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PROPAGATION OF PUMMELO (*CITRUS GRANDIS* L. OSBECK) BY AIR-LAYERING

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ABSTRACT

Different experiments were carried out in mid-hill (1000 m) and Terai (72 m) of Nepal to determine the effect of rooting media, branch positions, seasons, plant growth regulator and age of mother plant on rooting of pummelo layers. The layers made on Cocopeat as rooting medium produced highest percentage of rooted layers (88%) followed by Madhumas (84%). The one year old branches exposed to sun light gave highest success in rooting when layering was carried out in July (82%) and April (77%). The percentage of rooting decreased linearly with increased age of mother trees. The trees less than 20 years old gave better results in comparison to older trees. Success rate in layering was similar both in Terai and mid-hill conditions and application of auxin (Rootex-3) did not impart significant effect to increase the percentage of rooted layers. The conditions associated with higher percentage of rooted layers also produced higher number and longer roots. Statistically, the success rate in rooting was similar in the layers made in April and July but the layers prepared in April gave higher success rate (85%) than the layers prepared in July (67%) at nursery establishment.

Additional Key Words: *Citrus* spp., vegetative propagation, rooting media

INTRODUCTION

Pummelo or shaddock (*Citrus grandis* L. Osbeck syn. *C. maxima* J. Burman) has been regarded as one of the ancestral and first domesticated *Citrus* species. It is believed that the species was originated in south-east Asia and domesticated in southern China. Pummelo fruits of superior varieties are valued highly for fresh consumption in China, Japan and Thailand. At present, pummelo production is wide spread in warm and humid climate of Asia and Pacific regions. The principal countries for commercial production are Thailand, China, Taiwan, Japan, Malaysia and Indonesia. In other countries of the region pummelo is mainly grown in homestead gardens. Pummelo ranks third and contributes 11.2 percent of Thailand's total fruit export (Subhandrabandhu, 1992). Several parts of the plant such as leaves, flowers, fruits and seeds are used for the treatment of cough, fever and gastric disorders in many countries of south and south-east Asia (Niyomdham, 1991).

Pummelos are mono-embryonic in nature and produce only zygotic seedlings. Cross-pollinated crops like pummelo are heterozygous and do not normally produce true to type trees if raised from the seeds. Some pummelo varieties are self-incompatible and seedless. Vegetative propagation is the only way for sapling production and maintenance of such varieties. While a few countries have standardized the vegetative propagation methods of pummelo, many countries are still using seedling for planting. The common propagation method of pummelo is air layering in Thailand. It is mainly propagated by grafting. The effects of various rootstocks on fruit yield and quality have been studied in China, Taiwan (Wan et al., 1992; 1994; Xiao et al., 1992) and Japan (Okudai et.al. 1991). Chattopadhyay

and Swarnakar (1993) reported significant effect of seasons and climate on the success of grafting and budding in pummelo.

In Nepal, farmers have been growing pummelo for centuries in their homestead gardens for domestic consumption and religious purposes. All pummelo trees in Nepal are seed propagated, which come into bearing only after 5 to 8 years of planting. As farmers are cultivating highly variable trees, which are propagated from seed, quality of production is very low. No attempts have been made yet to standardize the vegetative propagation methods of pummelo in Nepal. Therefore, studies on air-layering were carried out to investigate the appropriate season, media, branch position and age of mother plant suitable for agro-ecological conditions of Nepal.

