length (14.37 mm) was recorded in P_2M_3 (softwood grafting under natural shade).

Interaction effect of propagation methods and varieties of scion was significant [Table 21(d)] with respect to leaf length. The highest leaf length (15.62 cm) was observed in epicotyl grafts of kotookonam Varikka (P_1V_3) which was on par with P_1V_2 (15.57 cm). The lowest leaf length (14.28 cm) was noted in softwood grafts of Kalapady (P_2V_1).

The data presented in table 22(e) indicated that there was significant difference due to interaction between modified environments and varieties of scion on leaf length 90 DAG. The highest leaf length (16.02 cm) was recorded in M_1V_2 (fan and pad system + Neelum), which was on par with M_1V_3 (fan and pad system + Kotookonam Varikka) (15.96 cm). The lowest number of leaves per graft (13.98 cm) was recorded in M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was found significant [Table 23(e)] with respect to leaf length 90 DAG. The highest leaf length (16.03 cm) was recorded in the treatment combination $P_1M_1V_2$ (epicotyl grafting in Neelum under fan and pad system), which was on par with $P_2M_1V_2$ (16.00 cm), $P_1M_2V_2$ (15.97 cm), $P_1M_1V_3$

(15.95 cm) and $P_2M_1V_3$ (15.94 cm). The least (13.95 cm) leaf length was noted in $P_2M_3V_1$ (softwood grafting in Kalapady under natural shade).

4.4.15 Leaf width (cm)

The data on leaf width 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.15.1 Effect of propagation methods

The methods of grafting showed significant effect on leaf width 90 DAG [Table 17(e)]. The epicotyl grafted plants recorded higher leaf width (3.36 cm) than softwood grafted plants (2.98 cm).

4.4.15.2 Effect of modified environments

The data recorded on leaf width [Table 18(e)] revealed that there was significant effect due to environmental conditions. Among three environmental conditions, fan and pad system (M_1) resulted in the highest leaf width (3.65 cm), followed by humid chamber (M_2) (3.33 cm). The least leaf length was recorded under natural shade (2.54 cm).

4.4.15.3 Effect of varieties of scion

The data regarding leaf width of different scion varieties of mango is presented in table 19(d). Significant difference between scion varieties were observed with respect to leaf width. The highest leaf width (3.64 cm) was observed in Kotookonam Varikka, followed by Neelum (3.26 cm). The lowest leaf width (2.93 cm) was observed in Kalapady.

4.4.15.4 Effect of interactions

Interaction effect of propagation methods and modified environments was non-significant for leaf width at 90 DAG [Table 20(d)].

Table 22(b).Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Graft height (cm)	Length of sprout (cm)	Spread of plant (cm)	
			N-S direction	E-W direction
M_1V_1	26.04	5.88	19.98	17.89
M_1V_2	26.60	5.64	21.82	20.88
M_1V_3	26.86	5.97	23.64	22.35
M_2V_1	24.94	5.48	18.41	17.54
M_2V_2	25.05	5.60	20.54	18.91
M_2V_3	25.06	5.47	22.12	21.01
M_3V_1	22.82	4.97	17.56	16.57
M_3V_2	24.21	5.03	18.99	18.58
M_3V_3	24.28	5.16	21.11	19.88
SE m(±)	0.05	0.02	N.S	0.09
CD (0.05)	0.14	0.06	N.S	0.27

Interaction effect of propagation methods and varieties of scion was significant [Table 21(d)] with respect to leaf width 90 DAG. The highest leaf width (3.71 cm) was observed in softwood grafts of Kotookonam Varikka (P_2V_3), which was on par with P_1V_3 (3.59 cm) and P_1V_2 (3.36 cm). The lowest leaf width (2.50 cm) was noted in softwood grafts of Kalapady (P_2V_1).

The data presented in table 22(e) indicated that there was no significant difference due to interaction between modified environments and varieties of scion on leaf width 90 DAG.

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(e)] with respect to leaf width 90 DAG.

4.4.16 Leaf area (cm^2)

The mean data on leaf area 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.16.1 Effect of propagation methods

The methods of grafting showed significant effect on leaf area 90 DAG [Table 17(e)]. The epicotyl grafted plants recorded higher leaf area (41.69 cm^2) than softwood grafted plants (41.28 cm^2).

4.4.16.2 Effect of modified environments

The data on leaf area [Table 18(e)] revealed that there was significant effect due to modified environmental conditions. Among the three modified environments, fan and pad system (M_1) resulted in grafts with the highest leaf area (44.98 cm^2), followed by humid chamber (M_2) (44.53 cm^2). The least leaf area was recorded under natural shade (34.94 cm^2).

4.4.16.3 Effect of varieties of scion

The data regarding leaf area of different scion varieties of mango is presented in table 19(d). Significant difference between scion varieties were observed with respect to leaf area. The highest leaf area (43.33 cm^2) was observed

in Neelum, which was on par with Kotookonam Varikka (42.78 cm^2). The least leaf area (39.26 cm^2) was observed in Kalapady.

4.4.16.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(d)] for leaf area 90 DAG. The highest leaf area (45.19 cm^2) was recorded in P_1M_1 (epicotyl grafting under fan and pad system) which was on par with P_2M_1 (softwood grafting under fan and pad system) (45.12 cm^2) and P_2M_2 (softwood grafting under humid chamber) (44.84 cm^2). The least leaf area (34.84 cm^2) was recorded in P_2M_3 (softwood grafting + natural shade).

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(d)] with respect to leaf area of mango grafts 90 DAG.

The data presented in table 22(e) indicated that there was significant difference due to interaction between modified environments and varieties of scion on leaf area at 90 DAG. The highest leaf area (47.88 cm^2) was recorded in M_1V_2 (fan and pad system + variety Neelum) followed by M_2V_2 (46.19 cm^2). The least leaf area (33.41 cm^2) was recorded M_3V_1 (Natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(e)] with respect to leaf area 90 DAG.

4.4.17 Number of nodes on scion

The data regarding number of nodes on scion 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.17.1 Effect of propagation methods

The methods of grafting showed significant effect on number of nodes 90 DAG [Table 17(f)]. The epicotyl grafted plants recorded higher number of nodes (21.63) than softwood grafted plants (20.81).

Table 22(c). Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Days taken for first sprouting (days)	Days taken for last sprouting (days)	Number of grafts sprouted at weekly intervals (%)		
			First week	Second week	Third week
M_1V_1	9.77	24.27	27.22	55.00	73.89
M_1V_2	14.60	21.73	36.11	65.56	82.78
M_1V_3	9.90	20.20	45.55	76.67	87.78
M_2V_1	14.63	24.50	23.89	51.11	71.67
M_2V_2	11.80	21.77	33.89	63.89	79.44
M_2V_3	11.50	19.83	44.43	75.56	84.48
M_3V_1	15.00	24.90	24.44	49.98	72.22
M_3V_2	13.07	23.03	34.45	63.89	79.44
M_3V_3	10.38	20.77	42.78	74.44	86.67
SE m(±)	0.18	N.S	N.S	N.S	N.S
CD (0.05)	0.52	N.S	N.S	N.S	N.S

4.4.17.2 Effect of modified environments

The data recorded on number of nodes as influenced by various environment conditions 90 DAG was significant [Table 18(e)]. The highest number of nodes were observed in grafts maintained under fan and pad system (24.14), which was on par with humid chamber (23.92). The least number of nodes on scion (18.32) was noted under natural shade.

4.4.17.3 Effect of varieties of scion

The perusal of data presented in table 19(e) showed that there was significant effect of varieties on number of nodes. Kotookonam Varikka recorded the highest number of nodes (22.69) which was on par with Neelum (21.31). The lowest number of nodes on scion (19.64) was recorded in Kalapady.

4.4.17.4 Effect of interactions

Interaction effect of propagation methods and modified environments was non-significant for number of nodes on scion 90 DAG [Table 20(e)].

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(e)] with respect to number of nodes on scion 90 DAG.

The data presented in table 22(f) indicated that there was significant difference due to interaction between modified environments and varieties of scion on number of nodes on scion 90 DAG. The highest number of nodes (25.08) were observed in M_1V_3 (fan and pad system + Kotookonam Varikka), which was on par with M_2V_3 (24.80). The least number of nodes (16.43) was recorded in M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(f)] with respect to number of nodes on scion 90 DAG.

4.4.18 Internodal length (cm)

The data on internodal length 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.18.1 Effect of propagation methods

The methods of propagation showed significant effect on internodal length 90 DAG [Table 17(f)]. The significantly higher internodal length (5.20 cm) was noted in epicotyl grafted plants than the softwood grafted plants (5.14 cm).

4.4.18.2 Effect of modified environments

The data presented in table 18(f) showed that there was significant effect of environmental conditions on internodal length 90 DAG. The highestinternodal length (5.48 cm) was observed under fan and pad system which was on par with humid chamber (5.46 cm), whereas the lowest internodal length (4.77 cm) was recorded under natural shade (75 % shade).

4.4.18.3 Effect of varieties of scion

The varieties of scion showed significant effect on internodal length 90 DAG [Table 19(e)]. The highest internodal length (5.38 cm) was noted in Kotookonam Varikka, followed by Neelum (5.23 cm), while the lowest internodal length (4.91 cm) was recorded in Kalapady.

4.4.18.4 Effect of interactions

The interaction effect between propagation methods and modified environments was significant [Table 20(e)] with respect to internodal length 90 DAG. The highest internodal length (5.58 cm) was recorded in P_2M_1 (softwood grafting under fan and pad system) followed by P_1M_1 (5.38 cm) and P_1M_2 (5.36 cm). The lowest internodal length (4.70 cm) was recorded in P_2M_3 (softwood grafting under natural shade).

Interaction effect of propagation methods and varieties of scion was significant [Table 21(e)] with respect to internodal length 90 DAG. The highest

internodal length (5.46 cm) was observed in epicotyl grafts of Kotookonam Varikka (P_1V_3), which was on par with P_2V_3 (5.42 cm). The lowest internodal length (4.86 cm) was in softwood grafts of Kalapady (P_2V_1).

The data presented in table 22(f) indicated that there was significant difference due to interaction between modified environments and varieties of scion on internodal length 90 DAG. The highest internodal length (5.70 cm) was recorded in M_1V_3 (fan and pad system+ Kotookonam Varikka) which was on par with M_2V_2 (5.66 cm). The least internodal length (4.38 cm) was recorded in M_3V_1 (natural shade + Kalapady).

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(f)] with respect to internodal length 90 DAG. The highest internodal length (5.80 cm) was recorded in $P_2M_1V_3$ (softwood grafts of Kotookonam Varikka under fan and pad system), whereas the lowest internodal length (4.05 cm) was noted in the treatment combination $P_2M_3V_1$ (softwood grafts of Kalapady under natural shade).

4.4.19 Root length (cm)

The data regardingroot length 180 DAG as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.19.1 Effect of propagation methods

The data on root length 180 DAG as influenced by different propagation methods was non-significant [Table 17(f)].

4.4.19.2 Effect of modified environments

The data presented in table 18(f) showed that there was no significant effect of environmental conditions on root length 180 DAG.

Table 22(d). Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Initial success percentage (%)	Percentage of graft establishment (%)	Days taken for leaf opening (days)	Number of leaves per graft
M_1V_1	72.78	61.67	13.83	14.67
M_1V_2	82.78	73.33	16.43	16.87
M_1V_3	88.33	80.00	14.97	15.50
M_2V_1	72.89	60.00	13.93	14.03
M_2V_2	79.44	71.11	16.93	15.73
M_2V_3	86.11	76.67	15.00	14.97
M_3V_1	71.67	51.11	14.28	12.67
M_3V_2	80.00	66.67	17.07	15.62
M_3V_3	83.89	68.91	15.73	13.70
SE m(±)	N.S	1.06	0.12	0.17
CD (0.05)	N.S	3.05	0.33	0.49

Table 22(e). Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
M_1V_1	15.01	3.38	42.20
M_1V_2	16.02	2.58	47.88
M_1V_3	15.96	3.99	44.85
M_2V_1	14.83	2.40	42.16
M_2V_2	15.80	2.83	46.19
M_2V_3	15.40	3.85	45.27
M_3V_1	13.98	2.85	33.41
M_3V_2	14.85	2.91	36.19
M_3V_3	14.43	3.14	35.21
SE m(±)	0.03	N.S	0.26
CD (0.05)	0.08	N.S	0.74

4.4.19.3 Effect of varieties of scion

The perusal of data presented in [Table 19(e)] showed that there was no significant effect of varieties on root length.

4.4.19.4 Effect of interactions

Interaction effect of time of propagation methods and modified environments was non-significant for root length 180 DAG [Table 20(e)].

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(e)] with respect to root length 180 DAG.

The data presented in table 22(f) indicated that there was no significant difference due to interaction between modified environments and varieties of scion on root length 180 DAG.

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(f)] with respect to root length 180 DAG.

Table 22(f). Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
M_1V_1	20.00	5.14	17.51	1.50
M_1V_2	23.37	5.27	17.08	1.93
M_1V_3	25.08	5.70	18.94	2.30
M_2V_1	22.57	5.23	22.40	1.30
M_2V_2	24.07	5.66	21.49	1.80
M_2V_3	24.68	5.51	20.83	2.03
M_3V_1	16.43	4.38	24.28	1.23
M_3V_2	18.50	4.89	23.74	1.43
M_3V_3	20.03	5.02	23.18	1.90
SE m(±)	0.15	0.02	N.S	N.S
CD (0.05)	0.43	0.06	N.S	N.S

4.4.20 Number of growth flushes per graft

The mean data on number of growth flushes per graft 90 DAG as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.20.1 Effect of propagation methods

The methods of grafting showed significant effect on number of growth flushes per graft at 90 DAG [Table 17(f)]. The significantly higher number of growth flushes per graft (1.76) was recorded in epicotyl grafted plants (P_1) than softwood grafted plants (P_2) (1.65).

4.4.20.2 Effect of modified environments

The data recorded on number of growth flushes per graft 90 DAG revealed that there was significant effect due to modified environmental conditions [Table 18(f)]. Among three different modified environmental conditions, fan and pad system resulted in production of more number of growth flushes per graft (1.91), followed by humid chamber (1.71). The least number of growth flushes per graft (1.52) was noted under natural shade (75 % shade).

4.4.20.3 Effect of varieties of scion

The close perusal of data [Table 19(e)] indicated that, there was significant differences in number of growth flushes per graft as influenced by different varieties of scion 90 DAG. The highest number of growth flushes per graft (2.08) was observed in Kotookonam Varikka, followed by Neelum (1.74). The least number of growth flushes (1.34) was in Kalapady.

Table 22(g).Interaction effect of different modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
M_1V_1	24.27	41.77	52.22
M_1V_2	31.50	49.10	61.11
M_1V_3	27.43	41.87	69.45
M_2V_1	23.47	42.00	50.56
M_2V_2	30.33	49.57	59.44
M_2V_3	25.97	42.33	67.33
M_3V_1	26.63	45.23	50.56
M_3V_2	30.03	50.80	62.78
M_3V_3	23.47	43.36	65.00
SE m(±)	N.S	0.16	N.S
CD (0.05)	N.S	0.46	N.S

4.4.20.4 Effect of interactions

The interaction effect of propagation methods and modified environments were significant [Table 20(e)] with respect to number of growth flushes per graft. Highest number of growth flushes per graft (2.02) was recorded in P_1M_1 (epicotyl grafting + fan and pad system), which was on par with P_2M_1 (1.89), while the least number of growth flushes per graft (1.47) was recorded in P_2M_3 (softwood grafting + natural shade).

Interaction effect of propagation methods and varieties of scion was non-significant [Table 21(e)] with respect to number of growth flushes per graft.

The data presented in table 22(f) indicated that there was non-significant difference due to interaction between modified environments and varieties of scion on number of growth flushes per graft.

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(f)] with respect to number of growth flushes per graft 90 DAG. The highest number of growth flushes per graft (2.33) was recorded in $P_1M_1V_3$ (epicotyl grafting + fan and pad system + Kotookonam Varikka), which was on par with $P_1M_2V_3$ (2.27) and $P_2M_1V_2$ (2.11). The lowest number of growth flushes per graft (1.20) was noted in $P_2M_3V_1$ (epicotyl grafting + natural shade + Kalapady).

Table 23(a). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Girth of rootstock (mm)			Girth of scion (mm)	Length of scion (cm)
	Initial (At the time of grafting)	Final	Girth increment		
$P_1M_1V_1$	3.30	4.15	0.84	4.96	15.68
$P_1M_1V_2$	3.55	4.36	0.91	5.21	16.36
$P_1M_1V_3$	3.64	4.63	0.99	5.27	16.80
$P_1M_2V_1$	3.39	4.17	0.66	4.91	15.25
$P_1M_2V_2$	3.48	4.38	0.83	5.15	15.47
$P_1M_2V_3$	3.51	4.46	0.93	5.13	15.59
$P_1M_3V_1$	3.23	3.79	0.55	4.98	15.03
$P_1M_3V_2$	3.33	4.10	0.77	4.92	15.25
$P_1M_3V_3$	3.39	4.21	0.82	5.15	15.28
$P_2M_1V_1$	6.42	7.24	0.88	7.15	15.48
$P_2M_1V_2$	6.60	7.52	0.97	7.79	15.55
$P_2M_1V_3$	6.92	7.94	1.07	7.45	16.19
$P_2M_2V_1$	6.34	7.15	0.81	7.12	15.16
$P_2M_2V_2$	6.48	7.21	0.86	7.69	15.34
$P_2M_2V_3$	6.74	7.73	0.99	7.49	15.96
$P_2M_3V_1$	6.28	7.06	0.76	7.10	14.89
$P_2M_3V_2$	6.32	7.15	0.86	7.59	15.13
$P_2M_3V_3$	6.34	7.36	0.88	7.35	15.36
SE m(±)	0.04	0.04	N.S	N.S	0.05
CD (0.05)	0.12	0.12	N.S	N.S	0.14

Table 23(b). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Graft height (cm)	Length of sprout (cm)	Spread of plant (cm)	
			N-S direction	E-W direction
$P_1M_1V_1$	24.21	5.67	19.48	17.31
$P_1M_1V_2$	24.57	5.98	21.17	20.72
$P_1M_1V_3$	24.96	6.05	23.11	22.31
$P_1M_2V_1$	23.53	5.68	18.25	17.65
$P_1M_2V_2$	23.77	5.60	21.86	21.07
$P_1M_2V_3$	24.07	5.88	20.14	18.87
$P_1M_3V_1$	22.17	5.03	17.26	16.27
$P_1M_3V_2$	22.46	5.00	18.80	17.85
$P_1M_3V_3$	22.93	5.39	20.73	19.37
$P_2M_1V_1$	26.99	5.39	20.47	19.85
$P_2M_1V_2$	27.50	5.35	22.46	21.03
$P_2M_1V_3$	28.76	5.52	24.17	22.39
$P_2M_2V_1$	25.05	5.80	18.57	17.43
$P_2M_2V_2$	25.81	5.43	20.94	18.95
$P_2M_2V_3$	26.34	5.59	22.38	20.95
$P_2M_3V_1$	23.47	4.90	17.85	16.88
$P_2M_3V_2$	24.82	4.94	19.18	17.94
$P_2M_3V_3$	25.50	5.03	21.49	20.39
SE m(±)	0.07	0.03	N.S	N.S
CD (0.05)	0.19	0.08	N.S	N.S

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Table 23(c). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Days taken for first sprouting (days)	Days taken for last sprouting (days)	Number of grafts sprouted at weekly intervals (%)		
			First week	Second week	Third week
$P_1M_1V_1$	9.53	19.07	24.44	51.11	74.44
$P_1M_1V_2$	15.12	24.00	37.78	68.89	83.33
$P_1M_1V_3$	9.80	19.27	47.78	82.25	92.22
$P_1M_2V_1$	10.20	20.73	28.89	55.56	77.78
$P_1M_2V_2$	15.00	24.80	40.00	71.11	90.00
$P_1M_2V_3$	10.00	22.27	51.11	84.45	94.44
$P_1M_3V_1$	10.29	21.60	21.11	47.78	72.22
$P_1M_3V_2$	14.67	24.53	34.44	65.56	81.11
$P_1M_3V_3$	13.00	20.60	46.67	77.78	85.56
$P_2M_1V_1$	12.27	21.93	26.67	62.22	71.11
$P_2M_1V_2$	14.27	24.47	33.33	54.45	77.78
$P_2M_1V_3$	11.33	20.33	42.22	73.33	83.33
$P_2M_2V_1$	10.36	22.73	25.56	54.44	70.00
$P_2M_2V_2$	15.36	24.53	32.22	60.00	75.56
$P_2M_2V_3$	13.13	21.13	40.00	68.89	81.11
$P_2M_3V_1$	10.42	23.80	24.44	48.89	69.99
$P_2M_3V_2$	15.44	25.00	31.11	58.89	75.55
$P_2M_3V_3$	12.80	21.20	37.78	66.67	81.11
SE m(±)	0.26	0.31	N.S	N.S	N.S
CD (0.05)	0.73	0.88	N.S	N.S	N.S

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Table 23(d). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Initial success percentage (%)	Percentage of graft establishment (%)	Days taken for leaf opening (days)	Number of leaves per graft
$P_1M_1V_1$	76.67	64.44	14.00	14.87
$P_1M_1V_2$	94.44	76.67	17.33	16.73
$P_1M_1V_3$	90.00	83.33	15.33	16.00
$P_1M_2V_1$	74.44	62.22	14.27	13.33
$P_1M_2V_2$	83.33	74.44	17.00	16.63
$P_1M_2V_3$	91.11	80.00	15.73	14.33
$P_1M_3V_1$	72.22	64.44	15.55	14.60
$P_1M_3V_2$	81.11	72.22	16.93	16.47
$P_1M_3V_3$	85.56	72.22	14.33	15.60
$P_2M_1V_1$	68.89	60.00	13.53	14.47
$P_2M_1V_2$	75.56	65.56	15.93	17.00
$P_2M_1V_3$	82.24	70.00	14.40	15.00
$P_2M_2V_1$	71.11	61.11	13.67	13.47
$P_2M_2V_2$	78.89	72.22	16.80	15.00
$P_2M_2V_3$	82.22	80.00	14.67	14.33
$P_2M_3V_1$	71.11	57.78	14.00	12.00
$P_2M_3V_2$	75.56	61.11	16.87	14.60
$P_2M_3V_3$	81.11	65.57	15.73	13.07
SE m(±)	N.S	N.S	N.S	N.S
CD (0.05)	N.S	N.S	N.S	N.S

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Epicotyl graft

Softwood graft

Plate 18. Graft union of epicotyl and softwood graft of Kotookonam Varikkaat 180 DAG

4.4.21 Number of days taken between grafting and first vegetative flush

The data regardingthenumber of days taken between grafting and first vegetative flush as influenced by different propagation methods and modified environments on different varieties of scion is presented below.

