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The Performance of Acid Lime (*Citrus aurantifolia* Swingle) Cultivars in Mass Propagation Using Hard Wood Cuttings under Shade Net

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Abstract

The experiment was carried out during (June–Sep) during (June–September) 2019–2020 and 2020–2021, respectively at College of Horticulture, UHS, Bagalkot, Karnataka, India to evaluate the mass propagation performance of different cultivars of acid lime (*Citrus aurantifolia* Swingle) *viz*. Kagzi lime, Sai Sharbati, Balaji, Vikram and Pramalini using a methodology involving hardwood cuttings, treatment with 500 ppm IBA, cocopeat as growing media under shade net condition. The statistical design employed was Completely Randomised Design (CRD). The results were significantly affected in all parameters. The lowest number of days taken for sprout initiation was recorded in cv. Balaji (8.5 days) and highest was in cv. Vikram (13.4 days), respectively. The highest and lowest sprout percent was recorded in cv.Vikram (91.66%) and cv. Kagzi lime (48.61%). Shoot length was increased up to 90 days and the highest shoot length was in cv. Vikram (13.10 cm) and lowest was in cv. Balaji (7.19 cm). The highest and lowest fresh and dry weight was recorded in cv. Saisarbati (8.54 g and 6.29 g) and cv. Pramalini (7.19 g and 3.67 g), respectively. The highest and lowest number of primary roots was recorded in cv. Vikram (4.2) and Sai sarbati (2.6), highest and lowest average root length was recorded in cv. Vikram (18.70 cm) and Kagzi lime (12.26 cm), respectively, highest and lowest rooting percent was recorded in cv. Vikram (88.88%) and Kagzi lime (45.83), respectively at 90 DAP. There was considerable variation in responses which genotype dependent. The results are helpful in future root stock studies as well as mass multiplication of planting material on commercial scale.

Keywords: Cuttings, IBA, acid lime, mass propagation, shadenet, kagzi

1. Introduction

The acid lime (*Citrus aurantifolia* Swingle) is an important member of *citrus* group of fruits that belongs to family Rutaceae. It is one of the most economically important fruits grown worldwide. It is originated in East Indies and hasspread in entire world in both tropical and sub-tropical areas. It is an ever greensmall tree with spines and branched irregularly. It bearssmall, elliptic to oblong leaves which are lightgreen. The flowers are white in color, small and produced auxiliary in clusters. The fruits are small to medium, round and thin-skinned with yellow in color with whitish pulp. It has high nutritive and medicinal values reduce inflammation; improve gastrointestinal function and health (Anand and Debashish, 2020). The juice is a rich source of vitamin C, carbohydrates, proteins, fat and contains a fair to good amount of minerals such as K, Ca, Fe, Mg, Na, S and P. Further, the peel contains volatile oil thatis used in the production of sent and various kinds of sweet. The lime is adjustable to a wide range of Agro-climaitic conditions and is hard enough to withstand harsh conditions compared to many other fruit crops. These qualities made lime an important and one of the most cultivated and used fruits of India.

Further, with increasing awareness about the nutritional security and faster development of processing industries throughout the globe, the demand of this crop has increased and that leading to area expansion under this.

Generally, acid lime is regenerated through seeds, but there is a problem of nonuniformity of progeny and high chance of viral disease contamination by this method (Babu, 2001). The vegetative multiplication through cutting is comparatively