MATERIALS AND METHODS

Three different experiments were carried out to determine the effect of rooting media, seasons, branch positions, plant growth regulators and climate on rooting of air-layers. In the first experiment, success in layering was evaluated using five types of rooting media namely moss, clay, clay + cowdung, Madhumas and cocopeat in mid-hill (1000 m) and Terai (72 m). Moss grown on the trunk of old trees was collected from natural forest and dried under sunlight. Before using moss for layering water was applied to moisten it. Clay soil was collected from uncultivated clean field. Small gravel and other unwanted materials were removed. Mud or paste strong enough to hold on stem was prepared by mixing with water. Paste of clay and cow dung mixture was prepared by mixing equal amount (v/v) of clay and well-decomposed cow dung with water. Madhumas (Lignocell Ltd. Sri Lanka), a vegetable-based medium with no added nutrients is available in the form of dried and compressed bricks in market. When Madhumas brick is soaked with water, it breaks up and volume increases. Cocopeat (GM Enterprises, Nepal) is a blended mixture of sterilized cocopeat, loam and bone meal supplemented with other essential nutrients. Second experiment was carried out in Terai and mid-hill regions using the branches from shade and sunny positions of trees in April, July and Sept. Cut portions of the layers were either treated with Rootex-3 or layering was carried out without any application of auxin. Effect of age of mother trees on success of rooting was evaluated in the third experiment. Mother trees with 5, 10, 20, 40 and 60 years of age were used for air-layering.

All experiments were carried out with the trees grown and maintained by farmers in their homestead gardens. Unless otherwise stated the layering was carried out during the third week of June using moss as rooting media and 15 years old trees. Healthy branches of pencil sized thickness were selected. A ring of bark about 3 cm wide was removed completely from selected branches at a point 20-25 cm from the tip end. The exposed wood was then bruised with grafting knife to ensure the removal of phloem and cambium. The ringed area was covered with handful of moist rooting medium and then wrapped up completely by a piece of polyethylene sheet. The ends of the plastic were tied up so that no loss of moisture occurs from the rooting media.

All experiments were laid in a randomised complete block design. One tree was considered as one block and there were 24 branches (layers) per experimental unit. The layers were cut from mother plants after ninety days of layering. The plastic sheet and rooting media were removed carefully without damaging roots. Data on the number of rooted layers, number of roots per layer and length of roots were recorded. Rooted layers made in April, July and Sept (Expt. # 2) were potted in polyethylene bags after the completion of data recording. Potting medium was composed of clay and well-decomposed compost in equal ratio. Potted layers were maintained in semi-shaded area and evaluated for establishment after

90 days.

The experimental data were analysed using statistical software, MSTAT. Percent data were transformed to Arcsine value and data containing small numbers (below 10) were transformed by square root method to normalize them before performing analysis of variance. Means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The type of media used for layering significantly affected the percentage of rooted layers, number of roots and root length ($P \leq 0.01$). However, the main effect of location and its interaction with media did not affect rooting and root characteristics significantly (Data not shown). The layers made on cocopeat produced highest percentage of rooted layers (88%) followed by Madhumas (84%). A significant positive correlation ($r = 0.69$) was found between the percentage of rooted layers and the number of roots. The media such as cocopeat and Madhumas associated with higher percentage of rooted layers also produced higher number of roots per layer (Table 1). Cocopeat and Madhumas possess high levels of water holding capacity and porosity for aeration. It was very likely that these media were free from pathogens because they were available in sterilized form in the market. Higher rate of success and production of better quality roots with Cocopeat and Madhumas may be associated with these characters. The air-layers made on the medium containing clay or on mixed media containing clay and cow dung were poor for all root characteristics. Higher rates of layer mortality were noted where clay alone or mixed medium containing clay and cow dung was used for layering. The primary reasons for the mortality of layers was rotting of shoots at cut portions and it is likely that rotting was caused by the pathogenic microorganisms present in clay and cow dung.

It was observed that the months of layering and branch positions were most critical for higher rate of success in layering. The percentage of rooted layers was the same in Terai and mid-hill of Nepal. This indicates that location specific recommendation might not be required and same set of practices may be equally applicable for both locations. Application of auxin (Rootex-3) increased the percentage of rooted layers and quality (number and length) of roots. Lack of effects of auxin could be attributed to the fact that pummelo branches used for layering were possessing sufficient amount of endogenous auxin required for root initiation and development. Dutta and Singh (1993) also obtained 100 percent rooted layers in *C. karna* and *C. jambhiri* without the use of growth regulators.