4.4.21.1 Effect of propagation methods

The methods of grafting showed significant effect on thenumber of days taken between grafting and first vegetative flush [Table 17(g)]. The earliness in first vegetative flushing (26.06 days) was noted in epicotyl grafted plants, while the highest numbers of days (27.95 days) were taken in softwood grafted plants for first vegetative flushing.

4.4.21.2 Effect of modified environments

The data presented in table 18(g) revealed that modified environment conditions had significant effect on thenumber of days taken between grafting and first vegetative flush. The significantly least number of days for first vegetative flushing (26.58 days) was recorded under fan and pad system, which was on par with humid chamber (26.71 days). The highest number of days for first vegetative flushing (27.73 days) was observed under natural shade (75 % shade).

4.4.21.3 Effect of varieties of scion

The data regarding the number of days taken between grafting and first vegetative flush of mango grafts as influenced by different varieties of scion is presented in table 19(f). The variety Kalapady recorded the least number of days for first vegetative flushing (23.73 days), followed by Kotookonam Varikka (26.68 days). The variety Neelum recorded the highest number of days for first vegetative flushing (30.62 days).

Table 23(e). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
$P_1M_1V_1$	15.71	3.47	42.36
$P_1M_1V_2$	16.03	3.71	44.96
$P_1M_1V_3$	15.95	4.02	48.04
$P_1M_2V_1$	15.10	3.32	41.53
$P_1M_2V_2$	15.97	3.65	45.76
$P_1M_2V_3$	15.38	3.95	44.38
$P_1M_3V_1$	14.93	2.62	33.09
$P_1M_3V_2$	14.51	2.72	35.07
$P_1M_3V_3$	13.97	2.76	36.35
$P_2M_1V_1$	13.99	3.28	42.04
$P_2M_1V_2$	16.00	3.44	44.75
$P_2M_1V_3$	15.94	3.97	47.73
$P_2M_2V_1$	15.70	2.00	42.78
$P_2M_2V_2$	15.41	2.96	46.17
$P_2M_2V_3$	14.91	4.10	46.63
$P_2M_3V_1$	13.95	2.03	33.73
$P_2M_3V_2$	14.36	2.07	35.35
$P_2M_3V_3$	14.77	3.06	36.04
SE m(±)	0.04	N.S	N.S
CD (0.05)	0.12	N.S	N.S

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Table 23(f). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of nodes on scion	Internodal length (cm)	Root length (cm)	Number of growth flushes per graft
$P_1M_1V_1$	20.67	5.09	18.54	1.73
$P_1M_1V_2$	22.00	5.45	18.21	2.00
$P_1M_1V_3$	23.07	5.61	18.89	2.33
$P_1M_2V_1$	23.07	5.11	17.62	1.29
$P_1M_2V_2$	24.33	5.20	17.07	1.87
$P_1M_2V_3$	25.70	5.11	17.31	2.27
$P_1M_3V_1$	16.73	4.70	16.32	1.27
$P_1M_3V_2$	18.87	4.82	15.77	1.87
$P_1M_3V_3$	20.20	4.98	16.02	1.60
$P_2M_1V_1$	21.97	5.30	28.16	1.33
$P_2M_1V_2$	23.80	5.58	28.94	2.11
$P_2M_1V_3$	24.47	5.80	29.67	1.93
$P_2M_2V_1$	19.33	5.17	25.68	1.27
$P_2M_2V_2$	20.73	5.35	24.59	1.67
$P_2M_2V_3$	22.87	5.49	27.19	1.93
$P_2M_3V_1$	16.13	4.05	20.85	1.20
$P_2M_3V_2$	18.13	4.96	20.21	1.93
$P_2M_3V_3$	19.87	5.08	21.27	1.73
SE m(±)	N.S	0.03	N.S	0.08
CD (0.05)	N.S	0.09	N.S	0.24

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4.4.21.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(f)] for number of days taken between grafting and first vegetative flush. The earliness in first vegetative flushing (25.44 days) was recorded in P_1M_1 (epicotyl grafting under fan and pad system) followed by P_1M_2 (26.02 days). The largest number of days for first vegetative flushing (28.73 days) were recorded in P_2M_3 (softwood grafting under natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was significant [Table 21(f)] with respect to the number of days taken between grafting and first vegetative flush. The least number of days for first vegetative flushing (23.04 days) was observed in epicotyl grafts of Kotookonam Varikka (P_1V_3), followed by P_2V_3 (24.42 days). The largest number of days for first vegetative flushing (31.89 days) was noted in softwood grafts of Neelum (P_2V_2).

The data presented in table 22(g) indicated that there was no significant difference due to interaction between modified environments and varieties of scion on number of days taken between grafting and first vegetative flush.

Interaction effect of different propagation methods, modified environments and varieties of scion was non-significant [Table 23(g)] with respect to number of days taken between grafting and first vegetative flush.

4.4.22 Number of days taken between grafting and second vegetative flush

The data on number of days taken between grafting and second vegetative flush as influenced by different propagation methods and modified environments on different varieties of scion is described below.

4.4.22.1 Effect of propagation methods

The methods of grafting showed significant effect on thenumber of days taken between grafting and second vegetative flush [Table 17(g)] (Table 35). Significantly lower number of days for second vegetative flush (44.98 days) was recorded in epicotyl grafted plants compared to softwood grafted plants (45.80 days).

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4.4.22.2 Effect of modified environments

The data presented in table 18(g) revealed that modified environments had significant effect on the number of days taken between grafting and second vegetative flush. The earliness in second vegetative flushing (44.96days) was recorded under fan and pad system, which was on par with humid chamber (45.11 days). Whereas the largest number of days for second vegetative flushing (46.12 days) was recorded under natural shade.

4.4.22.3 Effect of varieties of scion

The data regarding thenumber of days taken between grafting and second vegetative flush as influenced by varieties of scion were presented in table 19(f). The variety Kotookonam Varikka recorded the least number of days for second vegetative flushing (41.98 days) followed by Kalapady (44.28 days). Neelum recorded the largest number of days for second vegetative flushing (49.92 days).

4.4.22.4 Effect of interactions

The interaction effect of propagation methods and modified environments was significant [Table 20(f)] for number of days taken between grafting and second vegetative flush. The least number of days for second vegetative flushing (43.84 days) was recorded in P_1M_1 (epicotyl grafting under fan and pad system), followed by P_2M_1 (softwood grafting under fan and pad system) (45.02 days). More number of days for second vegetative flushing (46.31 days) were recorded in P_2M_3 (softwood grafting + natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was significant [Table 21(f)] with respect to the number of days taken between grafting and second vegetative flush. The earliness in second vegetative flushing (41.42 days) was observed in epicotyl grafts of Kotookonam Varikka (P_1V_3) followed by P_2V_3 (43.42 days). The largest number of days for second vegetative flushing (50.11days) was noted in softwood grafts of Neelum (P_2V_2).

The data presented in table 22(g) indicated that there was significant difference due to interaction between modified environments and varieties of

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scion on number of days taken between grafting and second vegetative flush. The least number of days for second vegetative flushing (41.77 days) were noted in M_1V_1 (Fan and pad system+ Kalapady) which was on par with M_1V_3 (41.87 days) and M_2V_1 (42.00 days). The largest number of days for second vegetative flushing (50.80 days) were recorded in M_3V_2 (Natural shade + Neelum).

Interaction effect of different propagation methods, modified environments and varieties of scion was significant [Table 23(g)] with respect to number of days taken between grafting and second vegetative flush. The least number of days for second vegetative flushing (40.87 days) was recorded in $P_1M_1V_3$ (epicotyl grafts of KotookonamVarikka under fan and pad system), which was on par with $P_1M_2V_3$ (41.50 days). Whereas the largest number of days for second vegetative flushing (50.47 days) was recorded in $P_2M_3V_1$ (epicotyl grafts of Kalapady under natural shade).

Table No. 23(g). Interaction effect of different propagation methods, modified environments and varieties of scion on vegetative and growth characters of mango grafts

Treatments	Number of days taken between grafting and first vegetative flush (days)	Number of days taken between grafting and second vegetative flush (days)	Survival percentage of grafts (%)
$P_1M_1V_1$	24.73	44.20	60.00
$P_1M_1V_2$	28.80	49.00	68.89
$P_1M_1V_3$	22.80	40.87	77.78
$P_1M_2V_1$	22.93	50.20	52.22
$P_1M_2V_2$	29.87	45.13	64.44
$P_1M_2V_3$	25.27	41.50	71.11
$P_1M_3V_1$	23.40	51.13	54.43
$P_1M_3V_2$	30.60	44.87	66.67
$P_1M_3V_3$	26.20	42.93	71.11
$P_2M_1V_1$	24.13	50.13	44.44
$P_2M_1V_2$	31.27	43.80	53.33
$P_2M_1V_3$	28.53	41.67	61.11
$P_2M_2V_1$	24.00	48.60	48.89
$P_2M_2V_2$	30.80	42.07	54.44
$P_2M_2V_3$	26.67	41.87	63.55
$P_2M_3V_1$	25.13	50.47	46.67
$P_2M_3V_2$	32.40	45.60	58.90
$P_2M_3V_3$	28.67	41.73	58.89
SE m(±)	N.S	0.23	N.S
CD (0.05)	N.S	0.65	N.S

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4.4.23 Survival percentage of grafts

The data regardingsurvival percentage of grafts 180 DAG as influenced by different propagation methods and modified environments on different varieties of scion is furnished below.

4.4.23.1 Effect of propagation methods

The methods of propagation showed significant effect on survival percentage of grafts 180 DAG [Table 17(g)]. The percentage of survival was higher (65.19 %) in epicotyl grafting compared to softwood grafting (54.47 %).

4.4.23.2 Effect of modified environments

The data presented in table 18(g) showed that there was significant effect of environmental conditions on survival percentage of grafts 180 DAG. The highest percentage of survival percentage of grafts (67.26 %) was observed under fan and pad system, followed by humid chamber (61.11 %). Whereas the least survival percentage of grafts (51.11 %) was noticed under natural shade (75 % shade).

4.4.23.3 Effect of varieties of scion

The varieties of scion did not have any significant effect on survival percentage of grafts 180 DAG [Table 19(f)].

4.4.23.4 Effect of interactions

The interaction effect between propagation methods and modified environments was significant [Table 20(f)] with respect to survival percentage 180 DAG. The highest survival percentage (68.89 %) was recorded in P_1M_1 (epicotyl grafting under fan and pad system) followed by P_1M_2 (64.07 %). The lowest survival percentage of grafts (52.96 %) was recorded in the treatment combination P_2M_3 (softwood grafting + natural shade).

It is evident from the data that the interaction between propagation methods and varieties of scion was non-significant [Table 21(f)] with respect to survival percentage of mango grafts 180 DAG.

The data presented in table 22(g) indicated that there was no significant difference due to interaction between modified environments and varieties of scion on survival percentage of mango grafts 180 DAG.

Interaction effect of different propagation methods, modified environments and varieties of scion was also non-significant [Table 23(g)] with respect to survival percentage of grafts 180 DAG.

Discussion

5. DISCUSSION

The results obtained from the investigations on "Evaluation of propagation techniques and rootstock studies of mango (*Mangifera indica* L.)" are discussed and furnished below under various sub-headings.

5.1: Screening of local mango varieties/ collections for polyembryony

5.1.1 Germination characters of mango genotypes

The germination behaviour of twenty mango genotypes were analysed and the probable causes for variation in germination characters are discussed below.

Among the twenty varieties, the highest germination percentage was recorded in Kappa Manga, while the least germination percentage was noted in Kotookonam Varikka (Fig. 1). The germination capacity of mango genotype appears to be related to its stone size. Larger the seeds, more efficient will be germination than the smaller ones. The Kappa Manga had more stone weight than others. Hence the Kappa Manga recorded the highest percentage of germination. In Kotookonam Varikka which is polyembryonic in nature, all the cotyledons within a seed differed in weight and size. The results are in confirmity with Barbosa *et al.* (2009) and Kumar *et al.* (2018) in mango.

It is evident from Fig. 2 that, among seventeen polyembryonic varieties, the number of seedlings/stone ranged from 1.67 (Pandi Manga) to 5.00 (Kotookonam Varikka). The phenomenon of polyembryony was of genetic nature and the frequency varied according to varieties. The intensity of occurrence of multiple seedlings is directly proportional to the number of embryos (Aron *et al.*, 1998). From the present study, it is clearly evident that the different categories (such as one, two, three, four and five plantlets per stone) varied significantly between cultivars. Sturrock (1968) and Khobragade *et al.* (2000) are also reported the variations in different poyembryonic mango varieties. The probable reason of variations in sprouts of polyembryonic varieties is the failure of few embryos to germination due to the temporary aberrations of embryos, which might be mediated through various extraneous factors (Barbosa *et al*, 2009).

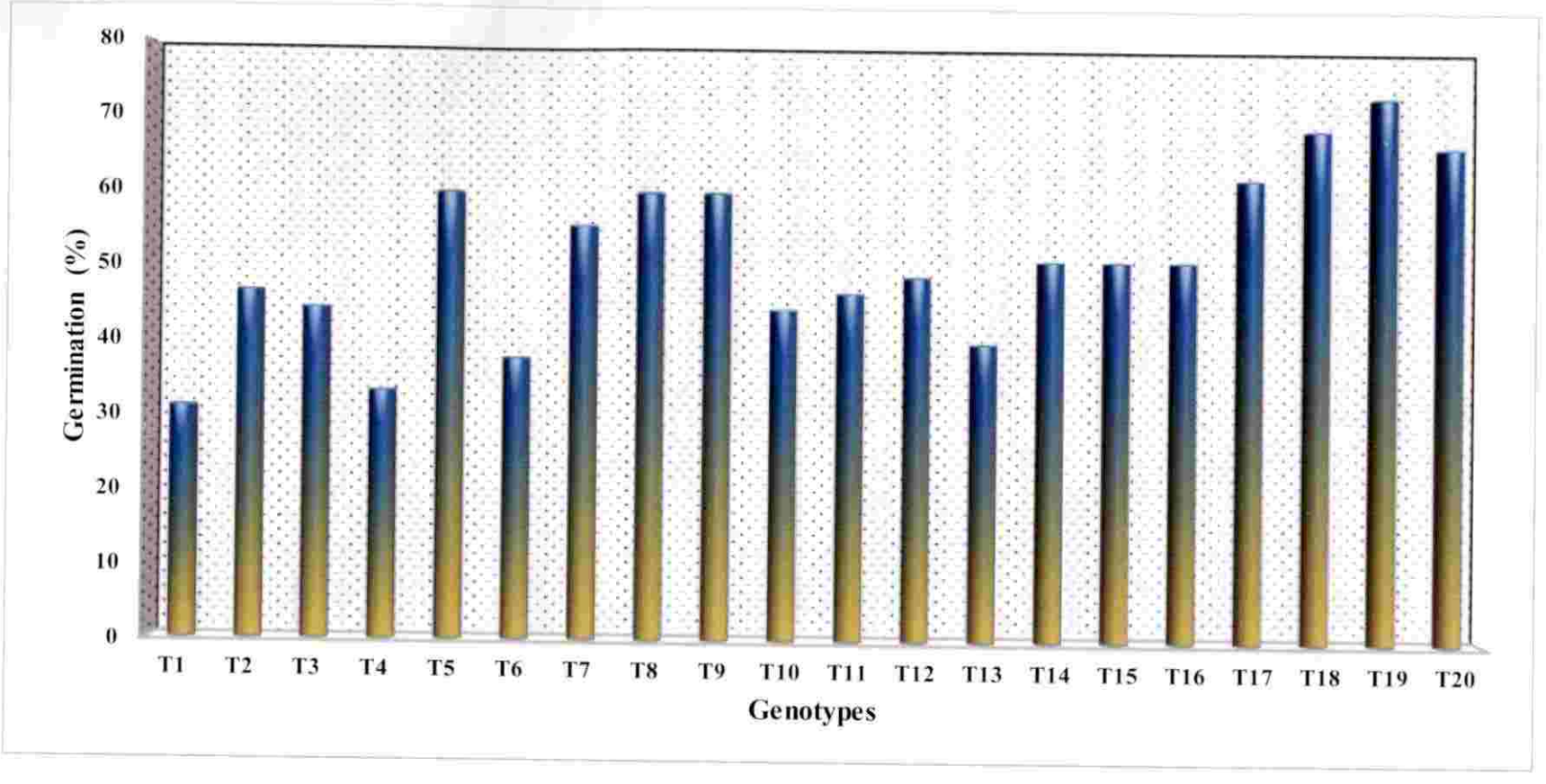

Fig. 1. Germination (%) of different mango genotypes

The extent of polyembryony was significant among the different mango varieties under study. The var. Kotookonam Varikka recorded the highest percentage of polyembryony whereas, the least was in Pandi Manga (Fig.3) The aberrant results obtained for percentage polyembryony in mango genotypes might be attributed to their genotypes and genotype-environment interactions (Kumar, 2015). The results are in confirmity with Rao and Reddy (2005b) and Sane *et al*, (2015) in mango.

The least mean germination time was noticed in Vellari Varikka, whereas the highest mean germination time was recorded in Kotookonam Varikka. The germination capacity of mango genotypes appears to be related to its stone size. Larger the seeds, more efficient will be germination and faster will be the radicle emergence than the smaller ones. This might be the probable cause of earliness in germination of var. Vellari Varikka. The results are in confirmity with the studies conducted by Kumar *et al.* (2018) in mango. The delay in seed germination of var. Kotookonam Varikka might be due to the presence of hard seed coat as well as the competition among the seedlings. Similar results were also reported by Khobragde *et al.* (2000) in mango.

The variety Kappa Manga recorded the highest germination index, whereas the lowest germination index was in Kotookonam Varikka. This significant variation among the genotypes with respect to germination index might be due to the differential germination per cent recorded by different genotypes (Abirami *et al.*, 2011a).

With respect to vigour index-I on growth basis, highest vigour index- I was recorded in Kappa Manga and the least was in Kochu Kilichundan. The greatest vigour index in Kappa manga might be due to the vigorous seedling growth as vigour index-I is the product of germination percentage and seedling length. Besides, being a monoembryonic variety, presence of more endosperm tissue as well as higher stone weight might be a probable cause for more seedling growth compared to other genotypes. The similar results were also reported by Rao and Reddy (2005b). The slow growth rate of seedlings in var. Kotookonam Varikka resulted in low seedling vigour index. In general, the probable reason for

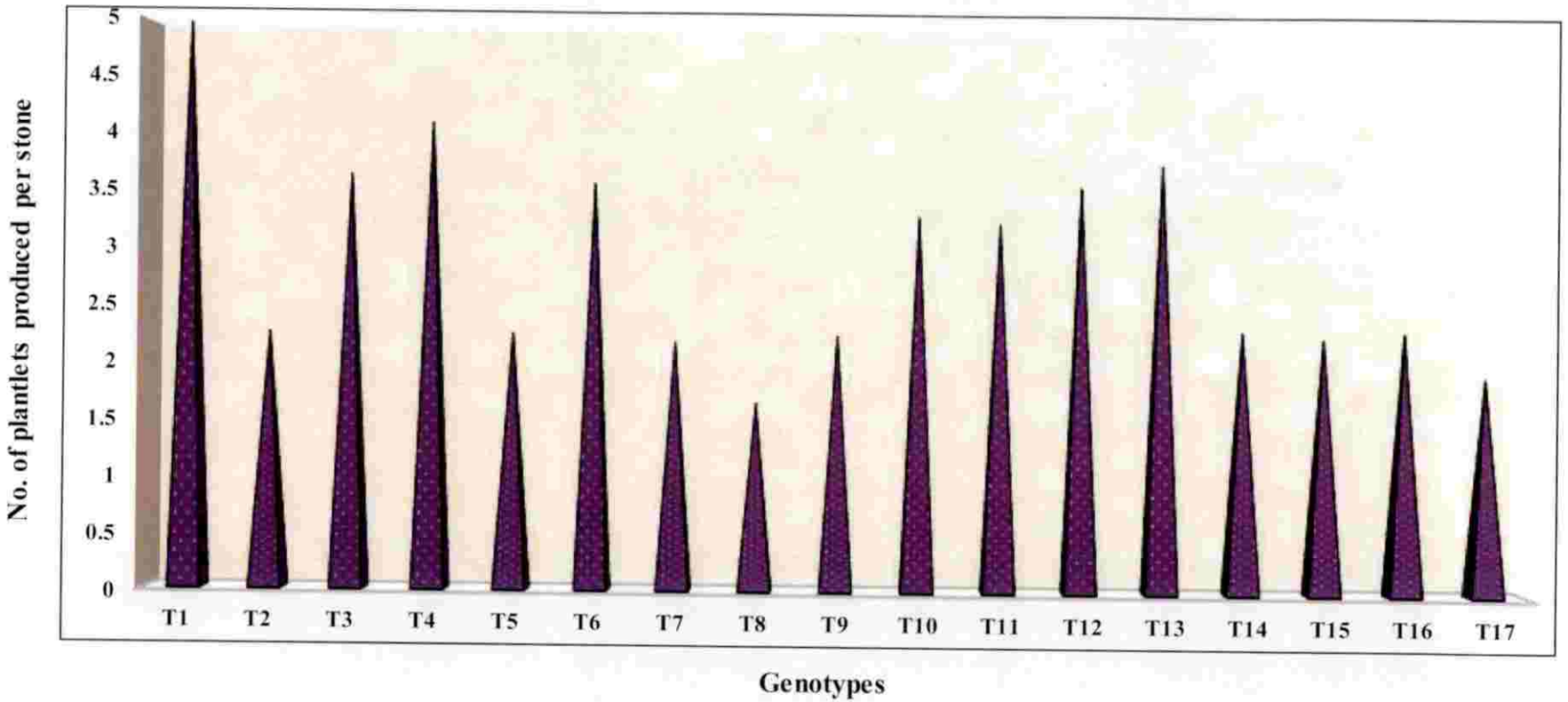

Fig. 2. Number of plantlets produced per stone of different mango genotypes

high and low growth potential of different genotypes might be due to their genetic constitution (Abirami *et al.*, 2011a).