cheap, simple, practicable and does not require the special techniques as required in other vegetative methods. Hence,it is widely used option for augmenting natural regeneration as well as for large scale cultivation programmes. However, the success of stem cuttings depend on many factors associated with plants such as age of the mother plant, parts used of tree, time of planting, rainfall, humidity, temperature, rooting media and after care (Frey et al., 2006; Das et al., 2021; Guchhait et al., 2024). Further, research studies reported varied success in various parameters during multiplication of acid lime and other members of citrus using cuttings viz., in lemon (*Citrus limon* Burm.) cv. pant lemon^{-1} (Bhatt and Tomar 2011) under polyhouse, in *Citrus aurantifolia* S. (Kagzi lime) using hardwood and IBA 500 ppm (Bhatt and Bhatt, 2014), in *Citrus medica* L. (Corsian) using semi hardwood and IBA (500 and 1000 ppm) (Kako et al., 2014), in citrus for improving bud grafting efficiency (Niedz and Bowman, 2023), in other citrus species (Pirlak and Cinar, 2020; Pokhrel et al., 2021; Solonia et al., 2020). It indicates necessity of proper standardization of procedures for mass multiplication of good quality planting material. Hence, considering the importance of cultivar or genotype, PGR and condition of incubation of cuttings for root induction we have standardized a procedure for mass multiplication of acid lime (*Citrus aurantifolia* S.) cv. KagziLime in our earlier studies (Prakasha et al., 2023).

However, as we understand the variability in root induction is highly genotype dependent, hence, we opt to assess the procedure standardized by us in acid lime Cv. Kagzi lime with commercial cultivars of acid lime before concluding the wider application of procedure. Hence,the present research was formulated to assess performance of four commercial acid lime cultivars (Vikram, Pramalini, Sai Sharbati and Balaji) in mass propagation using hard wood cuttings under shade net.

2. Materials and Methods

The present experiment was conductedduring the year 2019–21 [4 months (June-Sep) during 19–20 and 4 months (June–Sep) during 20–21] at College of Horticulture, Bagalkot, University of Horticultural Sciences, Navanagar, Bagalkot, Karnataka, India.The University was geographically, locating at 16.1635° North latitudes, 75.6172° East longitudes and at an altitude of 678 meter above the MSL.

In this study, best treatments from our earlier experiments have been pooled to make a procedure for evaluation of responses of various acid lime (*Citrus aurantifolia* Swingle) cultivars viz. Kagzi lime (standard check), Sai Sharbati, Balaji, Vikram and Pramalini.The procedure involves cocopeat as growing media, hardwood cutting, IBA 500 ppm and shade net as propagation condition. The study was laidoutin Completely Randomised Design (CRD) involving five (the cultivars) treatments with four replications. Different varieties of acid lime trees (of cultivars used in the study) grown in the orchard of the Department of Fruit Science, College of Horticulture, Bagalkot and MHREC, University of Horticultural Sciences, Bagalkot were selected for this experiment on the basis of their uniformity in appearance, growth habit, free from visual symptoms of pests and disease occurrence.

2.1. Preparation of hard-wood cuttings

The branches of pencil thickness from the past season growth having an age of more than nine months dark green bark were selected from the mother plant. All the leaves were removed by using secateurs. The length of the cuttings was about 15–20 cm with 6–8 nodes. The slant lower cut was made below a node at the lower end.

2.2. Preparation of cocopeat as growing media

It is a multipurpose growing medium made from coconut husk. The fibrous coconut husk was pre washed, machine dried, sieved and made free from sand and other contaminations such as animal and plant residue. The cocopeat is a very good alternative to traditional peat moss and rock wool. Its airfilled porosity and highwater holding capacity makes it, an ideal growing medium for the plant crops. It was 100% organic and eco friendly, free from soil borne pathogen and weed. It had a pH of 5.7–6.5, EC level <1 mS cm^{-1} is ideal for plant growth, purchased from certified nursery vendors. It was soaked overnight in water and filled into polythene covers of 4×5 inches for use in the study

2.3. Preparation of 500 ppm IBA solution

An accurate amount of 500 mg of IBA powder was weighed using precision balance, collected in a small glass beaker, dissolved in little quantity of 0.1 N NaOH and the volume was made up to one litre by adding distilled water.

2.4. Treatment applied and planting of cuttings

A node of cutting atbasal portion of the hardwood cuttings was dipped in aqueous solution of IBA (500 ppm) before planting in the polycovers by inserting just more than one-third portion. The cuttings weretilted slant in the medium to make sure of exposure of more surface area to rooting media and also to make sure to drain out of water droplets accumulated on cutting during irrigation. The planted cuttings were irrigated regularly depending upon soil moisture conditions.

