



# Root Induction Influenced by Strength of Tissue and Propagation Conditions in Acid Lime cv. Kagzi Lime

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## ABSTRACT

The investigation was conducted during the year 2019–2021 at College of Horticulture, UHS, Bagalkot in acid lime [*Citrus aurantifolia* (Christm.) Swingle] cv. Kagzi lime to ascertain influence of strength of tissue and propagation conditions on ability of rooting. The treatments included three types of cuttings (shoot tip, semi-hardwood and hardwood cuttings) and three propagation conditions (shade net, poly house and mist house). Coco peat filled portrays was used as the standard rooting media and container. The experiment was laid out in two factorial completely randomised design (FCRD) with three replications. There was significant influence of both strength of tissue and propagation conditions on shoot and root parameters. Combination of these two factors too had significant influence. Further, among the interactions, hardwood cutting+shade net had given maximum sprouting percentage (66.66%), number of sprouts at 30 DAP (3.67), length of shoots at 30 DAP (3.42 cm), fresh weight and dry weight (7.40 and 4.52 g), length of longest root (11.38 cm) and rooting percentage (64.81%). The interaction of hardwood cutting+mist house had produced maximum length of shoot at 60 and 90 DAP (5.46 and 9.15 cm), whereas, the minimum number of days taken for sprouting was recorded in the interaction of shoot-tip cuttings+shade net with 22.95 days. It also implied that the hardwood cuttings in combination with shade net were better to induce more shooting and rooting parameters under hot and dry weather conditions.

**KEYWORDS:** Kagzi lime, propagation, stem cuttings, IBA, propagation conditions

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

The acid lime [*Citrus aurantifolia* (Christm.) Swingle, family Rutaceae] is one of the most important fruit of India. It occupies the highest commercial importance among citrus group of fruits. It is very rich in vitamin C which is useful in wounds healing, developing strong blood vessels and gums, gives strength to bones and protection from cold and cough etc. It is mostly used as fresh fruits for the table purpose, processed into beverages, industrial and medicinal purpose.

In India, Citrus fruits are third most important with a production of 13.97 mt (2019-20) from 1.05 mha and area under it is increasing yearly. There is shortage in disease free citrus planting material due to strict quarantine procedures worldwide. (Wang, 2021; Vashisth et al., 2020; Folimonova, 2020). Good quality planting material is prerequisite of fruit growing community and hence, a rapid method for production of sufficient planting material is necessary. Though acid lime seeds are polyembryonic, difficulty of discarding zygotic seedlings, high gestation period and viral disease contamination are few of common problems in seed produced citrus plants. Citrus may be propagated by vegetative by Stem cuttings, grafting, budding, Layering and tissue culture techniques. Some of these techniques are time consuming, whole process may last for 2-3 years for multiplying plants for field planting (Alves et al., 2019; Widaryanto et al., 2019; Niedz and Bowman, 2023.). Further, plant multiplication by stem cuttings is a cheap, practically easy that does not require the special skills as in other vegetative methods. Therefore, it is widely used for augmenting large scale planting material production. Once standardized mass propagation technique through cuttings may be useful in production of uniform rootstocks for use in production of quality acid lime in advanced production techniques like high density planting. Many studies have been under taken to ascertain a proper set of conditions to enhance root induction and further responses in stem cuttings in various acid limes and other citrus spp. Those studies reported that the success of stem cuttings depend on many factors viz., age of the mother plant, plant parts used, time of propagation, rainfall, humidity, temperature, rooting media and after care (Beeson and Silva, 2017; Bowman and Albrecht, 2017; Pirlak and Cinar, 2020; Fadli et al., 2017; Siqueira et al., 2016; Pokhrel et al., 2021; Solonia et al., 2020; Guchhait et al., 2024). Furthermore, rooting success and survival varied with citrus species, region, strength of tissue, propagation condition employed (Singh et al., 2015). Hence, it appears type of cuttings and propagation conditions are very important contributing factors for successful multiplication of planting material through cuttings.

Among different commercial citrus species cultivated in India, sweet orange and acid lime are two most important and popular in India. These are cultivated in almost all agroclimatic zones of the country. Further, the acid lime has wider adaptability in respect to agro-climatic conditions and it is one of the main fruit crop grown in northern Karnataka. Generally, based on ontogenic maturity of tissues, the stem cuttings are divided into hardwood, semi hard wood and soft wood stem cuttings. Poly house, mist house and shade net are most commonly used propagation conditions for plant multiplication through stem cuttings. The low cost poly house provides moderate humidity with region dependent temperature inside it and mist chamber involves lot of investment and provides high humidity with region dependent temperature inside it. Whereas shade net house is cheaper in cost and provides shade only. Hence, it was found necessary to investigate response of cuttings of different tissue strength under different propagation conditions under northern dry zone of Karnataka having very high temperature and dry conditions. Hence, the present study was taken up on assessment of root induction influenced with the type of cuttings and propagation conditions in acid lime cv. Kagzi lime.

## 2. MATERIALS AND METHODS

The investigation was conducted during the year 2019–2021 at College of Horticulture, Bagalkot, University of Horticultural Sciences, Navanagar, Bagalkot, Karnataka 587104, India located at 16.16° North latitudes, 75.62° East longitudes and an altitudes of 678 meter above the from mean sea level to standardise the *hi-tech* propagation protocol in acid lime (*Citrus aurantifolia* Swingle) through cuttings.

### 2.1. Preparation of cuttings, media and PGRs

Acid lime (*Citrus aurantifolia* Swingle) stem cuttings were taken from healthy mother plants. In the present study hard-wood cuttings 15 to 20 cm long, containing about 6 to 9 buds were treated with PGR and planted in coco peat as a growing media.