5.1.2 Molecular characterization of zygotic and nucellar seedlings

In polyembryonic mango varieties, there is one zygotic embryo (sexual) and several nucellar embryos which have the entire genetic constitution similar to that of mother tree (Sauco *et al*, 2001). From the maternal nucellar tissue, the adventitious embryos are directly initiated, which surround the embryo sac containing a developing zygotic embryo (Aleza *et al.*, 2010). Hence, the identification of the zygotic embryo has great significance in mango. Furthermore, the nucellar embryos can be used to propagate disease free clonal rootstocks (Santos *et al.*, 2010). It is commonly believed that the most vigorous plantlets which arise from a polyembryonic seed are nucellar ones. Srivastava *et al.* (1988) ascribed that in polyembryonic mango seeds, the zygotic seedling might be the weakest and in lower proportion among the plantlet population on account of suppression of zygotic embryo with nucellar tissue or it perhaps degenerates due to the competition with nucellar plantlets.

There are many contradictory reports with respect to the identification of zygotic seedlings from polyembryonic mango genotypes. Cordeiro *et al.* (2006) revealed that the zygotic plantlet was most vigorous and later confirmed this with RAPD marker. In accordance with this result, Rocha *et al.* (2014) reported that the zygotic seedling need not be always weak. The zygotic plantlet will be vigorous in certain cases and grow healthy along with the vegetative (nucellar) plantlets. So that the visual identification of seedlings are not an efficient way and is inevitable to identify the zygotic plantlet at an early growth stage from the nucellar population.

Usually in classical progeny testing, comparison has been made between the mother plant and its offspring or evaluates the heterogeneity within progeny. Recently, various molecular markers have been adopted in many fruit crops for distinguishing the zygotic and nucellar seedlings (Rodriguez *et al.*, 2004; Rao *et al.*, 2008). Amongst various marker systems, the simple sequence repeats (SSRs) are very quick and more reliable to discriminate the zygotic and nucellar plantlets

from both selfing and interspecific crosses (Ruiz *et al.*, 2000). Hence in the present study, an attempt has been made to analyse the multiple seedlings by a more robust microsatellite marker.

The polyembryonic mango varieties 'Kotookonam Varikka' and 'Kochu Kilichindan' were selected for the analysis because of the occurrence of more different categories of seedlings ranging from one to five. The microsatellite analysis was done to compare the genomic DNA of mother tree with its offspring and SSR primers were evaluated for their ability to discriminate between zygotic and nucellar seedlings.

It is evident from the present study that all the seedlings obtained from the respective stones had SSR profile identical to the mother plant. The identical banding pattern between multiple seedlings and mother plant indicated the nucellar origin of seedlings having the similar genetic composition (Dhillon *et al.*, 1993). Generally, the offspring from polyembryonic varieties, especially the nucellar ones are expected to be true to type and genetically identical to the mother plant (Shareefa *et al.* 2009). Any deviation from the banding pattern of mother plant, either presence or absence of any band could assure the zygotic origin of plantlet.

Most polyembryonic mango varieties occasionally produce morphologically off-type plants that presumptively are zygotic in origin (Schnell and Knight, 1992). From the present study, it can be presumed that the zygotic seedling has ceased growth and degenerated at very early stage of growth. Hence the identical SSR profiles of seedlings and mother plants ensure the nucellar origin of the seedlings. The result of the present investigation confirms that all vigorous seedlings of the local polyembryonic mango varieties, Kotookonam Varikka and Kohcu Kilichundan can be used for clonal propagation to ensure homogeneity in orchards.

It could be presumed that among the seedlings the vigorous ones were of nucellar origin and the zygotic plantlet was weak and died off. The nucellar ones could produce more uniform rootstocks and they could be used to generate homogeneous grafted plants. The research works on genetic and morphological

evaluation to characterize the nucellar and zygotic seedlings in polyembryonic mango varieties are meagre. More extensive works including other polyembryonic mango varieties/collections should be carried out to confirm the present results. Hence more evaluations are needed under different climatic conditions and with different varieties in order to prove this supposition beyond doubt.

5.2: Effect of pre-sowing treatments, sowing position and age of stones after extraction from the fruit on germination of mango stones

5.2.1 Effect of sowing positions

The results obtained from the present investigation revealed that stalk end up position of sowing significantly gave most promising results with respect to all the parameters under study. The stones which were sown in stalk end up position took the least number of days for initiation of germination (Fig. 4) and 50 per cent germination, exhibited higher percentage of germination (Fig. 5), germination rate, seedling length, seedling dry weight, vigour index -I on growth basis (Fig. 6) and vigour index –II on weight basis (Fig. 7) compared to flat method of sowing.

Sowing of seeds at proper depth and position is one of the most important nursery operations as it affects both germination and subsequent growth of seedlings. To ensure good germination and rapid seedling emergence, seeds must be placed in a position and better environment that ensures the availability of nutrients and water from the soil (Garner and Chaudhri, 1976).

Germination commences with the uptake of water by dry seed (imbibition) and is completed when embryonic shoot and root, i.e., plumule and radicle, respectively, emerged. The amount of energy required to accomplish this task varies according to genotype and seed orientation on seed bed because of the quantity of stored nutrients, especially endosperm and positioning of micropyle, respectively (Hammed *et al.*, 2014). Naturally, the radicle has a positive geotropism whereas the shoot of the germinating seed has a negative geotropism. Supporting the upcoming response of the seedling to the stimulus (gravity) is highly correlated with the orientation of micropyle. The downward root curvature tipped towards the center of gravity is affected by various biochemical and

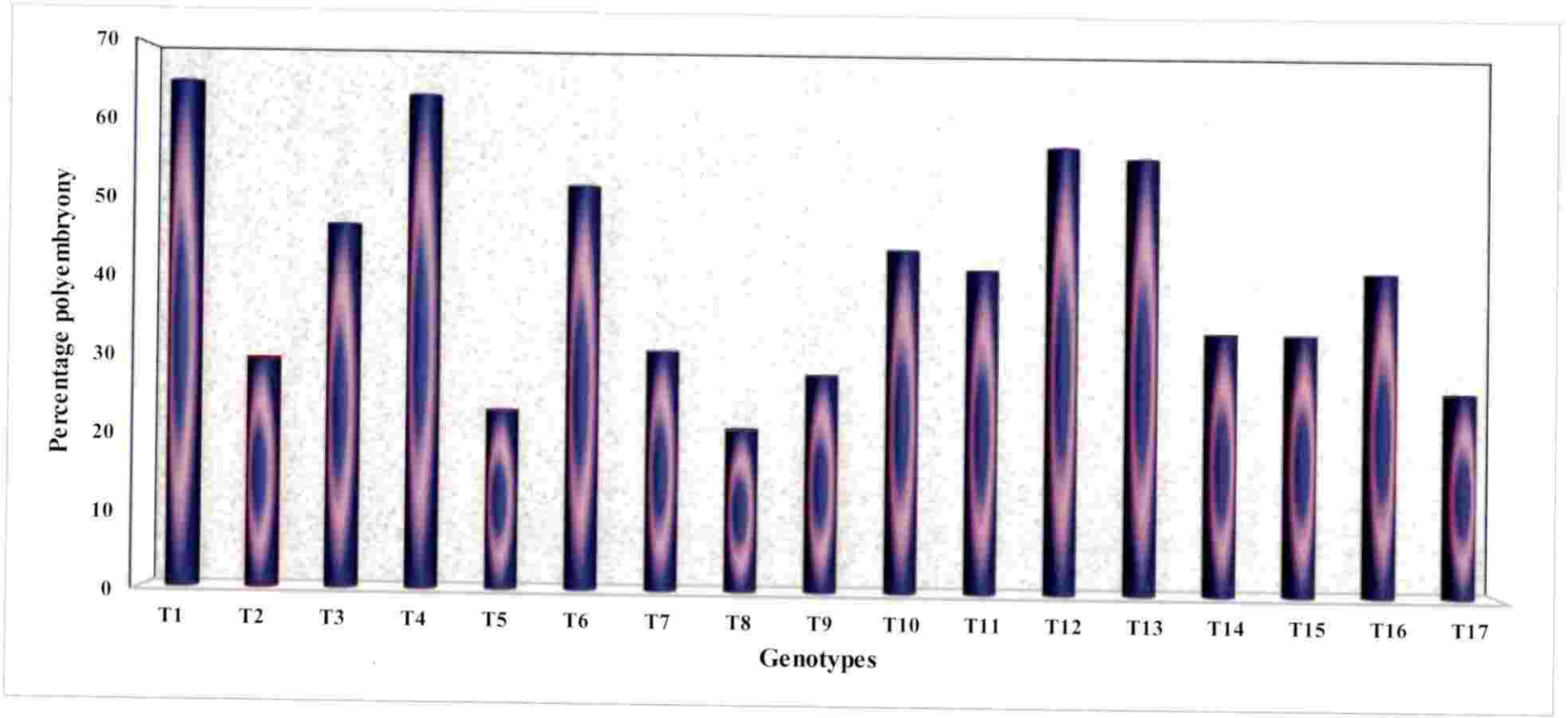

Fig. 3. Percentage polyembryony of different mango genotypes

environmental factors and is considered as a vital characteristic for plant survival (He and South, 2006). The tip of the root bends downward if the seeds oriented vertically upward with respect to micropyle. The roots of seed sown by micropyle in vertically upright position need to curve over the seed itself in order to grow in downward (normal) direction (Coutts, 1989).

Mango stones with stalk-end up position of sowing places the micropyle in the most suitable position, i.e., pointing downward, the roots of the seed grew easily and directly downward (does not require bending), which requires less energy for germination and radicle emerge from the embryo. Furthermore, stalk-end facing up (plumule up) position might heighten the accessibility to required oxygen for the initial metabolic process that produces energy for the emergence of radicle (Bewley, 1997). Hence the highest percentage of germination and earliness in germination is observed in stones sown by positioning the micropyle downward (Stalk end up). The stalk end up position of sowing enhance the production of more straight tap root system which facilitates less time and energy for germination, leading to the earliness in germination of mango stones than the flat position method of sowing. The results are in confirmity with Vijaya and Satyanarayana (2004).

In general, being positively geotropic and hydrotropic, the plant roots grow downward. Nutrient exploration from the soil pool and building up of the frame work for anchorage are also the advantages of properly growing roots. The micropyle positioning while sowing has direct implications on seedling quality as it determines the uniformity, speed as well as rate of germination. The seedlings whose roots grow properly without any curvature will establish well for its function and growth, which can ultimately improve the performance. The non-uniform germination pattern and curving roots at early growth stages have negative impact on seedling performance (Zewdie and Welka, 2015). Hence, stalk end up method (Micropyle pointing downward) resulted in the highest seedling length and dry weight which ultimately resulted in better seedling vigour indices, both on growth and weight basis than the flat method of sowing.

The improper orientation of seeds could impoverish the emerging embryo for needed quantum of oxygen which could lead to the synthesis of higher amount of ethanol and pyruvate in the plant system and finally leads to the death of the emerging embryo. This might be the probable reason for reduced germination and poor quantitative plant vigour in seeds sown in flat method (Bewley, 1997).

5.2.2 Effect of age of stones after extraction from fruit

There was a significant impact of age of stones after extraction from fruit on all the germination characteristics under study. The least number of days for initiation of germination (Fig. 4) and 50 per cent germination, the highest germination percentage (Fig. 5), highest rate of germination, seedling length, seedling dry weight, vigour index -I on growth basis (Fig. 6) and vigour index –II on weight basis (Fig. 7) were the best for the freshly harvested stones as compared to stones sown 10 days and 20 days after extraction from fruit.

The germination ability of a seed was directly related to its moisture content as well as the rate at which seeds lose its moisture thereby affecting the viability (Patil and Krishna, 2016). The reduction in viability and vigour were proportional to increased leaching of metabolites from seeds and decreased dehydrogenated activity of seeds. The leaching of metabolites increases with decreased seed moisture content during storage. Hence fresh stones required the least number of days for germination, the highest percentage of germination and germination rate. The freshly extracted seeds had considerable amount of post imbibition hydrolysis of non-reducing sugars and DNA-P (DNA polymerase) which resulting in initiation of protein synthesis, little or none could be observed as age advances (Chandra, 1980). The higher expression of DNA-P in imbibed seeds enhancing the protection against DNA damage and allows successful germination of seeds (Sihi *et al.*, 2015). These results are also in confirmity with Lima *et al.* (1985) in papaya, Warrier *et al.* (2009) in jack fruit and Chaudhari and Patel (2012) in mango.

It is evident from the data (Table 5) that seedling length, dry weight and seedling vigour indices (growth and weight basis) were higher in mango stones which were sown freshly after extraction from fruit. More seedling growth

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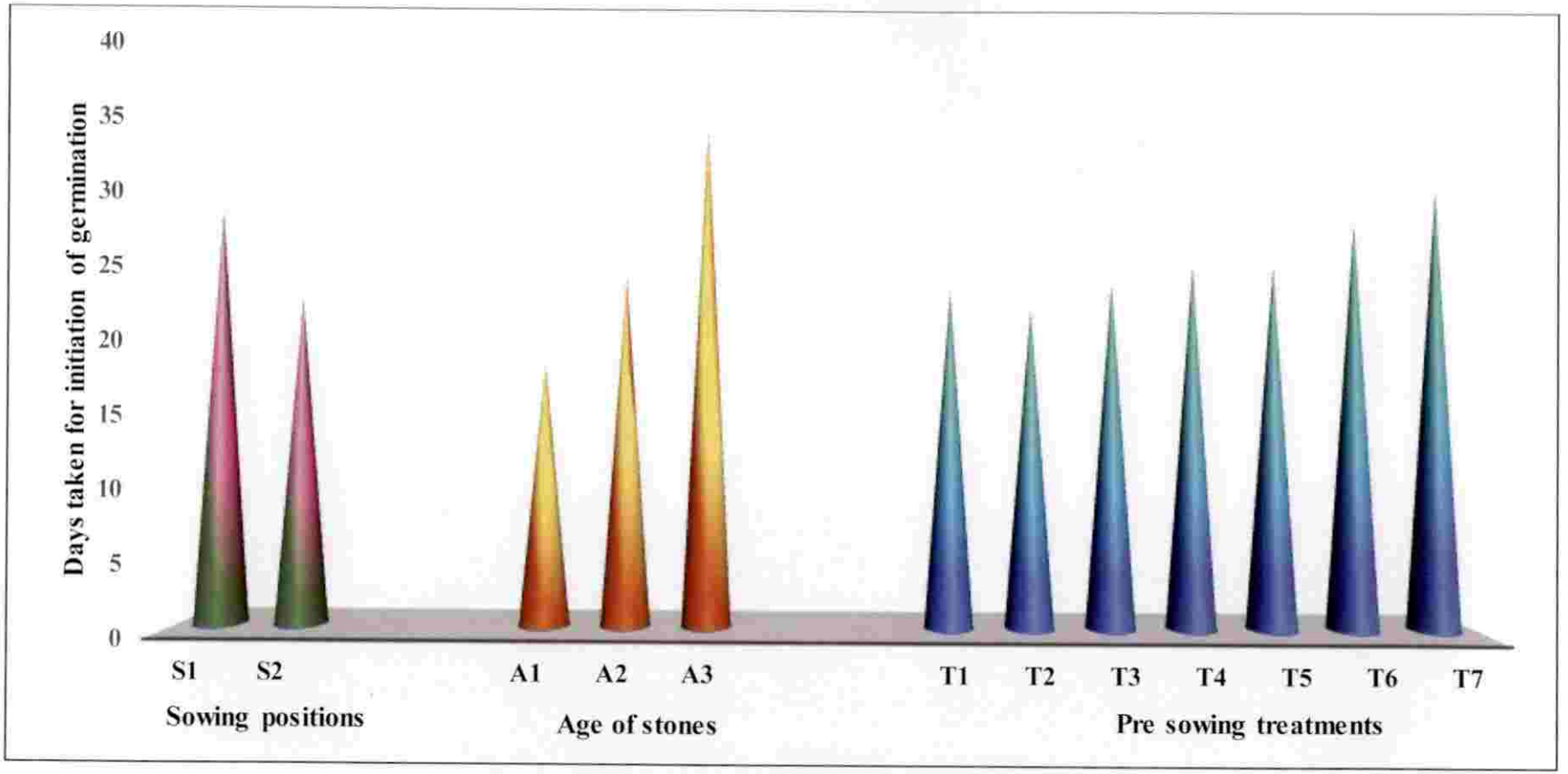

Fig. 4. Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on days taken for initiation of germination of mango stones

resulting from freshly harvested seeds is not only due to early germination but also highly conducive to hasten the physiological process which was needed for accelerating seedling growth. Germination became progressively slower as the age advanced. In case of stored seeds, the slow rate of physiological process, metabolic breakdown and increase in leaching of metabolites with decreased seed moisture content resulted in poor seedling growth (Ghosh and Sen, 1988). The results are in confirmity with Patil and Krishna (2016) in *Calamus spp.* and Kumar *et al.* (2018) in jamun.

5.2.3 Effect of pre sowing treatments

The results obtained from the current trial revealed that there was a significant impact of pre sowing treatments on all the germination parameters under study. The earliness in germination (Fig. 4) and the least number of days for 50 per cent germination was recorded in mango stones treated with 200 ppm GA_3 for 24 hours. The enzymatic reactions involved in the germination process might be affected and altered by application of GA_3. Early stone germination in GA_3 treatment might be due to the increased concentration of endogenous auxin content due to the GA_3 application. The increased level of auxin and enhanced enzymatic activities along with the repression of inhibitors might be the probable reasons for faster germination (Dabhi, 2000). GA_3 might have also triggered the starch hydrolysis and their translocation to the growing seedlings thereby inducing early germination (Rajmanickam *et al.,* 2004). Similar results were reported by Anjanaw *et al.* (2013) in papaya.

The stones pre-treated with 200 ppm GA_3 recorded the highest percentage of germination (Fig. 5). This might be due to the presence of GA_3 inside the seed which stimulates the imbibition process on subsequent seed germination. Pre-soaking treatment of GA_3 might have affected directly and altered various enzymatic reactions, synthesis of proteins and conversion of starch into sugars involved in the process of germination (Paleg, 1960). On the other hand, GA_3 also induces the *denovo* synthesis of proteolytic enzymes like ribonuclease and α-Amylase. The enzyme amylases successively hydrolyse the starch in endosperm

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thereby providing essential sugars for growth initiation processes and also liberate chemical energy which is utilized for RNA synthesis, activation of embryo as well as the suppression of inhibition which in turn resulted in higher germination (Copeland and Mcdonald, 1995). GA_3 treatment also have an ability to overrule the thermo-dormancy, photo dormancy, dormancy imposed by incomplete development of embryo, presence of various germination inhibitors as well as mechanical barriers (Diaz and Martin, 1971). Similar results were reported by Shaban (2010a) in mango, Al-Hawezy (2013) in loquat and Lay *et al.* (2013) in papaya. GA_3 directly acts on embryo, alleviating them from dormancy through synthesis of protein, elongation of coleoptiles and also helps in ethylene production. This ethylene bring about the synthesis of amylase, which favours the process of seed germination (Stewart and Freebairn, 1969). The difference in germination rate by various pre- soaking treatments (Fig. 10) might be due to the differential capability of these chemicals to reduce the time taken for initiation of germination as well as to remove the hindrance in embryo growth (Muralidhara *et al.*, 2015).