Table 2 shows the percentage of rooted layers (success in rooting) obtained in different months of layering and from different branch positions. The branches exposed to sunlight gave highest success in rooting when air- layering was carried out in July (82%) and April (77%).

Table 1. Effect of rooting media on root characteristics of layers.

Media	% Rooting	% Branch mortality	Root No.	Root length(cm)
Moss	70.0[b]	11.7[c]	4.46[b]	4.48[a]
Clay	67.8[b]	21.7[b]	4.17[b]	3.26[b]
Clay + cow dung	47.1[c]	40.2[a]	2.92[c]	2.79[b]
Madhumas	84.0[a]	2.4[d]	6.21[a]	4.97[a]
Cocopeat	88.3[a]	3.4[d]	5.05[b]	4.59[a]

Means followed by same letters within each column are not significantly different at 5% by DMRT

The continuous maintenance of moisture in rooting media, a most important requirement for higher success in air-layering was possible during this season because of regular rainfall and higher level of humidity in the atmosphere. The average temperature at experimental sites during this period ranged from 28 to 34^0 C in Terai and 24 to 28^0 C in mid-hill region. These temperature ranges are considered to be suitable to initiate rooting in most of the evergreen tree species (Hartmann and Kester, 1983). On the other hand, higher percentage (30%) of layers made from the branches of shady part of the canopy died when air-layering was carried out in April or July. The continuous presence of supra-optimal moisture in rooting media seems to be the reason for higher rate of mortality. Since layers prepared in April and July were with their mother plant during the rainy season, it is highly likely that the media received more moisture than they required. But, excess moisture could not evaporate from media due to low temperature and poor movement of air at shady positions. It is very likely that higher temperature coupled with more air movement facilitated the evaporation of unnecessary water from the media of the branches exposed to sunlight thereby maintaining optimum moisture level and reducing the mortality rate. Higher rate of mortality of layers was found irrespective of their positions when air-layering was carried out in Sept. Failure to maintain sufficient moisture in rooting media due to low rainfall during autumn season was most likelihood reason for lower rate of success of layer during this season.

The number of roots per layer was affected significantly by seasons ($P < 0.01$), locations ($P < 0.05$) and positions of branches ($P < 0.01$). The interaction of seasons, branch positions and locations was also significant for root number. The highest numbers of roots were produced in mid-hill when air-layering was carried out in April or July using branches from exposed position. However, layers of the same position and location gave low number of roots per layer (2.70) when air-layering was carried out in the month of Sept. (Table 3). In Terai, equal number of roots was produced from the layers of shade and sunny positions irrespective of months of layering. But in mid-hill, exposed layers produced more roots than from shaded layers if layers were made in April or July. In Terai, branches exposed to sunlight produced the longest roots (4.6 cm) in comparison to others. Longer roots were noticed on average from air-layers exposed to sun light in comparison to shaded layers and also in Terai than in mid-hill areas.

The percentage of rooted layers decreased with the older mother plants indicating the

age of mother plant on rooting ability of layers. The highest percentage of rooted layers (81.7%) was noted with five years trees, although, this value was not significantly different from the 10 year (77.9%) and 20 year (77.5%) old trees (Table 5). However, percentage of rooted layers in 40 and 60 years old mother plants were significantly lower than in 5, 10 and 20 year old trees. The age of the mother plants also affected the number and length of roots per rooted layer. The younger mother plants, such as 5, 10 and 20 years old produced significantly more and longer roots than 40 and 60 years old trees. The decreased ability to form adventitious roots with increasing age of the plants was also observed in apple, pear, and Douglas fir (Hartmann and Kester, 1983), and mango (Basu *et al.*, 1985). The cause of poor rooting when stock plant becomes older is not clearly understood. However, many researchers (Hartmann and Kester, 1983; Basu *et al.*, 1985) consider an increased level of root inhibitors and /or a decreased level of auxin cofactors as the major reason for poor rooting in older plants.

Table 2. Effect of month of air-layering and branch positions on the success rate.