2.5. Recording of observations

The observations were recorded for days taken for sprout initiation, sprouting percentage, sprout numbers, leaves number, length of shoot at 30, 60 and 90 DAP. Further, fresh weight (complete cutting), dry weight (of entire cutting after hot air oven driedat 36°C for 24 hours), number of primary roots, length of the longest root and rooting percentage was recorded after 90 days after planting. The observations were expressed in percent, cm, gram, day etc units as necessity.

The data recorded for all the parameters were statistically analyzed by following completely randomized design (CRD) at 5% level of significance. The analysis has been done in Web Agri-Stat Package (WASP 2.0) developed by ICAR Research

Complex, Goa. The standard error of mean was calculated and the critical difference at 5% level of probability was worked out to compare treatment means wherever F test was significant.

3. Results and Discussion

The performance of cuttings of different varieties of acid lime was influenced by the procedure involving growing media (cocopeat), type of stem cutting (hardwood cutting), plant growth regulators (IBA 500 ppm) and propagation condition (shade net) in present investigation.

3.1. Days taken for sprout initiation

Number of days taken for sprout initiation in five different varieties was significantly affected by procedure/protocol (Table 1). The minimum number of days taken for sprouting was recorded in T_3 (Balaji) with 8.50 days, whereas, the maximum number of days taken for sprout initiation was recorded in treatment T_5 (Pramalini) with 13.40 days. It might be because of variation in wood maturity in cuttings of different varieties which probably decides starch and sugar content and similar opinion was stated by Singh (2013).

3.2. Sprouting percentage

The percentages of sprouted cuttings in five different varieties were significantly affected by the protocol (Table 1). The highest sprouting percentage was the observed in treatment T_4 (Vikram) with 91.66% sprouting, whereas, lowest sprouting percentage was observed in treatment T_1 (Kagzi lime) with 48.61%. The difference in sprouting of cuttings of different variety may be directly linked with the difference in inherent growth regulator in combination with externally provided PGR to stimulate rooting and subsequent activities in the cutting. Similarly, the above findings are close in conformity with the finding of Pio et al. (2003) in rootstock regeneration of *Citrus* 'Flying Dragon' and 'Trifoliate'.

3.3. Number of sprouts cutting^{-1}

There were significant differences in number of sprouts cutting^{-1} at 30, 60 and 90 DAP among various treatments affected by protocol (Table 1). Further, the number of sprouts cutting^{-1} at 90 DAP was less as compared to cuttings observed at 30 DAP and 60 DAP as the vegetative growth of shoot increased, the number of shoot significantly got reduced (many of sprouts remained retarded and dropped off). The highest sprouts cutting^{-1} was obtained in the treatment T_2 (Sai Sharbati) with 4.40 sprouts which was on par with the treatment T_4 (Vikram) and T_5 (Pramalini) with 4.30 and 4.25 sprouts. The lowest number of sprouts cutting^{-1} obtained in treatment T_1 (Kagzi lime) with 2.3 sprouts.The genotype might have affected the cell division in the vascular cambium, cell expansion and control of differentiation into different types of cambial resulting in differential increase in number of sprouts which was also opinion of Cao et al. (2023). Similarly, Hussain et al. (2016) obtained highest number of sprouts cuttings (6.63) in softwood cuttings of sweet lime. Ahmad et al. (2013) recorded highest number of secondary branches cutting^{-1} (1.87) in rangpur lime cuttings treated with IBA 1000 ppm.