Among various treatments, the highest seedling length was recorded in 100 ppm GA_3 followed by I ppm KNO_3. The pre-soaking treatment of GA_3 would have increased the osmotic uptake of nutrients causing cell division, multiplication and cell elongation (internodal elongation) thus stimulating the vegetative growth resulting in the highest seedling growth (Shanmugavelu, 1966). These results are in agreement with results obtained by Shalini *et al.* (1999) and Kumar *et al.* (2008) in mango, Harshavardhan and Rajasekhar (2012) in jackfruit and Vasantha *et al.* (2014) in tamarind. The increased length of seedling was a result of increased photosynthetic activity, enhanced mobilization of photosynthates and change in the permeability of membrane (Shukla *et al.*, 1997). In general, the regulation of growth by gibberellins and potassium nitrate relates virtually to its stem elongation properties, especially due to the enhancement of metabolites responsible for cell division and enlargement of cell. These chemicals act exclusively on stem elongation by loosening the cell wall, increasing the concentration of solutes by increasing the extensibility of cell wall, stimulating

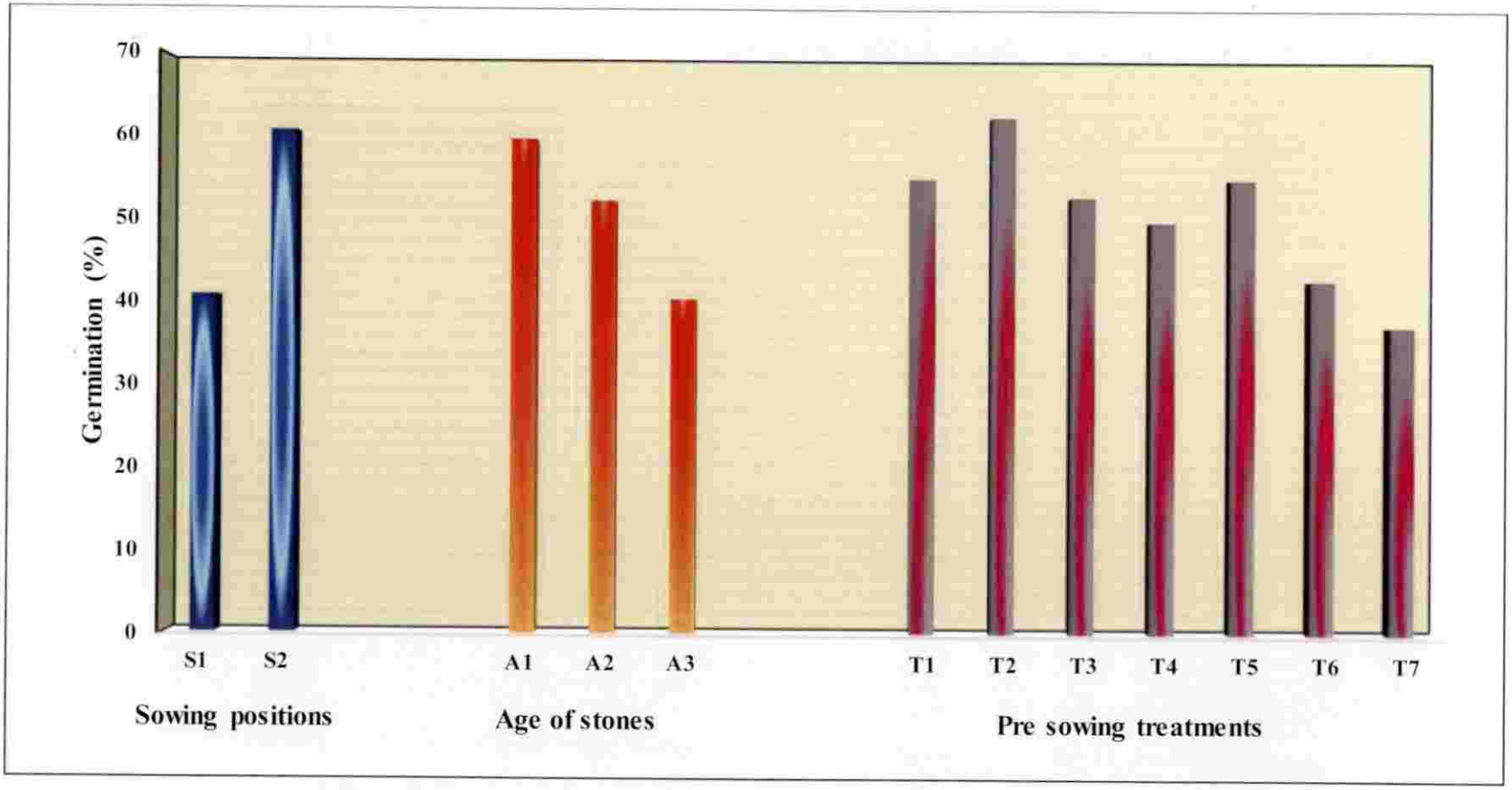

Fig. 5. Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on germination (%) of mango stones

cell wall synthesis, reducing the cell wall rigidity and by increasing cell division leading to more efficient growth. The indirect effect caused by these chemicals on stem elongation is by increasing the IAA synthesis that leads to more vegetative growth (Leopold and Krieddemann, 1983).

Among the treatments, the highest seedling dry weight was recorded in 100 ppm GA_3 followed by 1 ppm KNO_3. This result was mainly attributed to enhanced germination, early emergence of seedling as well as better seedling growth. Increase in dry weight of seedlings by GA_3 application might be due to the improved mobilization of the nutrients, which promotes the plant growth and development in better way. The GA_3 treatment might have resulted into higher production of photosynthates and their translocation through phloem tissue to the root zone might have led to increase in the production of lateral roots thereby increasing the root length (Vachhani *et al.*, 2014). The exogenous application of GA_3 also triggered the activity of gluconeogenic enzyme during the early stages of seed germination and this could be a probable reason for improved vigour characteristics which directly reflected on more production of lateral roots as well as increased root length, thereby improved the shoot growth (Vasantha *et al.*, 2014). This might have resulted in increased total dry weight of the seedling. The cumulative effect of better root and shoot growth as well as more production of lateral roots have led to overall assimilation and redistribution of photosynthates within the plant system, thereby promoting the better growth and development (Brian and Hemming, 1955). The identical results were obtained by Ameen and Imam (2007) in pistachio nut, Anjanaw *et al.*, (2013) in sapota, Brijwal and Kumar (2013) in guava, Kumawat *et al.* (2014) in papaya, Gurung *et al.* (2014) in passion fruit and Vasantha *et al.* (2014) in tamarind.

Significantly the highest vigour index- I (growth basis) and vigour index-II (weight basis) was recorded in mango stones pre-treated with 200 ppm GA_3 (Fig.6 and Fig. 7, respectively). The better results with respect to vigour indices might be due to the cumulative effect of higher germination percentage, shoot length, root length and seedling dry weight under GA_3 treatment (Abdul- Baki and Anderson, 1973). Similar results were put forth by Patil *et al.* (2012) in citrus.

5.2.4 Effect of interaction

The interaction effect of sowing positions and age of stones after extraction from the fruit was significant for the days taken for initiation of germination [Table 8(a)], days taken for 50 per cent germination [Table 8(a)], germination percentage [Table 8(a)], rate of germination [Table 8(a)], seedling length [Table 8(b)], dry weight of seedling [Table 8(b)], vigour index-I [Table 8(b)] and vigour index- II [Table 8(b)].The cumulative effect of viable and physiologically more active freshly harvested stones sown in micropyle pointing downward (stalk end up) position resulted in better results. Germination became progressively slower as the age advanced. The micropyle positioning while sowing has direct implications on seedling quality as it determines the uniformity, speed as well as rate of germination (Garner and Chaudhri, 1976). Hence the combined effect of sowing position and age of stones after extraction from fruit resulted in better results.

The interaction effect between sowing positions and pre sowing treatments was significant for days taken for initiation of germination [Table 9(a)], days taken for 50 per cent germination [Table 9(a)], rate of germination [Table 9(a)], seedling length [Table 9(b)], dry weight of seedling [Table 9(b)], vigour index-I [Table 9(b)] and vigour index- II [Table 9(b)]. This might be due to the combined effect of sowing position and pre sowing treatments. The GA_3 treated mango stones sown in micropyle pointing downward (stalk end up) position resulted in better germination as well as seedling vigour. The micropyle positioning while sowing has direct implications on seedling quality. The exogenous application of GA_3 has positive impact on induction of endogenous auxin content, *denovo* synthesis of proteolytic enzymes like ribonuclease and α-Amylase, conversion of starch to sugars involved in the germination process, protein synthesis and thus overrule the dormancy. Thus the cumulative effect of sowing positions and pre-sowing treatments resulted in superior results over other treatment combinations.

The interaction effect between age of stones after extraction from the fruit and pre sowing treatments was significant for days taken for initiation of germination [Table 10(a)], rate of germination [Table 10(a)], seedling length

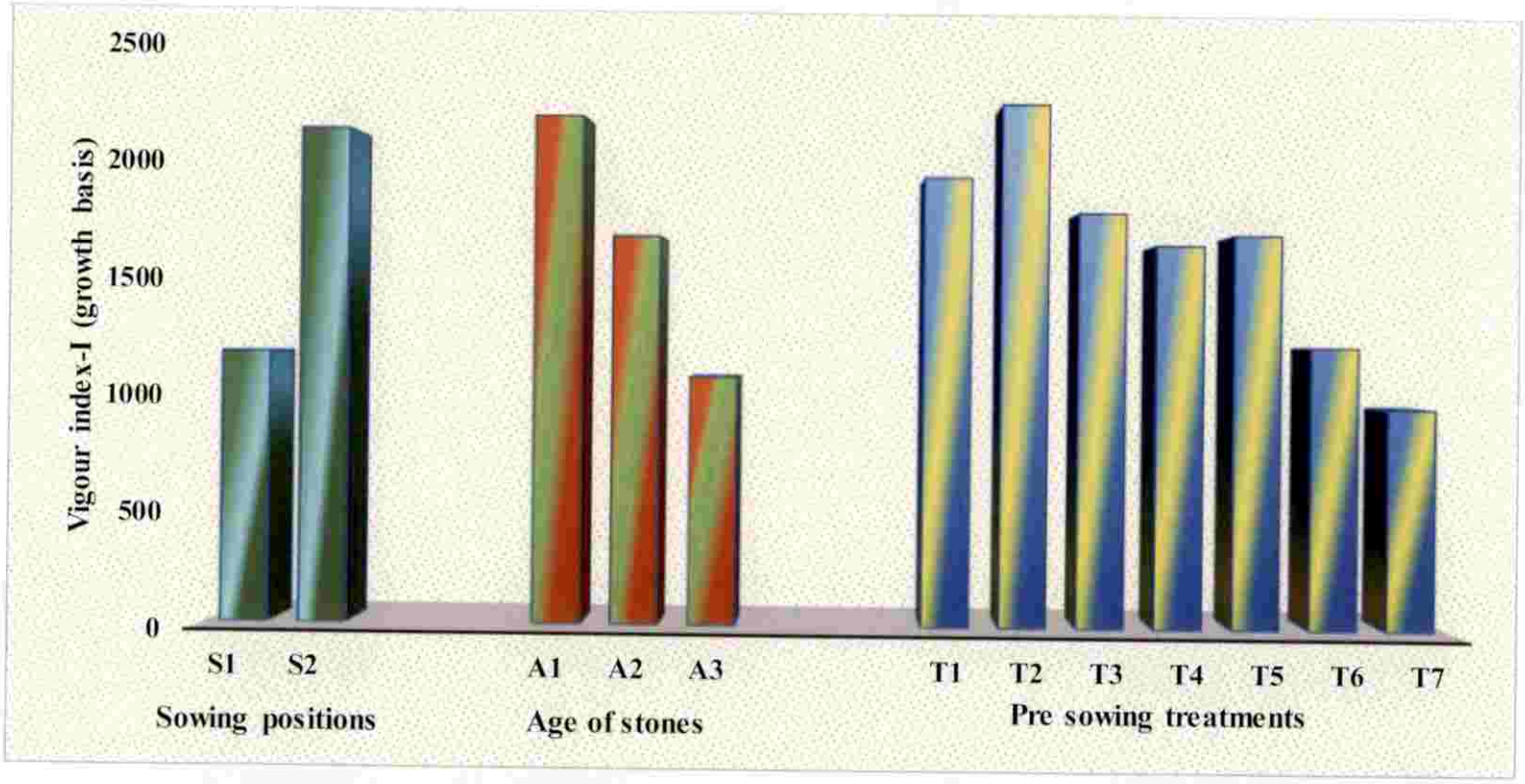

Fig. 6. Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on vigour index -I of mango seedlings

[Table 10(b)] and dry weight [Table 10(b)]. The cumulative effect of viable stones (freshly harvested stones) pre- treated with GA_3 recorded the promising results.

The interaction between sowing positions, age of stones after extraction from the fruit and pre sowing treatments was significant for days taken for initiation of germination [Table 11(a)], rate of germination [Table 11(a)], seedling length [Table 11(b)] and dry weight of seedling [Table 11(b)]. The coupled effect of freshly harvested stones, which were pre-treated with GA_3 and sown in micropyle pointing downward resulted in better germination and further growth of seedlings.

5.3 Screening of mango varieties for use as dwarfing rootstock

The results obtained from the present investigation regarding the screening of mango varieties for use as dwarfing rootstock are discussed below.

5.3.1 Germination characters

Among the ten genotypes, Kappa Manga recorded the highest germination percentage (71.11 %) and the least in Kotookonam Varikka (42.22 %). The germination capacity of mango genotype appears to be related to its stone size. Larger the seeds, more efficient and faster will be germination than the smaller ones. In Kotookonam Varikka which is polyembryonic in nature, all the cotyledons within a seed differed in weight and size. The Kappa Manga had more stone weight than others. Hence, the Kappa Manga recorded highest percentage of germination. The results are in confirmity with Barbosa *et al.* (2009) and Kumar *et al.* (2018) in mango.

The least seedling length was noted in Kochu Kilichundan and significantly the highest seedling length was recorded in Kappa Manga. It is evident from the data (Table 12) that seedling length has direct relation with the plant vigour. Abirami *et al.* (2011b) conducted an experiment to study the relationship between vegetative and physiological parameters of seedlings with vigour in some monoembryonic and polyembryonic mango genotypes. Among the polyembryonic genotypes, the highest seedling length was recorded in the vigorous rootstock Nekkare followed by Bappakai and the lowest length was

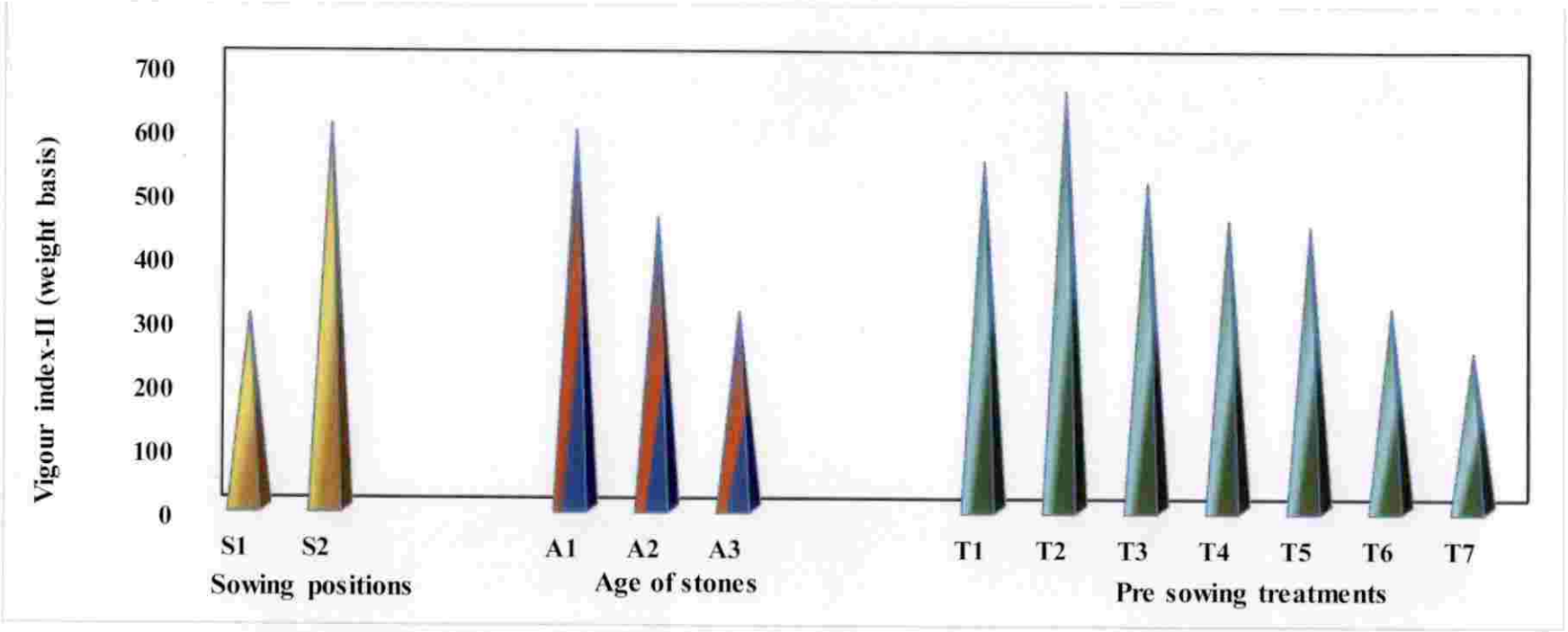

Fig. 7. Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on vigour index –II of mango seedlings

obtained from Kensington. The vigorous monoembryonic cultivar Bombay Green reported highest seedling length whereas Chausa reported the lowest value. The results obtained from the present study can be explained by the above results.

The analysis of the seedling dry weight revealed that the least dry weight was noted in Unda Varikka, while the highest was recorded in Vellari Varikka. The dry matter content had positive correlation with plant height (Singh *et al.*, 1986). The similar trend was also reported by Abirami *et al.* (2011b) in mango. The highest dry weight of seedling was obtained from vigorous polyembryonic mango rootstock Nekkare, followed by Bappakai. Among the various monoembryonic rootstocks, the highest dry weight was obtained from vigorous cv. Bombay Green followed by Pusa Arunima. The difference in dry weight might be attributed to the genetic characters.

The highest vigour index-I (growth basis) was recorded in var. Kappa Manga and the least was in Kochu Kilichundan (Table 12). Vigour index-I is a product of germination percentage and seedling length. Hence, the highest vigour index on growth basis might be due to highest germination percentage as well as the vigorous vegetative growth of var. Kappa Manga. The results are in agreement with the findings of Abirami *et al.* (2011b) in mango.

The highest seedling vigour index-II (weight basis) was recorded in var. Kappa Manga and the least was in Unda Varikka (Table 12). Vigour index-II is a product of germination percentage and dry weight. The dry matter content had positive correlation with the plant height (Singh *et al.*, 1986). Hence the highest vigour index on weight basis might be due to highest germination percentage as well as more seedling dry weight of var. Kappa Manga. The results are in agreement with the results of work by Abirami *et al.* (2011b) in mango.

5.3.2 Vegetative and growth characters

It is evident from Fig. 8 that the var. Kochu Kilichundan recorded the least plant height, followed by Unda Varikka, whereas vigorous variety Kappa Manga recorded the highest plant height. It is evident from the result that plant height has direct relation with plant vigour. Majumdar *et al.* (1972) reported that the vigorous rootstocks Goa, Kurukkan and Chausa resulted in the highest stem

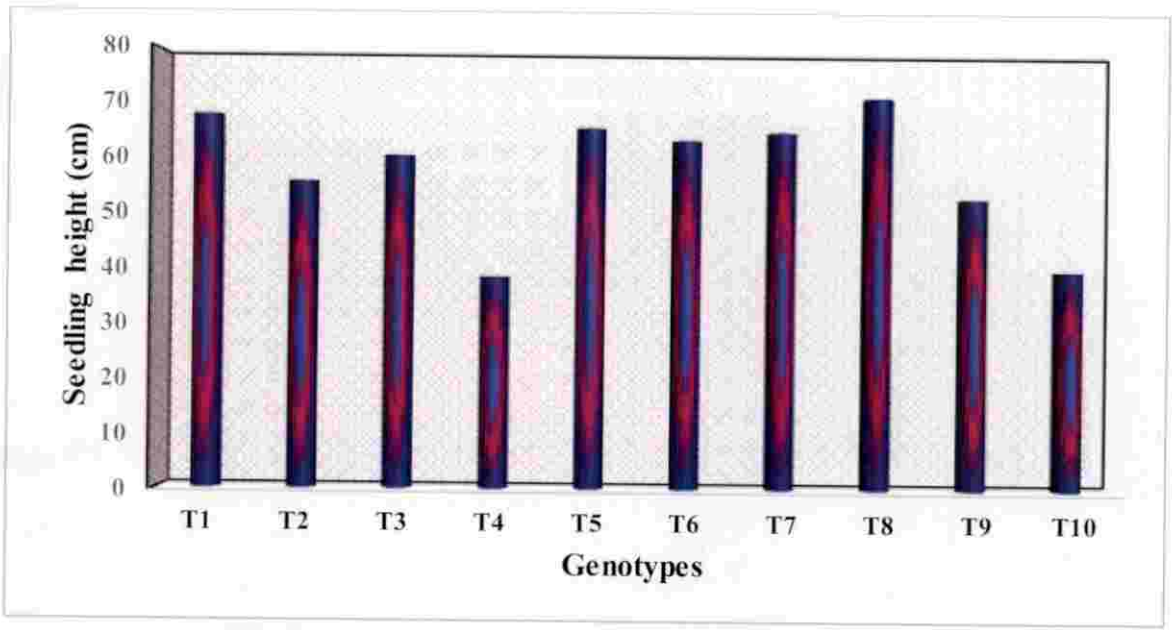

Fig. 8. Seedling height (cm) of different mango genotypes at 6 MAS

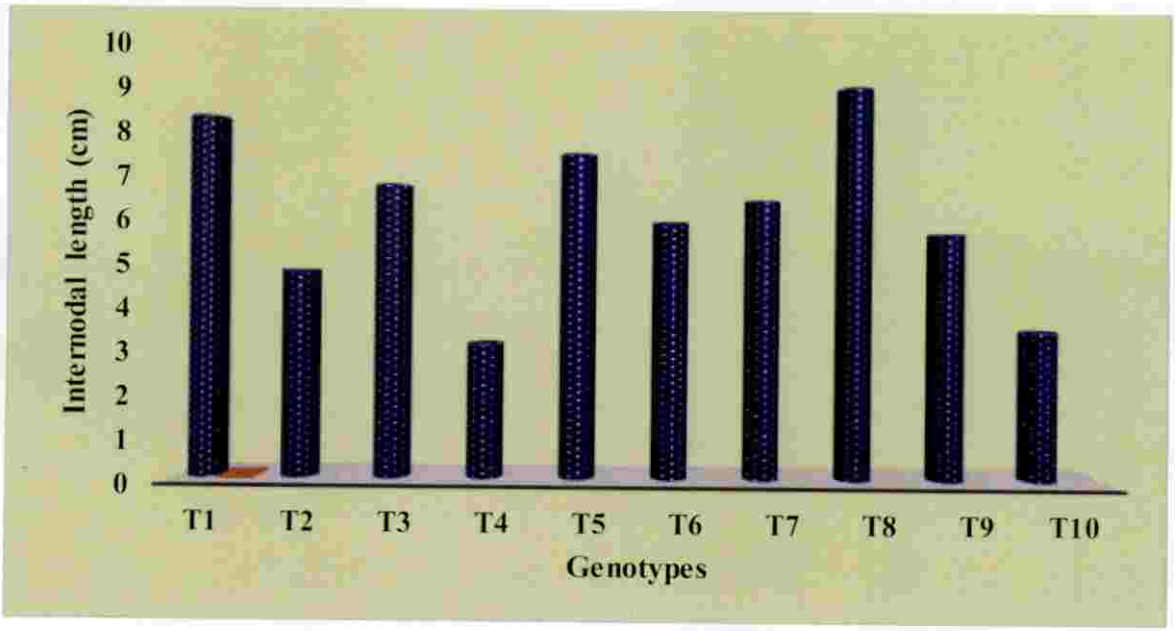

Fig. 9. Internodal length (cm) of seedlings of different mango genotypes at 6 MAS

growth whereas less vigorous cultivars Bombay Green and Totapuri Red Small recorded the least stem growth rate in mango. Similar results were put forth by Murti and Upreti (2003), Shenoy (2016), and Deepak *et al.* (2017) in mango.