Month of layering	Branch Position		Mean
	Shade	Sunny	(Month)
September (Autumn)	53.6b	25.3c	39.5
April (Spring)	49.9b	76.7a	63.3
July (Rainy)	53.9b	82.2a	68.0
Mean(Branch Position)	52.5	61.4	56.9

Means followed by same letters are not significantly different at 5% by DMRT.

Table 3. Effect of month of layering , locations and branch positions on number of roots per layer.

Month of layering	Hill		Terai		Mean
	Shade	Sunny	Shade	Sunny	(month)
Sept (Autumn)	3.0^{cd}	2.7^{d}	3.7^{cd}	3.1^{cd}	3.1
April (Spring)	4.7^{bc}	7.1^{a}	4.5^{bc}	4.8^{bc}	5.3
July (Rainy)	4.8^{bc}	6.7^{a}	4.3^{bc}	5.2^{b}	5.2
Mean	4.1	5.5	4.20	4.4	

Means followed by same letters are not significantly different at 5% by DMRT.

Table 4. Effect of age of mother plants on percentage of rooting and other root characteristics in air-layering of pummelo.

Tree age (years)	Percentage of rooting	Root Number	Root Length(cm)
Five	81.7[a]	6.05[a]	4.41[a]
Ten	77.9[a]	4.20[b]	3.92[a]
Twenty	77.5[a]	4.04[b]	3.70[a]
Forty	44.7[b]	2.2[c]	2.01[b]
Sixty	34.4[b]	1.9[c]	1.93[b]

Means followed by same letters are not significantly different at 5% by DMRT.

Propagation of plants by air-layering was accomplished in two stages. In the first stage, roots were formed on stems while they were still attached to the mother plant. Separation of rooted layers from mother plants and establishing them in the nursery was accomplished in the second stage. Most reports on air-layering generally do not include performance of layers in second stage. However, results of the present study revealed that the percentage of rooted layers may not be the indicator of success rate since all of them could not survive when established in the nursery. Table 6 shows a nursery establishment of layers prepared during April, July and Sept. The success rate in rooting was similar with layers made in April and July (Table 3), but layers prepared in April gave more success at nursery establishment than those prepared in July. Layers prepared in April were established at nursery in July and their rate of nursery establishment was 86 % in Terai and 84 % in mid-hills. The layers prepared in July were transferred to establishment nursery in September. The nursery establishment rate of these layers was 74 % in Terai and 60 % in mid-hill area. Most of the layers prepared in Sept. could not establish in the nursery when these layers were potted in Dec. The rate of nursery establishment of layers was better in Terai than in mid-hill area in all three seasons.

Table 5. Survival rate of layers during nursery establishment in different season

Month of layering	Month of nursery establishment	Location (Climate)	Number planted	Number established	Percentage of establishment
April	July	Hill	96	81	84
		Terai	100	86	86
				Mean	85
July	October	Hill	104	62	60
		Terai	103	76	74
				Mean	67
September	December	Hill	61	17	28
		Terai	67	29	43
				Mean	49.5

Survival rate of rooted layers during their establishment at nursery seems to be closely associated with atmospheric temperature and relative humidity of the season. Layers made in April were removed from the mother plant and potted for establishment in July. Three months, July, Aug. and Sept., were considered to be the best for establishment of these layers. In Terai, mean maximum and minimum temperatures were 33 and 26^0 C respectively during this period. Mid-hill region was also warm during this period with mean maximum temperature of 27^0 C and a minimum of 20^0 C. Furthermore, this is the main rainy season with frequent rainfall and high level of relative humidity in atmosphere. Layers prepared in July were potted in Oct. and those made in Sept. were potted in Dec. The temperature and precipitation were decreased gradually after Sept. in Terai and mid-hill regions and this reflects decrease in the success rate of layers during their establishment at nursery. In all seasons, layers from Terai region showed better performance for nursery establishment over the layers from mid-hill which may be attributed to warmer climate of the region.

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