Table 1: Days taken for sprout initiation, sprouting percentage and number of sprouts as influenced by combination of best type of cutting, media, PGR treatment and propagation condition

Sl. No.	Treatment	Treatment details	Days taken for sprout initiation	Sprouting percentage	No. of Sprouts cutting^{-1}		
					30 DAP	60 DAP	90 DAP
1	T_1	Kagzi lime	8.55	48.61	7.40	3.55	2.30
2	T_2	Sai Sharbati	11.35	76.39	11.25	5.55	4.40
3	T_3	Balaji	8.50	68.05	7.45	4.00	2.80
4	T_4	Vikram	11.20	91.66	9.75	5.40	4.30
5	T_5	Pramalini	13.40	80.55	11.70	5.45	4.25
SEm±			0.10	3.07	0.12	0.12	0.09
CD (*p*=0.05)			0.30	9.23	0.36	0.33	0.25

3.4. Number of leaves cutting^{-1}

There were significant differences in number of leaves at 30, 60 and 90 DAP in different varieties as affected by the protocol (Table 2). The number of leaves cutting^{-1} in different varieties was increased at 60 DAP and further, it was decreased at 90 DAP as the length was increasing and leaves became broader resulting dropping of leaves to promote more vegetative growth of shoots. The highest number of leaves cutting^{-1} was recorded in T_5 (Pramalini) with 13.75 leaves, whereas, the lowest number of leaves cutting^{-1} was recorded in the treatment T_3 (Balaji) 8.4 leaves. The difference in increase in number of leaves cutting^{-1} might be because of difference in diversion assimilates to the leaf buds for formation of leaves that are very important for vital processes like photosynthesis and respiration and similar opinion was stated by Wahab et al. (2001). Similarly, Hussain et al. (2016) obtained highest number of leaves shoot^{-1} (4.56) in semi-hardwood cuttings of sweet lime. Bhatt and Bhatt (2014) concluded that treatment 500 ppm IBA with polyhouse condition produced the maximum number of leaves on new sprout (14.33) in acid lime (*Citrus aurantifolia* Swingle).

Table 2: Number of leaves and shoot length as influenced by combination of best type of cutting, media, PGR treatment and propagation condition

Sl. No.	Treatment	Treatment details	Number of leaves cutting^{-1}			Shoot length (in cm)			Fresh weight (g)	Dry weight (g)
			30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP		
1	T_1	Kagzi lime	3.60	12.20	8.50	1.17	4.19	11.19	5.63	4.19
2	T_2	Sai Sharbati	6.65	14.65	11.60	1.25	5.39	8.04	8.54	6.29
3	T_3	Balaji	3.60	11.75	8.40	0.88	3.97	7.19	5.28	3.83
4	T_4	Vikram	6.30	14.60	11.30	1.41	6.23	13.10	8.10	5.50
5	T_5	Pramalini	8.45	16.50	13.75	1.14	4.93	10.11	4.87	3.67
SEm±			0.10	0.12	0.12	0.03	0.04	0.32	0.09	0.09
CD (*p*=0.05)			0.30	0.34	0.34	0.07	0.11	0.95	0.27	0.27

3.5. Shoot length (cm)

There was significant difference found in shoot length at 30 DAP among varieties as affected by the protocol (Table 2). There was increase in shoot length from 30 DAP to 90 DAP in different varieties may be due to better nutrient uptake by the cuttings and suitable temperature and relative humidity. The longest shoot was observed in the treatment T_4 (Vikram) with 13.10 cm, whereas, the shortest shoot was recorded in T_3 (Balaji) with 7.19 cm. This may be because of difference in levels and activity of IBA that caused hydrolysis and translocation of carbohydrates as well as nitrogenous substances in the cellular level at the base of cuttings of different cultivars which resulted in enhanced cell elongation and c division and similar opinion was expressed by Singh et al. (2015). Similarly, Bhatt and Bhatt (2014) obtained maximum length of longest sprout (6.59 cm) with 500 ppm IBA treatment in the cuttings of acid lime.Saranand Umadevi (2011) stated that stem cuttings of lemon (*Citrus limon* L.) treated with 2500 ppm of auxin (IAA) had longest shoot length of 5.73 cm.