It can be inferred from [Table 13(a)] that the variety Paiveli Local produced the least number of leaves (18.27), followed by Kochu Kilichundan (20.27) and Unda Varikka (23.20) six month after germination. Kappa Manga produced significantly higher number of leaves (34.60). Murti and Upreti (2003) found a positive correlation between number of leaves and plant height at nursery stage in mango. The highest number of leaves were found in vigorous cultivars Bappakai, Muvandan, Olour and Mylepelian whereas least number of leaves were observed inless vigorous cultivars Chandrakaran, Kurukkan, Vellaikolamban. The variations in vegetative behaviour of rootstocks might be attributed to vigorous growth, genetic factors and leaf producing capacity which enhance sun shine harvest in a better way by plants, in turn produce more number of leaves. The result obtained from the present investigation confirms the earlier findings of Murti and Upreti (2003), Srivastav *et al.* (2009), Shenoy (2016) and Deepak *et al.* (2017) in mango.

The leaves with the lowest leaf length (9.80 cm) and width (3.23 cm) were noticed in Kochu Kilichundan, whereas the vigorous var. Kappa Manga recorded the leaves with the highest leaf length as well as width. Similar trends in results were also reported by Shenoy (2016) in different mango varieties and revealed that the plant height is highly correlated with leaf length and width. Deepak *et al.* (2017) evaluated the vegetative growth performance of three different polyembryonic rootstocks *viz.,* Nekkare, Olour and Vellaikolamban at nursery stage. The highest leaf length and width were recorded in mango rootstock Olour. The least length and width of leaves were noted in cv. Vellaikolamban and revealed that the highest leaf length and width was associated with plant vigour. The difference in vegetative growth of seedlings might be due to stone characters as well as quicker germination.

The least average leaf area and total leaf area were noted in var. Kochu Kilichundan, while Kappa Manga recorded the highest values [Table 13(b)]. Kurian and Iyer (1997) conducted an experiment for the identification of morphological traits related to vigour management in mango and revealed that lower leaf area was a good indicator for imparting dwarfness. Murti and Upreti (2003) found a positive correlation between leaf area and plant vigour in mango. Positive correlation between leaf area and plant vigour was also reported by Agarwal (1986) in trifoliate orange; Muralidhara *et al.* (2014); Shenoy (2016) and Deepak *et al.* (2017) in mango.

As shown in Fig. 9 the least internodal length was recorded for Kochu Kilichundan, whereas the highest internodal length was observed in var. Kappa Manga. Iyer and Subramanyam (1986) found a positive correlation between internodal length and dwarfness. The less vigorous cv. Creeping showed the shortest internodal length (2cm) in mango. Plants with shorter internodal length had dwarf stature in papaya (Lim and Hawa, 2007). Similar results were put forth by Srivastav *et al.* (2009), Shenoy (2016) and Deepak *et al.* (2017) in mango.

The var. Unda Varikka produced less number of roots with the least root length while Kotookonam Varikka produced more number of roots and the var. Vellari Varikka recorded the highest root length. The number of secondary roots might be used as a good indicator of dwarfness (Mukherjee and Das, 1976). Shaban (2010b) reported a positive correlation between the root length and plant height in polyembryonic mango rootstocks. The positive correlation between number of roots and root length with plant height was also reported by Shenoy (2016) in mango.

The var. Unda Varikka recorded the least dry matter of shoot as well as root (Fig. 30), whereas Vellari Varikka recorded the highest shoot and root dry matter. Singh *et al.* (1986) reported that dry matter content of shoot and root could be used as a tool for the mango classification in relation with vigour and had a positive correlation between them. The results are in confirmity with Shenoy (2016) in mango.

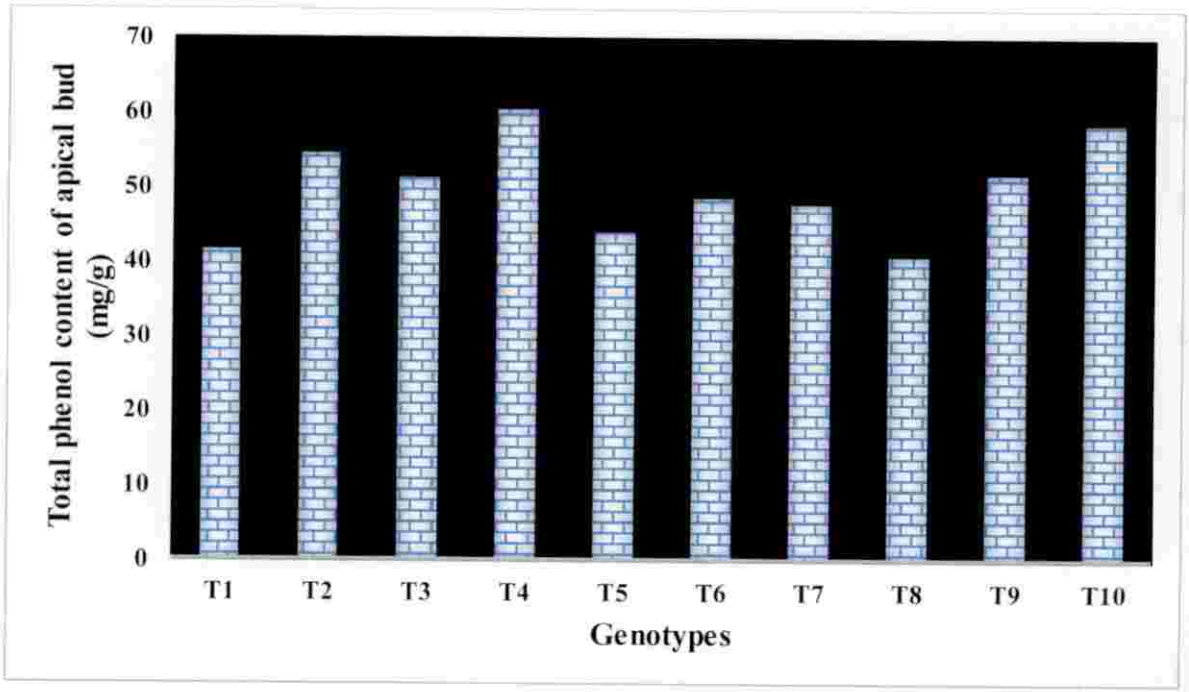

Fig. 10. Total phenol content of apical bud (mg/g) of different mango genotypes at 6 MAS

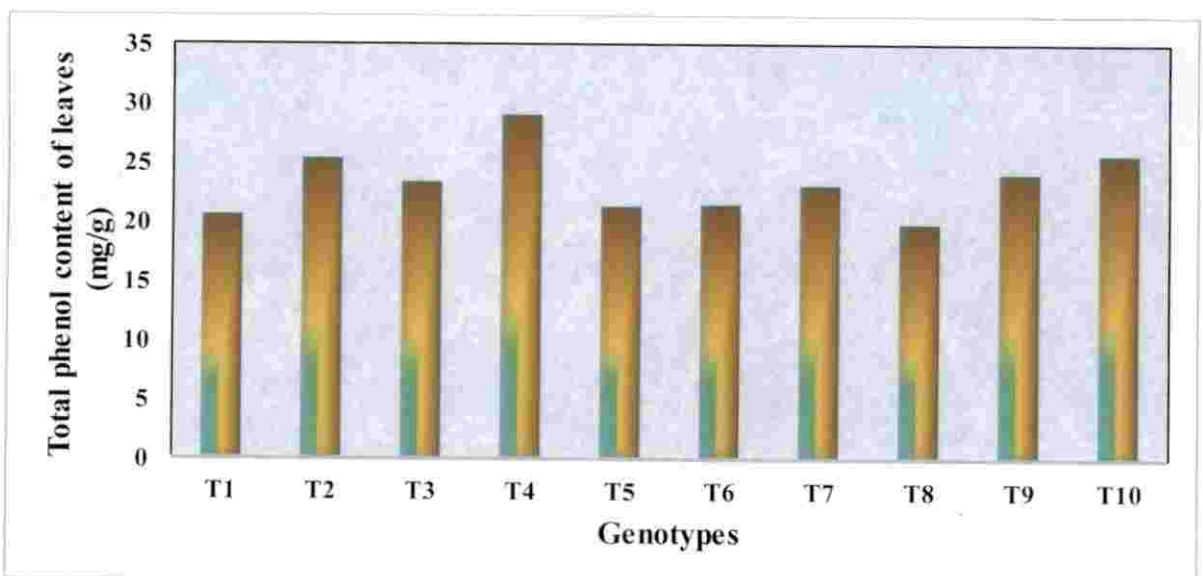

Fig. 11. Total phenol content of leaves (mg/g) of different mango genotypes at 6 MAS

5.3.3 Physiological and anatomical characters

The lowest stomatal density was noted in Unda Varikka while the highest was in Vellari Varikka. The maximum distribution of stomata was found in vigorous rootstocks than the dwarf ones (Eckerson, 1908). Chakladar (1967) adopted the stomatal density technique for the first time in mango for assessment of vigour at nursery stage and reported that this parameter might be a useful tool for easy forecasting of growth potentials at early stage. It has been evident from the research by Pathak *et al.* (1977) that the photosynthesis and stomatal density are mutually dependant and may increase the photosynthetic efficiency resulting in more accumulation of photosynthates which might be the probable reason for higher plant vigour in plum rootstock. The similar results were reported by Marie (2001) in mango, Pandit *et al.* (2004) in apple and Shenoy (2016) in mango.

It is evident from [Table 14(a)] that the var. Kasthuri recorded the highest starch content, whereas the vigorous var. Kappa Manga recorded the least starch content in leaves. According to Beakbane and Thompson, (1939), since the dwarfing rootstocks had highest bark percentage and high proportion of wood ray than the vigorous ones, they possess a larger volume of cells that were capable of storing carbohydrates relative to their size. Mendel and Cohen (1967) reported a negative correlation between starch content and tree vigour in citrus. Reduced cell growth and metabolism are the general characteristics of dwarfing rootstocks before any visible changes are apparent in either scion or rootstock. Usually lipid, amino acid and cell wall biosynthesis are found to be down regulated, while degradation pathways of these mentioned compounds are up regulated. In dwarfing rootstocks, cellulose and lignin biosynthesis pathways are highly down regulated, accordant with reduced cell wall synthesis. The imbalanced allocation of carbon highly influences the growth and development, generally the starch reserves of roots are found to be catabolized when glycolysis pathway or carbon for metabolic pathways are limiting. Foster *et al.* (2017) reported more accumulation of starch in stem and roots as well as reduced levels of glucose and fructose was found in apple dwarfing rootstock 'M9' relative to 'Royal Gala'.

The less vigorous Kochu Kilichundan recorded the highest phenol content in both apical bud (Fig. 10) as well as leaves (Fig. 11) followed by Unda Varikka, whereas, the vigorous variety Kappa Manga recorded the least values. Babu *et al.* (1985) found a negative correlation between total phenol content and vigour in mango. Murti *et al.,* (2000) reported that total phenols play a very significant role in the vigour restriction of mango seedlings. The highest phenol content of leaves were recorded from less vigorous polyembryonic cv. 'Vellaikolamban' followed by cv. 'Kurukan', whereas the vigorous cvs. 'Bappakai', 'Muvandan' and 'Alphonso' had lowest phenol content. The similar results were reported by Marie (2001), Murti and Upreti (2003), Srivastav *et al.* (2009), Abirami *et al.* (2011b) and Shenoy (2016) in mango.

Some special phenolic compounds such as coumarin and phloridzin are known to exert an inhibitory effect on cell division and cell elongation processes (Swensson, 1971). Greater the bark thickness, auxin degradation by IAA-oxidase, peroxidase and the phenolic compounds will be more. The reduction in auxin supply ultimately resulted in reduced production of cytokinin that alters the normal growth of a plant. The presence of thick bark has been associated with the low vigour in apple and also interfered with the phenolic compounds (Lockard and Schneider, 1981; Weibel, 2008). The mono phenols have antagonistic effect on IAA, meanwhile has a co-factor effect on peroxidase enzyme. The interaction between phenolic compounds and bark tissue has a reducing effect on plant vigour (Sulusoglu, 2014). This might be the probable reason for negative correlation between phenolic content and plant vigour.

The less vigorous var. Kochu Kilichundan has significantly the highest bark percentage of root (Fig. 12) and shoot (Fig. 13), whereas the vigorous var. Kappa Manga recorded the least values. The bark percentage of root was closely associated with dwarfness in mango (Mukherjee and Das, 1980). Negative correlation between plant height and bark percentage was reported by Saroj *et al.* (1997) in guava, Marie (2001), Abirami *et al.* (2011b) and Shenoy (2016) in mango.

The greater bark thickness in dwarf trees are mainly associated with higher degradation of auxins by enzymes such as peroxidase, IAA-oxidase and the phenolic compounds that are present in the bark. As a result of the reduction in auxin supply, there is a reduced production of cytokinin by roots, which alters the normal growth pattern of the tree (Lockhard and Schneider, 1981). This might be the probable reason for decreased vigour of plant with respect to increased bark percentage. These results are in confirmity with Jimenez and Priego (1987) in avocado.

The membrane stability index [Table 14(a)], relative water content [Table 914(a)], transpiration rate [Table 14(b)] and leaf temperature [Table 14(c)] were non-significant for predicting the vigour of mango seedlings at nursery stage.

The lowest xylem area of stem (1.93 mm^2) was noted in Kochu Kilichundan, followed by Unda Varikka (2.28 mm^2). The lowest percentage of xylemin stem (27.09 %) was noted in Unda Varikka. The highest values for stem xylem area was noted in Kappa Manga (6.07 mm^2) and xylem percentage in Vellari Varikka (54.46 %). The var. Unda Varika recorded the lowest root xylem area (1.34 mm^2) as well as percentage (26.84 %). The var. Kotookonam Varikka recorded the highest xylem area in root (10.86 mm^2) and significantly the highest xylem percentage was recorded in Kappa Manga (78.73 %). Goncalves *et al.* (2007) found a positive correlation between xylem conduit and plant vigour in sweet cherry tree.

Significantly higher phloem area of stem (3.62 mm^2) was noted in Unda Varikka, followed by Kochu Kilichundan (3.48 mm^2). The var. Kochu Kilichundan recorded the highest root phloem area (6.60 mm^2). The lowest stem phloem area was noted in Kappa Manga (1.52 mm^2) and Kotookonam Varikka recorded lowest phloem area in root (0.56 mm^2). The highest percentage of phloem in stem (33.53 %) as well as root (36.22 %) was in Unda Varikka. The lowest phloem percentage of stem was noted in Kotookonam Varikka (21.22 %) and that of root was in Kappa Manga (9.79 %). The vigorous citrus rootstock

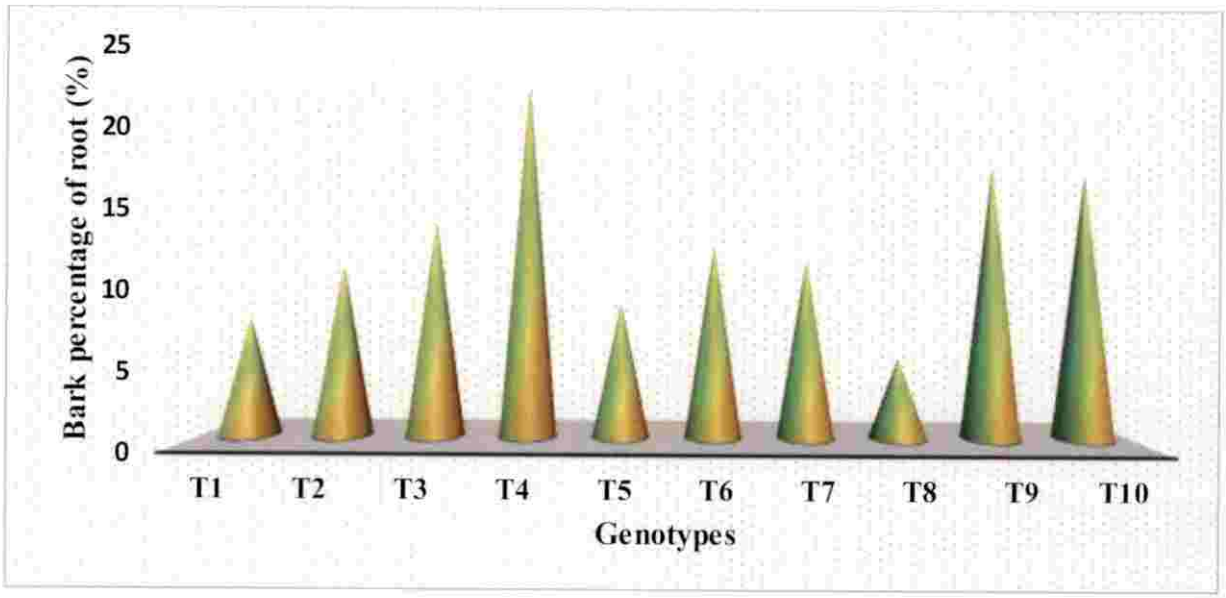

Fig. 12. Bark percentage (%) of root of different mango genotypes at 6 MAS

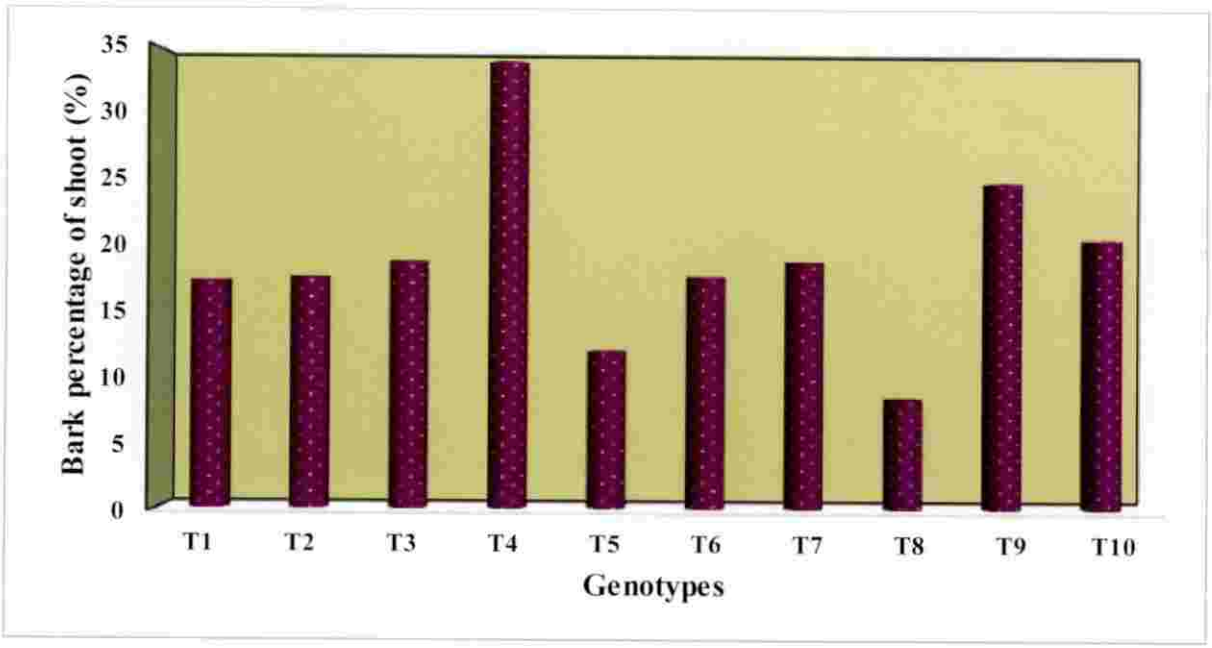

Fig. 13. Bark percentage (%) of shoot of different mango genotypes at 6 MAS

rough lemon possessed lower percentage of phloem in stem compared to less vigorous rootstock, *Poniciru strifoliata* (Saeed *et al.*, 2010).

Significantly the highest phloem/ xylem ratio of stem (0.99) as well as root (1.35) were noted in Unda Varikka. The lowest phloem/ xylem ratio of stem was noted in Vellari Varikka (0.39) and that of root was in Kappa Manga (0.13) (Fig. 14). According to Kurian and Iyer (1992) the higher primary phloem to xylem ratio of young mango shoots were associated with low vigour.

The lowest total conduit area of stem (4.38 mm^2) was noted in Thali Manga whereas, the var. Unda Varika recorded the lowest root total conduit area (6.38 mm^2). The highest stem total conduit area was noted in Kappa Manga (7.59 mm^2) while the var. Kotookonam Varikka recorded the highest total conduit area of root (14.43 mm^2) (Fig. 15). The vigorous avocado cv. Fuerte and Hass had higher total conduit area than dwarf Colin V-33 (Santamaria *et al.*, 2002).

Plant height had a negative correlation with the number of vessel elements in the xylem, xylem area and percentage of stem and root. More vigorous rootstocks tended to have fewer xylem vessels as well as comparatively narrower phloem both in stems and roots than less vigorous ones. Usually leaves are known to entice and influence the development of vascular tissues along the plant axis via steady polar flow of auxins (Saeed *et al.*, 2010).

Auxins control the cell enlargement rate by affecting the cell wall extensibility. The vessel density is positively correlated with auxins, which decreases from shoot to roots. Presence of relatively higher levels of auxin near the young leaves induce formation of numerous xylem vessels and these remain small because of their rapid differentiation rate. Lower concentration of auxins towards the plant base resulted in slower cell differentiation and hence resulted in fewer but larger vessels (Aloni, 1988).

The less vigorous rootstocks were characterised by smaller xylem vessel elements in the stem and root. It has been suggested that lower auxin/ higher cytokinin favours phloem differentiation (Digby and Wareing, 1966) whereas, higher auxin/ lower cytokinin favours xylogenesis or xylem differentiation

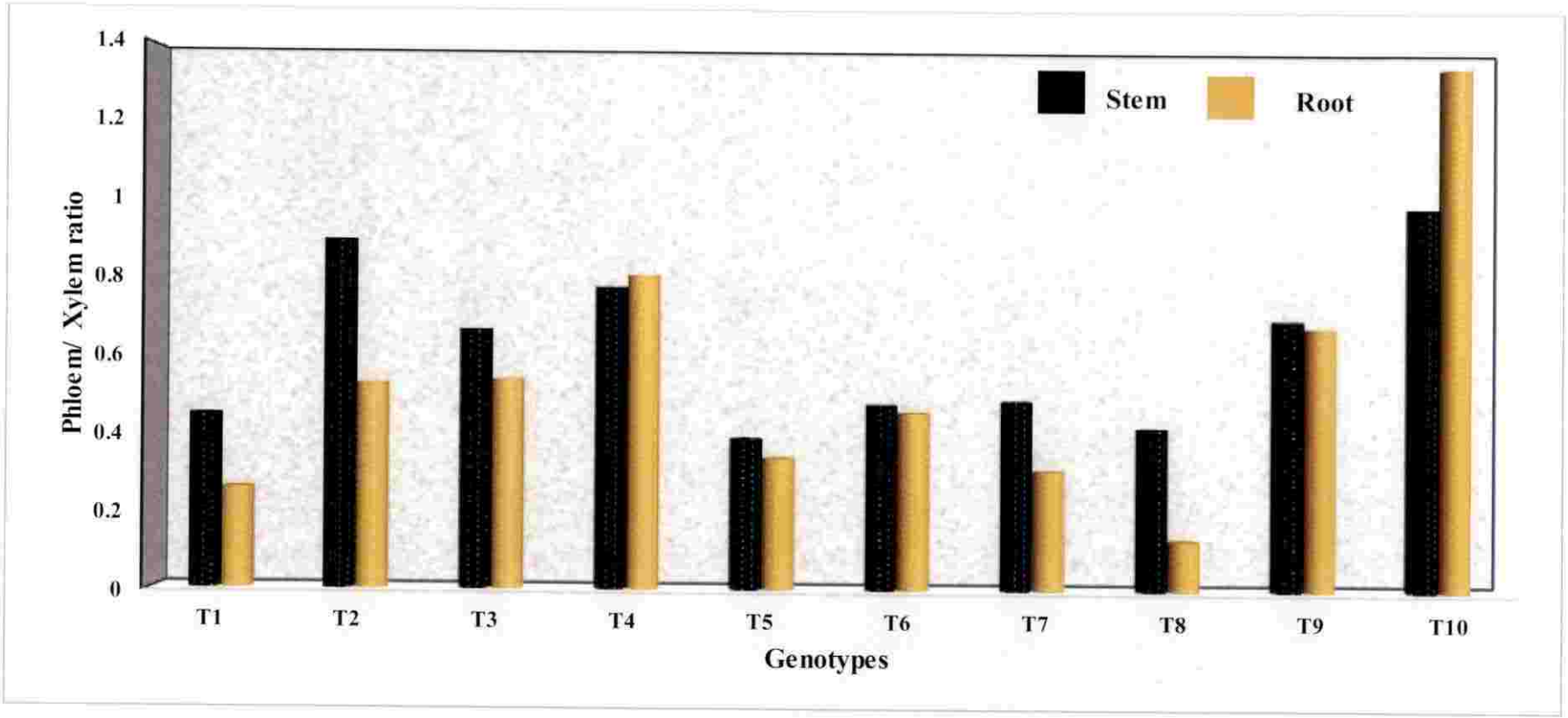

Fig. 14. Phloem/ Xylem ratio of stem and root cross sections of different mango genotypes at 6 MAS

(Roberts, 1969). In invigorating rootstocks, transport of auxin is consistent with higher ratios of xylem to phloem while less vigorous rootstocks have higher phloem to xylem ratios in stem as well as root.

Generally the dwarfing rootstocks possess a high bark: wood ratio and parenchymatous xylem as well as phloem. Hence they contain more living tissues per unit volume of stem as well as root than invigorating rootstocks (Beakbane, 1952). These parenchymatous tissues have a storage function and thus might be expected to have a high rate of respiration and heavy demand for essential nutrients. Hence the metabolic requirements of roots may vary according to the rootstock. Therefore, in dwarf trees, the competition is liable to occur between sites at which carbohydrates are utilised, and the roots may utilise relatively greater proportion of the total assimilates than in vigorous trees (Beakbane, 1956). The results are in agreement with those of Hegazi *et al.* (2013) in olive and Rashedy *et al.* (2014) in mango.

5.4: Effect of propagation methods and modified environments on different varieties of scion

5.4.1 Effect of propagation methods

From the results obtained, it was inferred that the efficacy of the epicotyl grafting with respect to vegetative and growth characters was better than softwood grafting. The equal and optimal level of metabolic activities in stock and scion have led to proper graft union and thus resulted in better success with respect to epicotyl grafting (Upadhya *et al.,* 2014). The high relative humidity and moderate temperature determines the success of grafts (Litz, 1997). The better growth of cambium both in rootstock and scion under favourable climate aided for the better success as well as vegetative growth of grafts in stone grafting (Singh *et al.,* 2014). The other possible reason might be due to the firmness with which the scion stick is held with the stem of stock without any interference from the plant tissue (Roy *et al.,* 1999). The scion maturity is also one of crucial factors which decides the success of grafting in any fruit crop. Softwood scion recorded more success in a very short period of time than hardwood scion (Baskaran *et al.,* 2008).

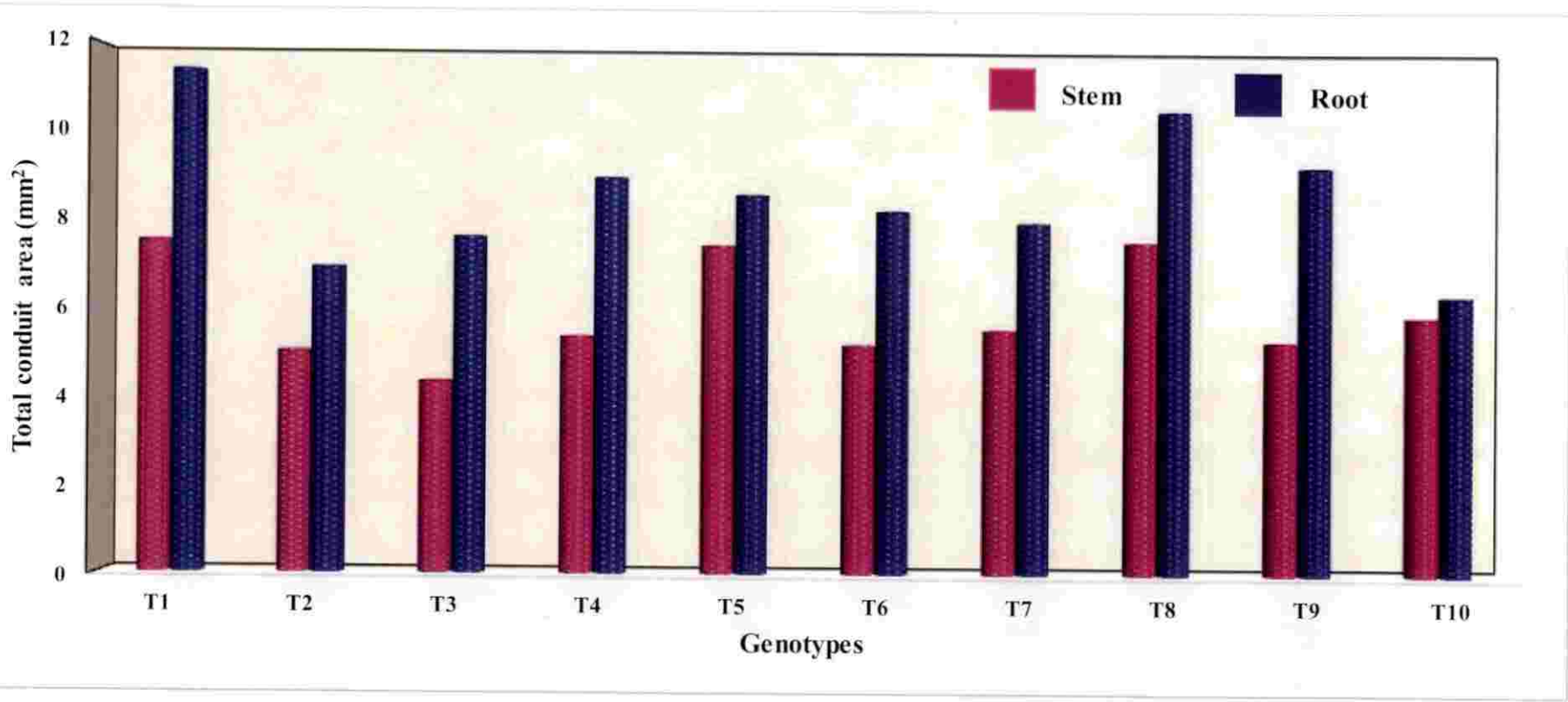

Fig. 15. Total conduit area (mm^2) of stem and root cross sections of different mango genotypes at 6 MAS

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The highest rootstock girth, scion girth, graft height (Fig. 16) and spread of the plant were observed in softwood grafts than epicotyl grafts. This might be due to the fact that the grafting operation was done at a greater height (around 14-16 cm) compared to epicotyl grafting (around 5-6 cm). The better combination of stock and scion with proper xylem and phloem tissue development from the callus resulted in higher values. The similar findings were also reported by Sivudu *et al.* (2013) in mango.

The earliness in sprouting, early leaf opening, lesser number of days for first vegetative flush (Fig. 22) and second vegetative flush (Fig. 23) in epicotyl grafting might be due to early and better contact of cambial layers of rootstock and scion, which in turn resulted in early callus (parenchymatous tissue) formation as well as rapid graft union process in epicotyl grafting. The rapid callus formation allows translocation of essential biochemical compounds between rootstock and scion, which resulted in the earliest sprouting (Kulwal and Tayde, 1989). Besides, the juvenile phase of young rootstock having the embryo attached and lack of effect of apical dominance might have contributed to it (Gunjate, 1989). The results are in agreement with Ram and Sirohi (1989), Singh *et al.* (2012) and Upadhya *et al.* (2014) in mango.

Higher initial graft success (Fig. 19) and graft establishment percentage (Fig. 20) were recorded in epicotyl grafts. This might be due to the complete and stronger union of epicotyl grafted before bud sprouting. The rapid callus formation, greater wound periderm formation and accumulation of resinous material in addition to lucid vascular continuity might be a probable reason for higher initial graft success. Besides, the preservation of higher amount of food materials in cotyledons as well as the actively growing stage of rootstock might also be a reason for higher initial graft success in epicotyl grafting (Sampath *et al,* 2017).

The best results with respect to scion length, sprout length (Fig. 17), number of leaves, leaf length, leaf width, leaf area, number of nodes on scion, and number of growth flushes/ graft (Fig. 21) were in epicotyl grafts. This might be due to the optimum temperature and relative humidity that prevailed during the

course of investigation, which resulted in early union of cambium layers of stock and scion, early formation of callus and initiation of subsequent growth of grafts.

The significantly higher graft survival was observed in epicotyl grafting (Fig. 24). The production of new xylem and phloem tissues permits the vascular connection between stock and scion. The space in the region of graft union between stock and scion favours good connection that subsequently would pass water and nutrients through the callus towards the scion for feeding it to stay alive, resulting in better graft survival (Omima *et al.,* 2012). Similar results were reported by Brahmachari *et al.* (1997) in mango, Singh and Singh (2006) in jamun and Harshavardhan (2011) in jackfruit. The lesser survival percentage of grafts in the case of softwood grafting might be due to the weaker graft union, which might have got involved in the nutrient uptake and translocation of food materials to the scion (Sivudu *et al.*, 2013).

5.4.2 Effect of modified environments

From the data analysed regarding the effect of modified environment, the better growth and survival of mango grafts were observed under fan and pad system. The high relative humidity in fan and pad system promotes low transpiration rate, which keeps the guard cells turgid and the stomata become open, thereby resulting in earlier production and accumulation of carbohydrates and proteins in a better way. Besides, these have significant impact on the completion of other physiological processes involved in rapid callus formation between the rootstock and scion. This might have resulted in early graft union between stock and scion, thus influencing the earliness in sprouting, opening of leaves, first vegetative flush (Fig. 22) as well as second vegetative flush (Fig. 23). Similar findings were also reported by Dhungana *et al.* (1989) in mango.

The modified micro climate inside the fan and pad system enhanced more hormonal activities in the terminal portion of grafts thereby encouraged the emergence of new growth flushes (Fig. 21). The results are in agreement with Karnachuk and Golovatskaya (1998) and Mahesh (1996) in lemon.

More vegetative growth *viz.,* stock and scion girth, graft height (Fig. 16), sprout length (Fig. 17) and plant spread under fan and pad system might be

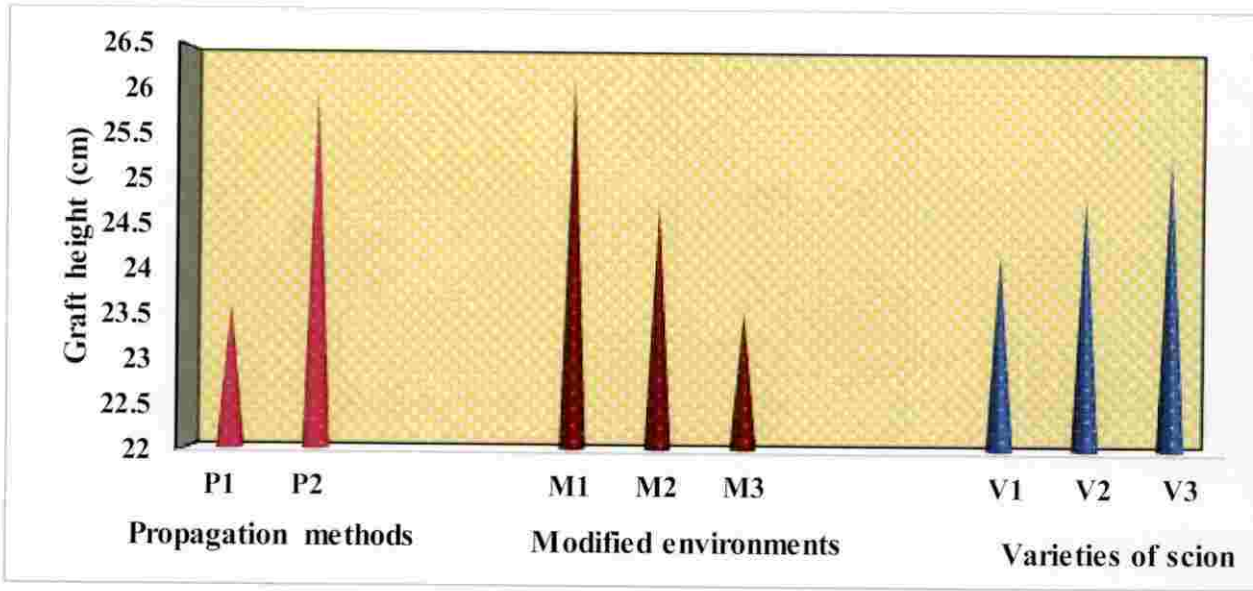

Fig. 16. Effect of propagation methods, modified environments and varieties of scion on graft height (cm) of mango at 90 DAG

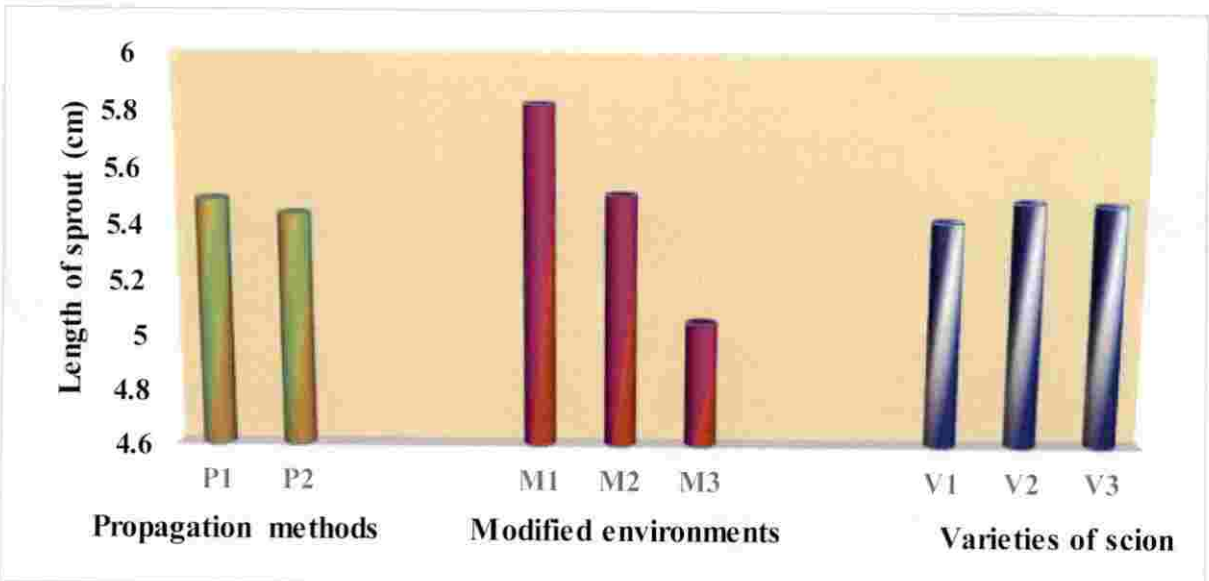

Fig. 17. Effect of propagation methods, modified environments and varieties of scion on length of sprout (cm) of mango grafts at 90 DAG

attributed to more production and accumulation of carbohydrates as well as increased hormonal activities resulting in higher photosynthesis. This ultimately resulted in rapid cell division and elongation of the existing cells by enlargement of the vacuoles. The results are in agreement with Chattopadhyay (1994) in mango.

Production of more number of leaves per graft, highest leaf length, and width, coupled with high leaf area were recorded under fan and pad system. This might be due to the combined effect of quick and strong graft union formation and better nutrient uptake which in turn caused better sprout growth. The higher humidity that prevailed inside the system during the period of investigation might have acted as a driving force for cell elongation and multiplication, which favoured more leaf expansion than in other plant growth structures (Sivudu *et al*, 2013).Similar results were also reported by Chovatia and Singh (2000) in jamun and Gadekar *et al.* (2010) in jamun. The fan and pad system provides relatively high percentage of moisture and also allows free exchange of gases, optimum light intensity as well as temperature. This favours more shoot growth and CO_2 enrichment resulted in production of more photosynthates. This might be the probable reason for better results. Similar results were also reported by Singh and Srivastava (1982) and Savani (2009) in mango.

The optimum temperature and water availability plays a vital role in photosynthetic activity as well as in bud sprouting, which increases the rate of photosynthesis, leading to production of more food materials, thereby facilitating improved growth and development of sprouts in grafted plants. The presence of thin water film in the callusing surface was observed to be more congenial for ample production of callus and better union, thus facilitating better initial success percentage (Fig. 19) and graft establishment (Fig. 20) under fan and pad system (Sivudu *et al.*, 2013).