3.6. Fresh and dry weight (g)

There was significant difference in fresh weight of cuttings at 90 DAP in different varieties as affected by the protocol (Table 2). The highest fresh weight of cuttings at 90 DAP was recorded in the treatment T_2 (Sai Sharbati) with 8.54 g, whereas, lowest fresh weight was recorded in T_5 (Pramalini) with 4.87 g. Similarly, Singh et al. (2015) recorded average fresh weight of cutting of about 12.24 g in lemon (*Citrus limon* Burm.) cv. pant lemon^{-1} planted in Soil+Sand+Cocopeat media. These findings are in confirmation with the study by Ahmad et al. (2016) in stem cuttings of Rangpur lime (*Citrus limonia* Osbeck). There was significant difference found in dry weight at 90 DAP among the varieties as affected by the protocol. The highest dry weight of cuttings at 90 DAP was recorded in the treatment T_2 (Sai Sharbati) with 6.29 g and the lowest dry weight was recorded in T_5 (Pramalini) with 3.67 g (Table 2). Similarly, Bowmana and Albrecht (2017) reported total plant dry weight for stem cuttings of the six citrus rootstock cultivars 3208 mg plant^{-1} on an average, age ranging from 12 to 20 weeks. However, the maximum root weight (both fresh and dry) was because of the fact that the combination of auxins naturally (differs with cultivar) occurring and exogenously applied causes initiation as well as growth of roots. Further, auxin activity and degradation of auxin by auxin destroying enzyme led to the formation and vigoursness of roots growth which might be differs with genotype due to the difference in reserved food in the cuttings and similar reasons were also quoted by Singh et al. (2013)

3.7. Number of primary root

There was significant difference found in number of primary roots at 90 DAP among the varieties as affected by the protocol (Plate 1). The highest number of primary root cutting^{-1} was recorded in T_4 (Vikram) with 4.20 primary roots (Figure 1), whereas, the lowest number of primary root cutting^{-1} was recorded in the T_2 (Sai Sharbati) with 2.60 roots. Reduction in number of primary roots in the present study may be because of difference in crop, weather conditions etc factors. Similarly, Ahmad et al. (2013) found that IBA 1000 ppm treatment gave the best response with respect to maximum number of roots cutting^{-1} (3.93) in rangpur lime cuttings.

3.8. Length of longest root (cm)

There was there was significant difference found in longest root length at 90 DAP among the varieties as affected by the protocol. The average longest root length cutting^{-1} was recorded in T_4 (Vikram) with 18.70 cm, whereas, the shortest average roots length was observed in the treatment T_1 (Kagzi lime) with 12.26 cm (Figure 1). Similarly, views were held by Murkute et al. (2009) in propagation study on trifoliate orange (*Poncirus trifoliata*). The difference in increase in root length in different cultivar may be due to the varied number of sprouts shoot^{-1} whose tips produces more auxin which results in root elongation, and the effect of metabolites translocation and carbohydrates metabolism. Similarly, Bhatt and Tomar (2010) also found increase in root length in Citrus *aurantifolia* with increasing IBA concentration.

3.9. Rooting or survival (%)

The percentages of rooted cuttings at 90 DAP in five different varieties were significantly affected by the protocol. The

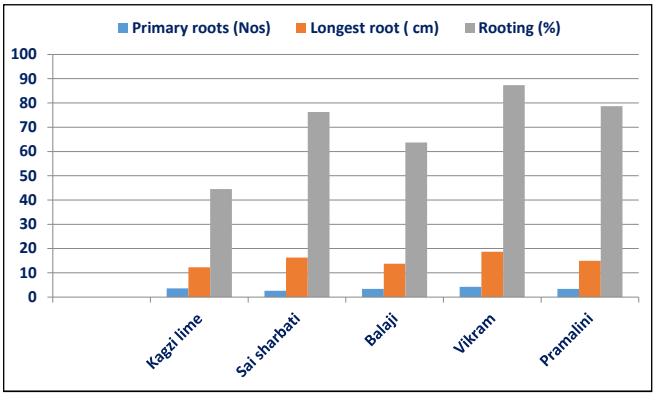

Figure 1: Number of primary root numbers, longest root (cm) and rooting (%) at 90 DAP as influenced by the procedure of mass propagation