The high level of relative humidity maintained in the humidity chamber reduced water loss from the scion and thus resulted in successful graft union. An examination of corresponding data on humidity reveals the fact that higher humidity level might have acted as driving forces for cell elongation and

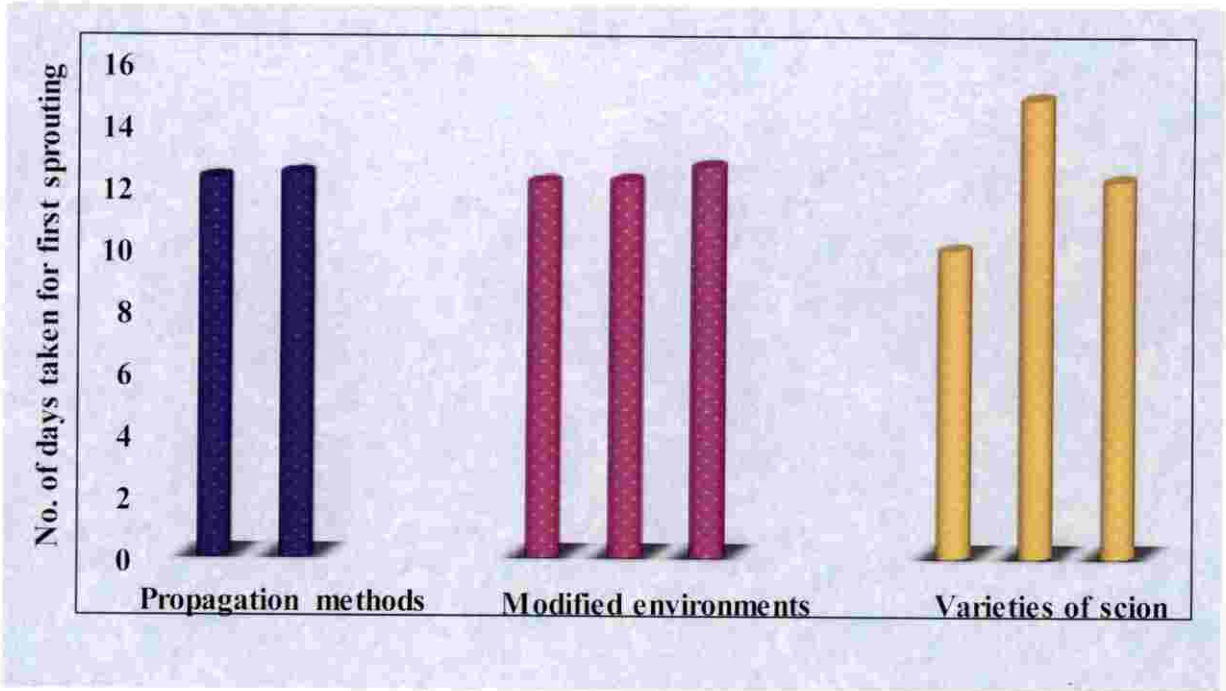

Fig. 18. Effect of propagation methods, modified environments and varieties of scion on days taken for first sprouting of mango grafts

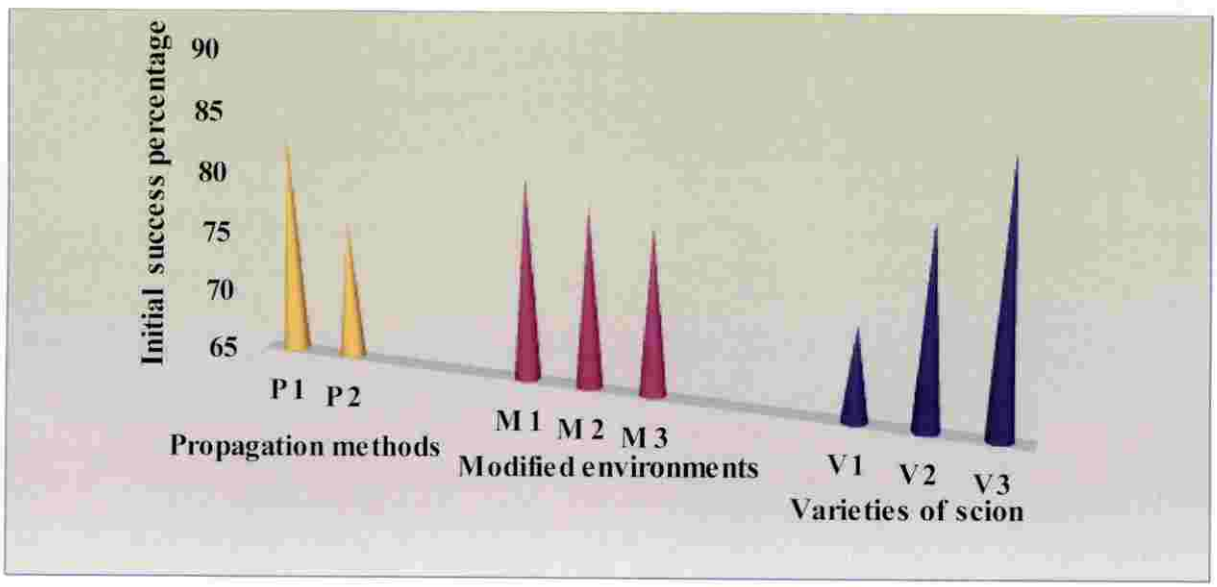

Fig. 19. Effect of propagation methods, modified environments and varieties of scion on initial success percentage of mango grafts at 30 DAG

multiplication that favoured production of more number of nodes as well as the higher internodal length under fan and pad system. The results are in agreement with those of Savani (2009) in mango.

The fan and pad system provides relatively high percentage of humidity as well as temperature. This optimum temperature maintained under this structure might be suitable for proliferation of new parenchymatous callus between stock and scion (Hartmann *et al.* 1997). The presence of high relative humidity favours the good callus bridge formation. It also have an impact on early completion of other physiological processes involved in the rapid callus formation between rootstock and scion. These favourable conditions resulted in early graft union between stock and scion as well as higher growth of grafts. Besides, the high relative humidity helps in avoiding the graft buds from drying, resulting better graft survival (Fig.24). These findings are in consonance with those of Baghel *et al.* (2002) and Savani (2009) in mango and Iqbal *et al.* (2004) in walnut. The low humidity and temperature under natural shade might be the probable reason for low survival of grafts (Reddy and Kohli, 1988).

The poor growth performance and survival of grafts under natural shade might be due to low humidity and temperature which have resulted in retarding the physiological process of graft union. (Chattopadhyay, 1994).

5.4.3 Effect of varieties of scion

In the present study, it was inferred that the graft success may vary depending on the genotype of scions. The better graft union in variety Kalapady might have easily furnished the required quantity of nutrients to the grafts resulting in earlier sprouting. The variation also might be due to the difference in endogenous substances *viz.,* phenols and latex. In other varieties, presence of more concentrated latex and hardiness of rootstock might have hindered the process of graft union resulting in delay in initiation of sprouting (Pandiyan *et al.,* 2011).

The difference in graft height (Fig. 16) and internodal length in Kotookonam Varikka might be due to the variation in vigour of different varieties

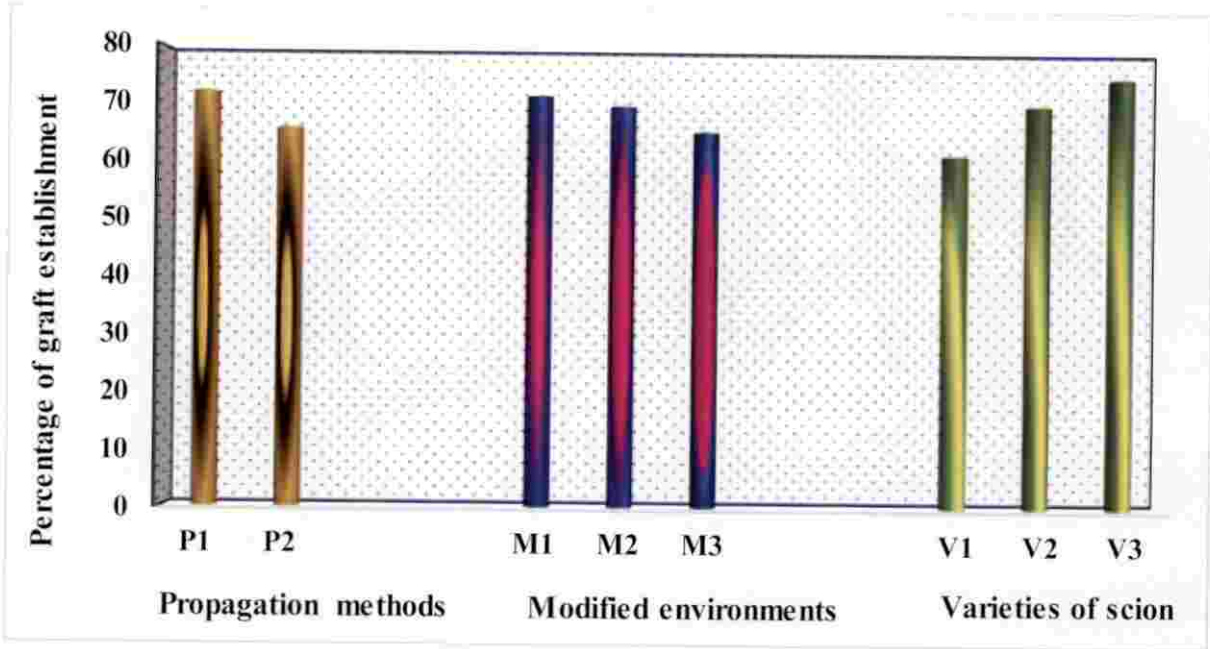

Fig. 20. Effect of propagation methods, modified environments and varieties of scion on percentage of graft establishment of mango at 60 DAG

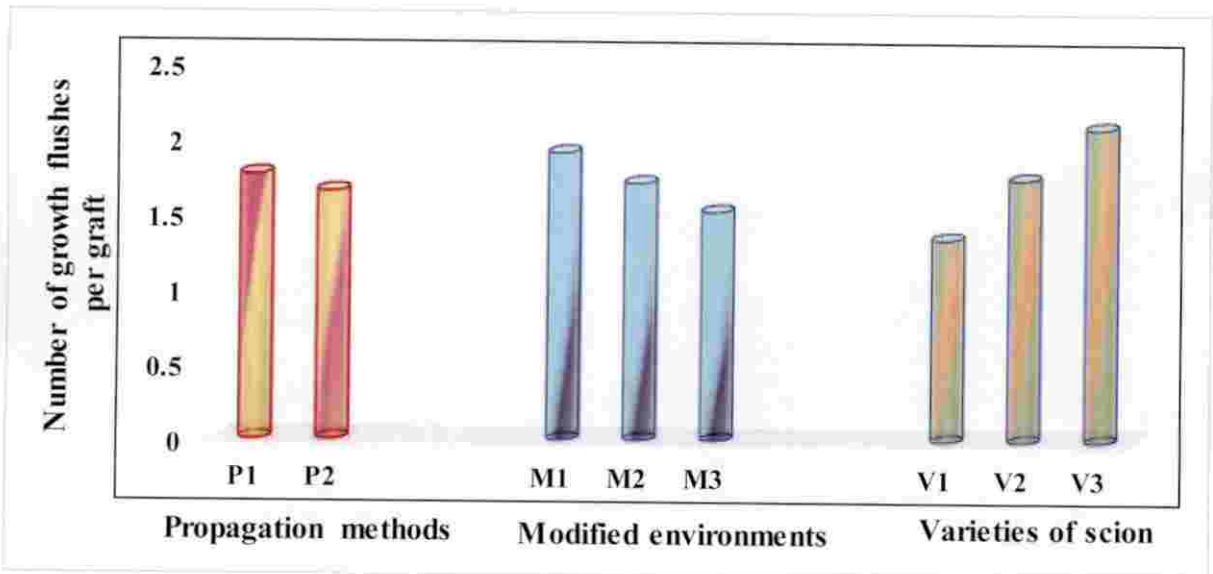

Fig. 21. Effect of propagation methods, modified environments and varieties of scion on number of growth flushes per graft of mango at 90 DAG

and early sprout emergence of grafts (Singh and Suryanarayana, 1996). The highest sprout length (Fig. 17), leaf length, leaf width and leaf area in var. Neelum might be attributed to earlier sprouting of grafts with more number of functional leaves, resulting in production of more carbohydrates required for proper growth and development of grafts. Similar results were also reported by Madalageri *et al.* (1989) and Baghel *et al.* (2002) in mango.

Better vegetative growth in terms of stock and scion girth, graft height (Fig. 16), plant spread and number of nodes in Kotookonam Varikka might be due to the variations in the genetic make-up of the varieties under study, which might influence the histological as well as physiological developments within the scion shoots in distinct ways (Alam *et al.,* 2006). The greater mechanical fit of the two cambium layers and fairly optimum level of hormones in the tissue, variation in callus formation and vascular continuity might be a probable cause. These findings were in agreement with Sivudu *et al.* (2013) and Ram *et al.* (2015) in mango.

The var. Kotookonam Varikka has produced more number of growth flushes (Fig. 21) compared to others probably due to the early callus formation, better bridging of graft union along with well-developed conducting tissues. The variations also attributed to difference in cell division as well as differential capacity in different scion varieties, which might have occurred due to the growth of meristematic cells coupled with physiological processes like photosynthesis and respiration. The results are in agreement with those of Asante and Barnett, (1977) in mango.

The production of more number of leaves in Neelum might be attributed to genetic make-up of the variety (Sivudu *et al.*, 2013). Besides, the better uptake of nutrients and water would increase the photosynthetic efficiency leading to more production of functional leaves, resulting in better growth of the grafts (Prajapati *et al.,* 2014). The results are in agreement with the results obtained by Bajpai*et al.* (1988) in mango. The highest number of leaves in Neelum may lead to production of more quantum of carbohydrates that are required for proper growth and development of the grafts. This favours quicker callus formation resulting in rapid

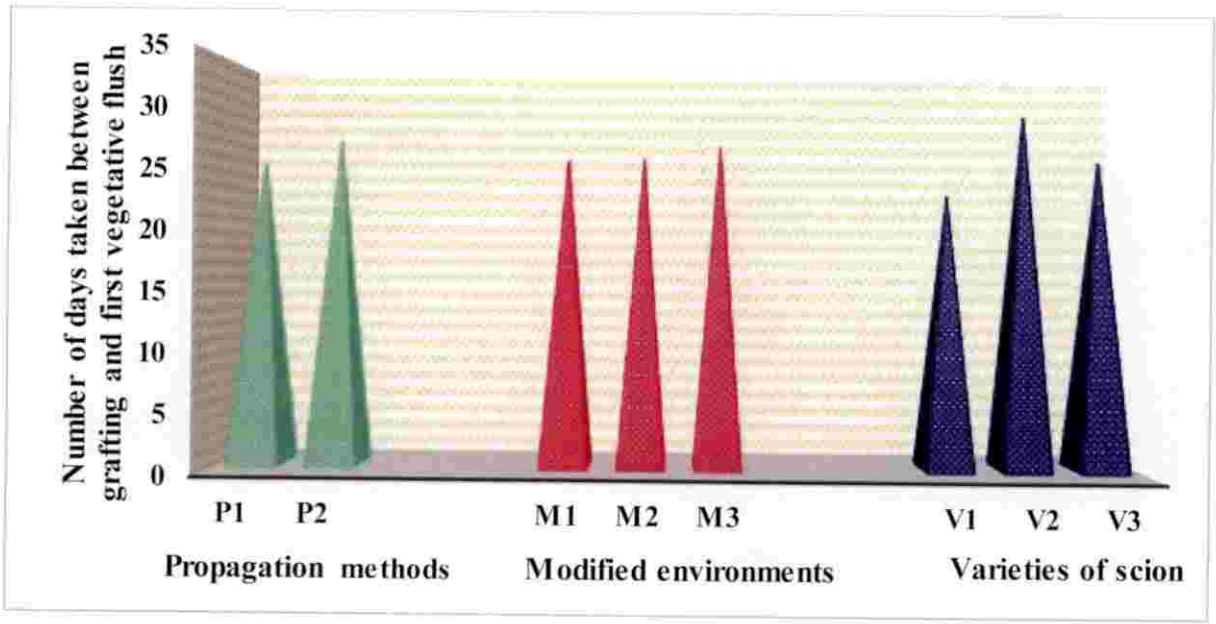

Fig. 22. Effect of propagation methods, modified environments and varieties of scion days taken between grafting and first vegetative flush of mango grafts

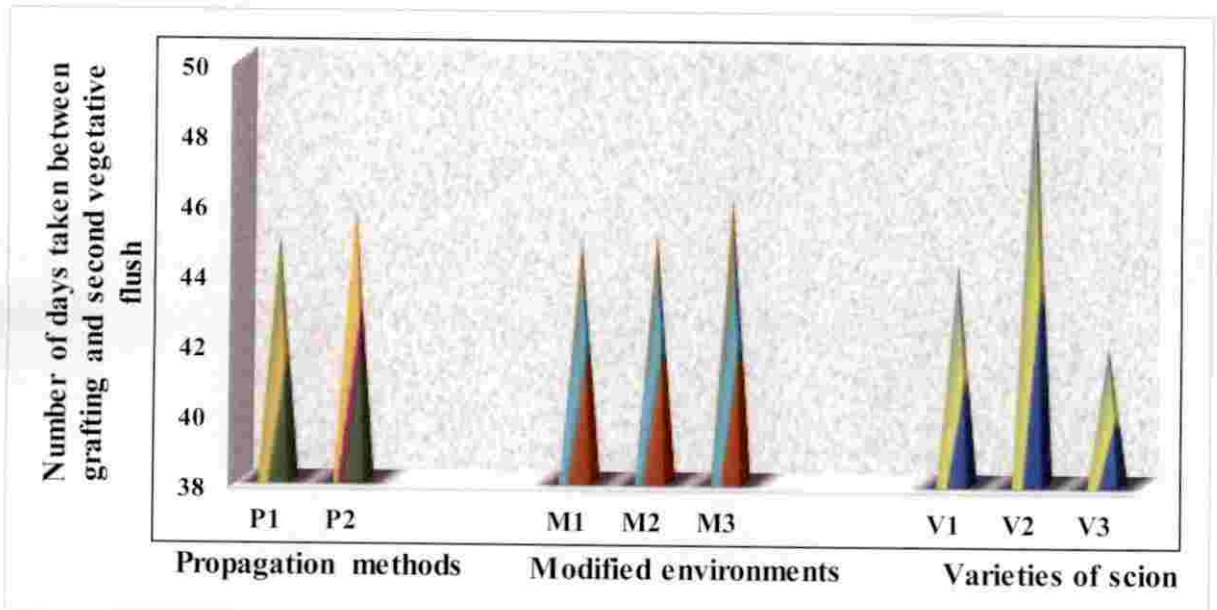

Fig. 23. Effect of propagation methods, modified environments and varieties of scion on days taken between grafting and second vegetative flush of mango grafts

wound healing in graft as a result of rapid cell division and cell elongation. These results are in agreement with results obtained by Alam *et al.* (2000) and Gurudatta *et al.* (2004) in mango.

The highest initial graft success (Fig. 19) and graft establishment percentage (Fig. 20) were noted in var. Kotookonam Varikka. This variation with respect to varieties of scion might be attributed to the differences in endogenous phenolic components in the selected genotypes. Besides, greater formation of wound periderm would also be a probable cause for higher initial success (Abd El-Zaher, 2008). These findings are in confirmity with those of Prashanth *et al.* (2006), Alam *et al.* (2006), Prajapati *et al.* (2014) and Ram *et al.* (2015).

The rapid physiological development and accelerated activities in the meristematic tissues of the varieties Kalapady and Kotookonam Varikka might have induced the early healing of the graft union which ultimately led to earlier production of first (Fig. 22) as well as second vegetative flushes (Fig. 23)than other varieties. The results are in agreement with those of Hanumantrao (2012) in mango.

5.4.4 Effect of interactions

In the present investigation, most of the growth parameters were appreciably increased under the interaction of epicotyl method of grafting and fan and pad system. This might be due to the congenial environmental conditions for cambial and intermediate tissue contact, which heals the wound in graft very rapidly and makes a strong union between stock and scion. This resulted in more nutrient uptake and translocation of food materials in a better way, which increased the graft growth. These results are in confirmity with results obtained by Gurudatta *et al.* (2004) in mango.

The promising results obtained due the interaction of modified environments as well as varieties of scion might be attributed to the genetic makeup of the variety along with enhanced vegetative growth by activated physiological process, which may be accountable for rapid multiplication of callus cells, quick and uniform proliferation of parynchymatous tissues, that supports the healing of wounds, resulting in early and proper graft union under the favourable

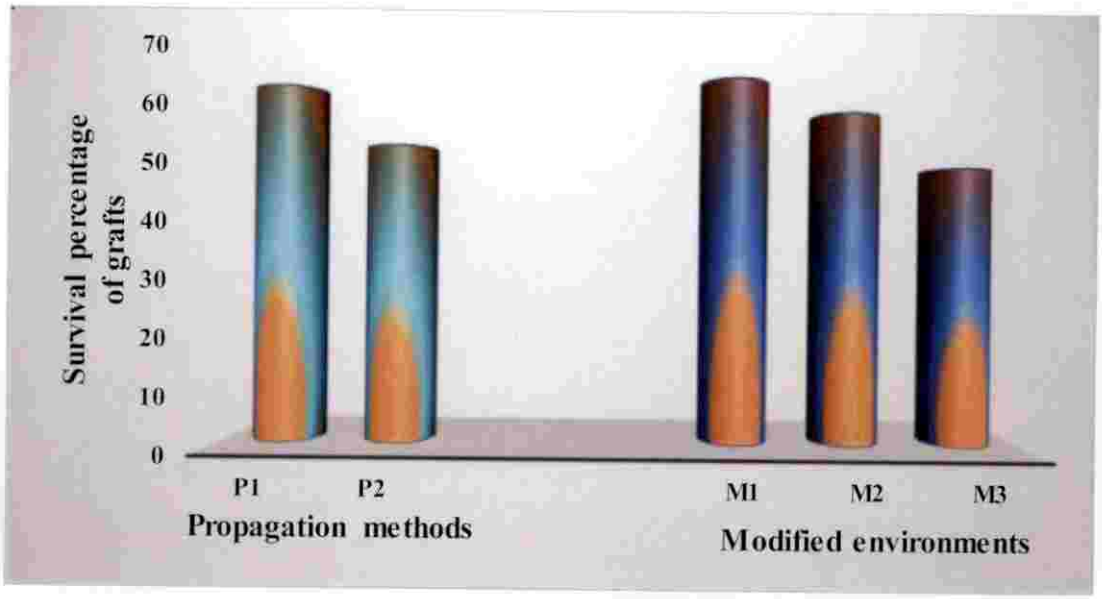

Fig. 24. Effect of propagation methods, modified environments and varieties of scion on survival percentage of mango grafts at 180 DAG

environmental conditions. These results are in agreement with the findings of Gunjate (1989), Patil *et al.* (2008) and Jawre (2012) in mango.

The best results obtained from the interaction effect between propagation methods and varieties of scion might be attributed to the early and better contact of cambial layers of stock and scion resulting in early callus (parenchymatous tissue) formation as well as rapid graft union process. The rapid callus formation allows translocation of essential biochemical compounds between rootstock and scion, which resulted in better growth of grafts. The better combination of stock and scion are also influenced by the genetic makeup of both scion as well as stock plants. The results are in agreement with Sampath *et al.* (2017) in mango. The histological studies on graft anatomical features of jackfruit varied in accordance with type of grafting as well as compatibility between stock and scion as reported by Abd El-Zaher (2008).

The coupled effect of relevant propagation methods with proper selection of genotype of scion under favourable environmental conditions resulted in better vegetative growth in mango grafts.

Summary

6. SUMMARY

Attempts were made at the Department of Pomology and Floriculture, College of Agriculture, Vellayani during 2016-2019 to evaluate the propagation techniques and rootstock studies of mango (*Mangifera indica* L.). The salient findings of the investigations are summarized below.

Twenty local mango varieties were collected from different parts of Thiruvananthapuram district of Kerala and screened for polyembryony. Among the 20 varieties, 17 were polyembryonic and 3 were monoembryonic. The germination characters of different genotypes revealed that the var. Kappa Manga performed best in terms of germination per cent, germination index and seedling vigour index-I. The mean germination time was the least in Vellari Varikka. Kotookonam Varikka recorded the highest percentage polyembryony and produced more number of plantlets per stone.

Out of seventeen polyembryonic mango varieties screened, molecular characterization of all the plantlets from two varieties *viz.,* Kotookonam Varikka and Kochu Kilichundan that exhibited the highest percentage polyembryony were done using SSR primers in comparison with their mother plants. The outcome of SSR marker to distinguish zygotic and nucellar seedlings depend on the degree of plant heterozygosity and the number of loci detected. The selected polyembryonic mango varieties were screened using 20 SSR primers. It is evident from the present study that all the seedlings obtained from the respective mother plant had SSR profile identical to the mother plant. The identical banding pattern of multiple seedlings and mother plant indicated the nucellar origin of seedlings having the similar genetic composition. It can be presumed that the zygotic seedling has ceased growth and degenerated at very early stage of growth and the nucellar seedlings that remain are all vigorous ones. The seedlings of Kotookonam Varikka and Kochu Kilichundan could produce more uniform rootstocks and they can be used to generate homogeneous grafted plants.

The fruits of 'Kotookonam Varikka' variety of mango were selected for stone extraction. The treatments comprised of different combinations of two

sowing positions (flat and stalk end up), three different age groups of stones after extraction from fruit (freshly extracted stone, 10 days after extraction, and 20 days after extraction) and seven pre-sowing treatments (GA_3- 100 and 200 ppm, KNO_3 -1 and 2 ppm, cow dung slurry, water and control). Among different sowing positions, stalk end up sowing (micropyle pointing downward) was superior with respect to minimum number of days for initiation of germination and 50 per cent germination, the highest percentage of germination, germination rate and seedling vigour indices (on both growth and weight basis) compared to flat method of sowing. The highest germination percentage, the least number of days for initiation of germination and 50 per cent germination, the highest rate of germination, seedling vigour indices on growth basis and weight basis were the best for the freshly harvested stones as compared to stones sown at 10 days and 20 days after extraction from fruit. With regard to the pre-sowing treatments, the earliness in germination, the least number of days for 50 per cent germination, the highest germination percentage, rate of germination, vigour index-I (growth basis) and vigour index- II (weight basis) were recorded when mango stones were soaked in 200 ppm GA_3 for 24 hours. Whereas 100 ppm GA_3 treated stones produced seedlings with the highest length as well as dry weight.

The interaction effect of sowing positions and age of stones after extraction from the fruit showed the least number of days taken for initiation of germination and 50 per cent germination, the highest germination percentage, rate of germination, seedling length, dry weight, vigour index-I and vigour index- II were recorded for freshly extracted stones sown in stalk up position. The interaction effect between sowing positions and pre sowing treatments revealed that the least number of days taken for initiation of germination and 50 per cent germination, the highest germination rate, vigour index-I and vigour index- II were recorded 200 ppm GA_3 pre-treated mango stones sown in stalk end up position, whereas, the highest seedling length and dry weight were recorded in 100 ppm GA_3 pre-treated mango stones sown in stalk end up position. The freshly extracted stones treated with 200 ppm $GA_3$for 24 hours proved to be the best with respect to the least number of days taken for initiation of germination and the

highest germination rate, whereas the greatest seedling length and dry weight were obtained from freshly extracted stones sown after treatment with 100 ppm GA_3.The freshly harvested stones, which were pre-treated with 200 ppm GA_3 and sown in stalk end up method resulted in the least number of days taken for initiation of germination and the highest rate of germination, whereas the greatest seedling length, and dry weight were obtained from freshly harvested stones pre-treated with 100 ppm GA_3 and sown in stalk end up method.

Ten local mango varieties/collections were screened at seedling stage for use as rootstocks in order to impart dwarfiness. The varieties selected for the present study included Kotookonam Varikka, Kasthuri, Thali Manga Manga, Kochu Kilichundan, Vellari Varikka, Pallikkal Local, Kili Manga, Kappa Manga, Paiveli Local and Unda Varikka. The morphological, physiological and anatomical features associated with dwarfing potential of the selected indigenous mango varieties were studied.

At 4 MAS, the lowest seedling length was noticed in Kochu Kilichundan, followed by Unda Varikka and the highest seedling length was in Kappa Manga. Moreover, the germination percentage of Kochu Kilichundan was on par with all the varieties except Kappa Manga along with the least vigour index-I and vigour index- II was on par with Unda Varikka. However, the least dry matter of seedling was recorded in Unda Varikka.

At 6 MAS, majority of the morphological features were the highest in Kappa Manga. The highest starch content was estimated in Kasthuri. The Kochu Kilichundan and Unda Varikka exhibited dwarfism with lower plant height, but the former had the least internodal length. The higher values for number of leaves, leaf length, leaf width and average leaf area were in Unda Varikka than Kochu Kilichundan. However, higher number of roots, root length, dry weight of root and shoot and stomatal density were recorded in Kochu Kilichundan. Total leaf area of the two varieties were on par. Moreover, Kochu Kilichundan had the highest phenol content in apical bud and leaves, bark percentage of root and shoot than all the varieties. The anatomical studies revealed the highest phloem-xylem ratio both in stem and root and the least total conduit area of root in Unda Varikka whereas the least total conduit area of stem was in Kochu Kilichundan, indicating

the dwarfing potential of both Kochu Kilichundan and Unda Varikka. The membrane stability index, relative water content, transpiration rate and leaf temperature were not significant as a useful criterion for the assessment of dwarfing potential of the selected mango genotypes.

The varieties were classified based on growth potential as vigorous, semi vigorous and dwarfing based on various morphological, physiological and anatomical characters. Among ten varieties, Kappa Manga, Kotookonam Varikka and Vellari Varikka were grouped under vigorous rootstocks, whereas Kili Manga, Kasthuri, Thali Manga, Paiveli Local and Pallikkal Local were grouped under semi vigorous group. The variety Kochu Kilichundan and Unda Varikka were identified as promising rootstocks to impart dwarfiness and Kochu Kilichundan was more superior in most of the morphological and physiological characters.

Epicotyl and softwood grafting were done on three scion varieties *viz.*, Kalapady, Neelum, and Kotookonam Varikka. The vegetative and growth performances of respective grafts were examined under different modified environments such as fan and pad system, humidity chamber and natural shade (75 % shade). Epicotyl grafts performed better in terms of most of the vegetative and characters. Higher values for scion length, sprout length, number of grafts sprouted at weekly intervals, initial success percentage, graft establishment percentage, the least number of days for first sprouting and last sprouting, days for leaf opening, higher number of leaves, leaf length, leaf width, leaf area, number of nodes on scion, intermodal length, number of growth flushes per graft, lower number of days taken between grafting and first vegetative flush as well as between grafting and second vegetative flush and the highest survival percentage were recorded for epicotyl grafts. Higher values for stock and scion girth, graft height and plant spread were observed in softwood grafted plants. The propagation methods had no significant effect on root length.

The micro climate controlled by fan and pad system produced the most conducive conditions for vegetative growth of mango grafts. The varieties of scion differed significantly with respect to the growth performance and success

percentage of grafts. Among different scion varieties, the highest stock and scion girth, scion length, graft height, plant spread, number of grafts sprouted at weekly intervals, initial success percentage, percentage of graft establishment, leaf width, number of nodes on scion, internodal length, number of growth flushes per graft and earliness in second vegetative flushing were observed in Kotookonam Varikka. Higher values for sprout length, more number of leaves, highest leaf length and leaf area were noticed in Neelum. The earliness in sprouting, leaf opening and first vegetative flushing were recorded in Kalapady. The varieties of scion did not influence the root length and final survival of grafts.

The interaction of propagation methods and modified environments revealed that the epicotyl grafts maintained under fan and pad system produced significantly better vegetative growth of grafts. The interaction effect of propagation methods and varieties of scion indicated that epicotyl grafts of Kotookonam Varikka performed best in terms of vegetative characters and establishment of mango grafts. An interaction of modified environments and varieties of scion revealed that the Kotookonam Vaarikka grafts kept under climate controlled fan and pad system resulted in more vegetative growth, success as well as establishment of grafts. The epicotyl grafts of Kotookonam Varikka under controlled climate using fan and pad system recorded better graft establishment, survival and vegetative and growth parameters.

Future line of work

On the basis of the results obtained, the following suggestions are made for future line of work.

- Identification of more numbers of SSRs are required in future for ascertaining the origin of plantlets and hybridity testing of progenies in polyembryonic mango varieties.
- The identified dwarf varieties can be included in further studies to impart dwarfness in commercial varieties and hybrids.
- Further research is needed to ascertain dwarfism induced through grafting with these rootstocks and economic viability for long term HDP system.

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Appendices

APPENDIX-I

Fruit characters of different mango genotypes

Sl. No.	Genotypes	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit circumference (cm)	Fruit volume (ml)
1	Kotookonam Varikka	216.40	11.87	8.12	15.84	139.24
2	Thali	94.80	9.14	6.48	11.64	101.60
3	Vellari	187.80	11.50	8.26	17.27	180.42
4	Kochu Kilichundan	193.20	8.98	6.43	13.18	90.36
5	Unda Varikka	113.00	9.78	7.69	17.26	81.25
6	Paiveli Local	84.60	8.5	6.32	14.68	60.86
7	Vazhapazhiti	76.86	7.15	4.89	9.24	49.27
8	Pandi Manga	150.40	8.42	6.78	16.74	86.32
9	Champa Varikka	161.60	11.76	6.22	13.24	135.8
10	Kili Manga	115.40	10.12	5.10	11.24	53.86
11	Peraykka Manga	140.65	9.24	7.14	16.54	121.41
12	Sreekaryom Local	215.60	12.47	7.85	18.23	88.62
13	Mylapoo	150.60	9.56	6.12	14.25	63.84
14	Kasthuri	144.62	10.24	5.89	15.64	61.44
15	Attanari	127.68	6.89	5.42	14.26	96.34
16	Pakalkkuri Local	196.43	10.34	6.14	17.89	88.80
17	Kuttara Local	226.80	11.63	6.34	18.25	168.44
18	VellariVarikka	309.00	12.48	8.24	21.28	150.28
19	Kappa Manga	552.52	15.85	9.84	28.24	257.42
20	Nattumavu	63.46	7.42	5.26	13.24	34.60

APPENDIX-II

Stone characters of different mango genotypes

Sl. No.	Genotypes	Stone length (cm)	Stone breadth (cm)	Stone weight (g)
1	Kotookonam Varikka	6.78	3.98	29.34
2	Thali	5.98	3.52	28.14
3	Vellari	7.22	4.23	35.54
4	Kochu Kilichundan	5.54	3.24	19.92
5	Unda Varikka	5.21	4.02	27.88
6	Paiveli Local	4.96	3.68	17.96
7	Vazhapazhiti	4.90	3.58	16.76
8	Pandi Manga	5.68	4.36	31.02
9	Champa Varikka	6.92	4.02	30.57
10	Kili Manga	6.54	3.56	31.72
11	Peraykka Manga	7.66	4.46	39.54
12	Sreekaryom Local	5.94	4.47	39.12
13	Mylapoo	5.78	4.10	33.24
14	Kasthuri	5.14	3.78	19.36
15	Attanari	5.34	3.64	15.44
16	Pakalkkuri Local	6.88	4.65	35.94
17	Kuttara Local	6.78	4.46	25.82
18	Vellari Varikka	6.87	5.23	19.27
19	Kappa Manga	10.12	5.72	52.46
20	Nattumavu	4.36	3.12	15.56

APPENDIX-III

Weather data during the grafting period

Month	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max	Min	Max	Min	
June-18	32.22	24.34	93.34	82.33	12.34
July-18	31.54	23.78	91.54	78.76	9.87
August-18	30.65	23.45	93.32	79.54	10.23
September-18	32.00	23.67	91.67	77.54	9.78
October-18	31.65	24.12	93.54	82.12	11.13
November-18	30.13	23.87	94.56	83.45	15.43
December-18	30.32	22.56	93.55	76.66	8.89
January-19	31.00	20.12	92.65	74.87	0
February-19	32.12	22.67	91.76	76.43	0
March-19	33.43	24.87	91.54	73.76	0
April-19	34.67	26.87	86.45	72.10	0

APPENDIX-IV

Benefit Cost Ratio of mango graft production

Fan and pad system area	= 500 m^2
No. of mango stones sown	= 20000
Germinated mango stones (@ germination 82.22 %)	= 16600
Pre sowing treatments	= 200 ppm GA_3
Grafting method	= epicotyl grafting
Modified environment	= fan and pad system

A) Material cost

Mango stones (@ Rs. 1 /stone)	= 20000
Poly bags (@ Rs. 2 /polybag)	= 33200
Pro trays (@ Rs. 15 /polybag)	= 6000
Pro tray media	= 10000
Sand (10 ton)	= 30000
Soil (10 ton)	= 5000
Cow dung (10 ton)	= 20000
Chemicals (Plant growth regulators + plant protection)	= 3000
Total material cost	= 127200

B) Labour cost (@ Rs. 750 /day/person)

Polybag filling	= 45000
Sowing of stones	= 11250

Lifting of seedlings	=11250
Grafting	= 120000
Planting	= 45000
After care of grafts	= 55500
Total labour cost	= 288000

C) Fixed cost of fan and pad polyhouse per annum

Annual usage	= 360 days
Life span of fan and pad polyhouse (n)	= 15 years
Interest rate (i)	= 11 % per annum
Cost of installation of fan and pad polyhouse (S)	= 120000
Fixed cost of fan and pad polyhouse	$= \frac{i(i+1)^n}{(i+1)^n-1} \times S$
	$= \frac{0.11\ (0.11+1)^{15}}{(0.11+1)^{15}} \times 120000$
	= 166878

D) Variable cost per annum

Maintenance cost of fan and pad system	= 2 % of cost of installation of fan and pad polyhouse
	= 24000

E) Cost of energy

Total energy requirement per annum (@ Rs. 6/kWh)	= No. of days x electricity charge/ kWh x energy/ day)
	= 360 x 6 x 5
	= 10800
Total expenditure	= A +B+C+D+E
	= 616878

Total number of epicotyls grafts obtained (@ 77.00 % survival rate)	= 12660
Total benefit (@ Rs. 75/graft)	= 949500
Net profit	= 332622
Benefit Cost Ratio	**= 1.54**

EVALUATION OF PROPAGATION TECHNIQUES AND ROOTSTOCK STUDIES OF MANGO (*Mangifera indica* L.)

by

RESHMA. U. R
(2016-22-001)

Abstract of the thesis

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ABSTRACT

An investigation entitled "Evaluation of propagation techniques and rootstock studies of mango (*Mangifera indica* L.)" was carried out during 2016–2019 at Department of Pomology and Floriculture, College of Agriculture, Vellayani. The investigation aimed to screen local mango varieties/ collections for polyembryony, to study the pre-sowing treatments, sowing positions and age of stone after extraction from fruit on germination of mango stones, to screen local mango varieties for use as dwarfing rootstocks and to study the effect of two propagation methods in three modified environments on three varieties of scions.

Out of twenty local mango varieties collected from different parts of Thiruvananthapuram district of Kerala, seventeen were polyembryonic while three were monoembryonic. The mango var. Kappa Manga (T_{19}) recorded the highest germination per cent (73.33 %), germination index (2.41) and seedling vigour index on growth basis (2795.20). The mean germination time (17.50 days) was the least in Vellari Varikka (T_{18}). Kotookonam Varikka recorded the highest per cent polyembryony (65.13 %) and produced the highest number of plantlets per stone (5.00). Microsatellite analysis of all the plantlets from two varieties *viz.,* Kotookonam Varikka and Kochu Kilichundan that exhibited the highest percentage of polyembryony were done using 20 SSR primers and the products were compared with their respective mother plants. All the seedlings obtained from the respective stones had identical SSR profile to the mother plant, which indicated nucellar origin of seedlings having similar genetic composition to the mother plant. The zygotic seedling might have degenerated at very early stage of growth and the remaining nucellar seedlings were all vigorous.

To study the effect of pre-sowing treatments, sowing positions and age of stone after extraction from the fruit on germination of mango stones, an experiment was laid out in completely randomized design with 42 treatment combinations replicated thrice. The treatments comprised two sowing positions *viz.,* flat (S_1) and stalk end up (S_2), three age of stones after extraction from fruit, *viz.,* freshly extracted (A_1), 10 days (A_2) and 20 days after extraction (A_3) and seven pre-sowing treatments *viz.*, 100 ppm GA_3 (T_1), 200 ppm GA_3 (T_2), 1 ppm KNO_3 (T_3), 2 ppm KNO_3 (T_4), cow dung slurry(T_5), water (T_6), control [without treatment (T_7)] and their combinations. The variety Kotookonam Varikka was

utilized for the study. The stalk end up sowing method and freshly extracted stones proved to be the best with respect to germination and vigour of mango seedlings. The stones treated with 200 ppm GA_3 required minimum number of days for initiation of germination (22.62 days), 50 % germination (31.78 days), exhibited the highest germination percentage (62.59 %), rate of germination (0.48), vigour index on growth basis (2310.02) and weight basis (657.09). Treatment with 100 ppm GA_3 produced the highest seedling length (35.70 cm) and dry weight (10.39 g) at 4 month after sowing (MAS). Interaction effects also indicated that the freshly extracted stones sown by stalk end up method after treatment with 200 ppm GA_3 for 24 hours resulted in significantly the highest germination rate (0.74) and the least number of days for initiation of germination (13.00 days).

An attempt was made to identify the local mango varieties for use as dwarfing rootstock based on morphological, physiological and anatomical features. The experiment was laid out in completely randomized design (CRD) with ten genotypes replicated thrice. At 4 MAS, the lowest seedling length (29.48 cm) was noticed in Kochu Kilichundan (T_4), followed by Unda Varikka (T_{10}) and the highest seedling length (56.11 cm) was in Kappa Manga (T_8). Moreover, the germination percentage of Kochu Kilichundan (46.67 %) was on par with all the varieties except Kappa Manga (71.11 %) along with the lowest vigour index-I and vigour index- II was on par with Unda Varikka. However, the least dry matter of seedling (9.66 g) was recorded in Unda Varikka.

At 6 MAS, majority of the morphological features were the highest in Kappa Manga. The highest starch content (8.53 %) was estimated to be in Kasthuri (T_2). Kochu Kilichundan and Unda Varikka exhibited dwarfism with less plant height (38.77 cm and 40.20 cm respectively), but the former had the least internodal length (3.16 cm). The highest values for number of leaves (23.20), leaf length (12.59 cm), leaf width (4.07 cm) and average leaf area (22.57 cm^2) were recorded in Unda Varikka compared to Kochu Kilichundan. However, the highest number of roots (28.53), root length (35.02 cm), dry weight of root (3.10 g), dry weight of shoot (1.46 g) and stomatal density (51.68) were recorded in Kochu Kilichundan while total leaf area of the two varieties were on par. Moreover, Kochu Kilichundan had the highest phenol content in apical bud (60.57 mg/g) and leaves (29.03 mg/g) and bark percentage of root (23.69 %) and shoot (34.02 %) of

all the varieties. Membrane stability index, relative water content, transpiration rate and leaf temperature were non-significant. The anatomical studies revealed the highest phloem-xylem ratio both in stem (0.99) and root (1.35) and the least total conduit area of root (6.38 mm^2) in Unda Varikka while the least total conduit area of stem (5.42 mm^2) was in Kochu Kilichundan, indicating the dwarfing potential of both Kochu Kilichundan and Unda Varikka.

To study the effect of propagation methods and modified environments on different varieties of scion, an experiment was laid out in completely randomized design with eighteen treatment combinations replicated thrice. The treatments comprised two propagation methods *viz.,* epicotyl (P_1) and softwood grafting (P_2), three modified environments *viz.,* climate controlled [fan and pad (M_1)], humid chamber (M_2) and natural shade [75 % shade (M_3)] and three varieties of scions, Kalapady (V_1), Neelum (V_2) and Kotookonam Varikka (V_3) and their combinations. The grafts produced by epicotyl grafting resulted in significantly higher scion length (15.80 cm), had the least number of days for leaf opening (15.07days) , first (12.19 days) and last sprouting (22.02), higher number of grafts sprouted at weekly intervals, higher initial success percentage (83.21 %), graft establishment percentage (72.22 %), number of leaves per graft (15.40), leaf length (15.27 cm), leaf width (3.36 cm), leaf area (41.69 cm^2), number of nodes on scion (21.63), internodal length (5.20 cm), number of growth flushes per graft (1.76), lower number of days taken between grafting to first vegetative flush (26.06 days) as well as to second vegetative flush (44.98 days) and higher final survival of grafts (65.19 %). The micro climate controlled by fan and pad system produced most conducive conditions for vegetative growth of mango grafts. Among the different varieties of scions, Kotookonam Varikka recorded the highest girth of rootstock, girth of scion, length of scion, graft height, spread of plant in N-S direction and E-W direction, number of grafts sprouted at weekly intervals, initial success percentage, percentage of graft establishment, leaf width, number of nodes on scion, internodal length, number of growth flushes per graft and the lowest number of days taken between grafting and second vegetative flush. Kalapady recorded the least number of days for first and last sprouting, leaf opening and for first vegetative flushing. The greatest sprout length, number of leaves, leaf length and leaf area were recorded in Neelum. The scion did not

influence the final survival of grafts. The treatment combinations had no influence on root length. Interaction effects also confirmed that epicotyl grafting method with Kotookonam Varikka variety as scion under controlled conditions using fan and pad system resulted in grafts with higher scion length (16.80 cm), sprout length (6.05 cm), more number of growth flushes per graft (2.33) and earlier second vegetative flush (40.87 days).

Based on the above findings it could be concluded that the highest percentage of polyembryony was in Kotookonam Varikka and microsatellite analysis revealed the nucellar origin of plantlets and confirmed higher vigour of nucellar seedlings over sexual seedling. Stalk end up sowing of freshly extracted stones treated with 200 ppm GA_3 for 24 hours recorded better germination and vigour of mango seedlings. The mango variety Kochu Kilichundan and Unda Varikka were identified as promising rootstocks to impart dwarfness and Kochu Kilichundan was superior in most of the morphological and physiological characters. The epicotyl grafts of Kotookonam Varikka under controlled climate by fan and pad system recorded better graft establishment, survival, vegetative and growth parameters.

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