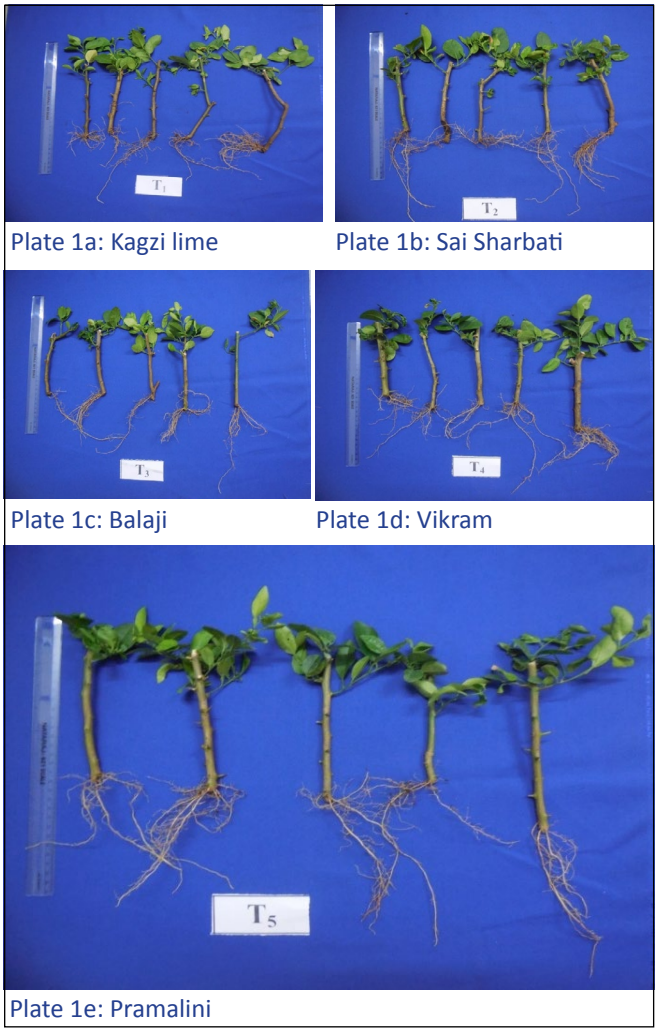

Plate 1a: Kagzi lime

Plate 1b: Sai Sharbati

Plate 1c: Balaji

Plate 1d: Vikram

Plate 1e: Pramalini

Plate 1: Shoot and rooting in different varieties of acid limeas influenced by the procedure of mass propagation

highest rooting percentage was the recorded in treatment T_4 (Vikram) with 88.88% rooting, whereas, lowest rooting percentage was observed in treatment T_1 (Kagzi lime) with 45.83% (Figure 1, Plate 1). The survival of the cuttings treated with high concentrations of IBA may be directly linked with the capacity of the growth regulator to stimulate the generation of adventitious roots system and increase in number and length of roots cutting^{-1} as influenced by the uptake of mineral nutrients and water from the soil, which helps in the survival of the cuttings (Reddy et al., 2008). The reason might be that presence of IBA has been found to stimulate cambial activity thereby resulted in the mobilization of reserve food material to the site of root initiation. Mariano (2022) has found similar kind of rooting responses and survival in selected citrus species. The enhanced hydrolytic activity in presence of applied IBA might be responsible for the increased percentage of rooted cuttings. Similarly, Bowmana and Albrecht (2017) reported that the citrus rootstalk genotype US-802 had the highest success in establishing growing plants at 8 weeks, with 82 to 91% of single node cuttings successfully rooted and growing out of six important rootstock cultivars, Swingle, Cleopatra, US-802, US-812, US-897, and US-942.

4. Conclusion

The procedure involving hardwood cutting, cocopeat, 500 ppm IBA had resulted in root induction in all the commercial acid lime (*Citrus aurantifolia* Swingle) cultivars CultivarVikram was best to perform better in shooting and rooting parameters.

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