#### 2.1.1. Plant growth regulators

500 ppm of IBA prepared by mixing 500 mg quantity of IBA powder in little quantity of 0.1 N NaOH and stirred thoroughly until the power gets dissolved completely. Later the final volume was made up to one litre by adding distilled water. The potting soil, or medium in which a plant grows, must be of good quality.

#### 2.1.2. Plant materials

Acid lime cv. Kagzi lime trees 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.

#### 2.1.3. Planting of cutting

The prepared cuttings were ready to be treated and for planting. One third basal portion of the cuttings were dipped in aqueous solution of IBA according to concentration and planted in the pro trays and garden pots by inserting two-third portion of it. The planted cuttings were irrigated regularly depending upon soil moisture conditions.

#### 2.2. Propagation conditions

The experiment was carried out under propagation structures. The different levels of factors were types of cuttings (Shoot tip, semi-hardwood and hardwood cuttings) and propagation conditions (Shade net, poly house and mist house).

#### 2.3. Containers

For the purpose of planting of cutting for rooting, 18 cavity plastic pro trays were being used having a thickness of 1 mm to contain the required media. The garden pots filled with desirable media were also used for planting the different types of stem cuttings.

#### 2.4. Observation recorded

The observation recorded under the present study were Days taken for sprout initiation, sprouting percentage, Number of sprouts cutting<sup>-1</sup>, Number of leaves cutting<sup>-1</sup>, Shoot length, Fresh weight, Dry weight, Number of primary roots, Length of the longest root and Rooting percentage at monthly interval. The observations were recorded in per cent, cm, gram, day etc units.

#### 2.5. Design and statistical analysis

The present study has been laid out in two factorial completely randomised design (FCRD) with sixty treatments and twice replications, respectively. The different levels of factors were types of cuttings (Shoot tip, semi-hardwood and hardwood cuttings) and propagation conditions (shade net, polyhouse and misthouse). Every treatment contained 18 kagzi lime cuttings. The data were statistically analyzed by following FCRD design at 5% level of significance using Web Agri-Stat Package (WASP 2.0) moderated by ICAR Research Complex, Goa. The critical difference (CD) at 5% level of probability was calculated to compare treatment means wherever F test was significant.

### 3. RESULTS AND DISCUSSION

Vegetative propagation is broadly followed to multiply plants of desired constitution and maintain their purity for commercial exploitation in many fruit crops. The art of propagation by vegetative method has gained popularity

in the field of horticulture in recent years. Many of the horticultural crops which are found to be difficult to root are made to root easily by using plant growth regulators, growing media and propagation conditions. Among the methods of vegetative propagation, use of cuttings is one of the important practices. The performances influenced by different types of cutting and propagation conditions in present investigation are presented.

#### 3.1. Days taken for sprout initiation

The days taken for sprout initiation were significantly affected by individually and by interaction of type of cutting and propagation condition. The minimum number of days of 23.48 was taken in shoot tip cuttings which were on par with hardwood cuttings and 23.23 was taken in mist house which was on par with shade house (Table 1). The minimum number of days of 22.95 was recorded for sprouting initiation in interaction of shoot-tip cuttings + shade net (Table 2). Further, shoot tip cuttings were early than others which may be due to prevention of down-word translocation of carbohydrate and accumulation of higher level of endogenous and exogenous auxins. It was early in hard wood cuttings may be because of availability of stored food for sprouting of buds. Further, both mist house and shade net was the best for early sprout initiation compared to poly house as the temperature was lesser due to the mist in mist house and aeration in shade net. Though there was high humidity in poly house, temperature was going very high compared to shade net and this might be one of the reasons for the kind of responses seen in different cuttings and propagation conditions. Similarly, in the study on propagation of six citrus rootstocks by Bowman and Albrecht (2017) sprouts were initiated 1–2 weeks in 2–5 weeks old single node cuttings. Meanwhile, Albrecht et al. (2020) has emphasized that nutrition plays very important role in growth and development of cuttings in *Citrus sinensis*

#### 3.2. Sprouting percentage

The percentage of sprouted cuttings was significantly affected by individually and by interaction of type of cutting and propagation condition (Table 1 and 2). The maximum sprouting of 43.21 and 45.06 was recorded in hardwood cuttings and shade net, respectively. In the interaction, the highest sprouting percentage was observed in hardwood cutting + shade net with 66.66% and the lowest sprouting percentage was observed in treatment in shoot-tip cuttings+poly house with 5.55%. Overall, highest percentage of sprouting was recorded in the hardwood cuttings compared to other type of cuttings which may be due to better utilization of stored carbohydrate, nitrogen and other factor with the aid of growth regulator. Shade net was best condition to record highest sprouting compared to other conditions due to optimum temperature and

Factors	Days taken for sprout initiation	Sprouting percentage	No. of sprouts cutting <sup>-1</sup> (90 DAP)	No. of leaves cutting <sup>-1</sup> (90 DAP)	Shoot length (cm) (90 DAP)	Fresh weight (g)	Dry weight (g)	No. of primary root	Length of the longest root (cm)	Rooting (%)
C <sub>1</sub> : Shoot-tip cuttings	23.48	11.11	4.43	9.84	4.6	2.11	1.29	3.56	6.21	10.49
C <sub>2</sub> : Semi-hardwood cutting	25.06	34.56	3.22	9.49	4.8	3.80	2.81	3.89	8.92	32.71
C <sub>3</sub> : Hardwood cutting	24.24	43.21	3.36	11.40	6.9	6.21	4.24	4.01	10.34	41.35
SEm±	0.30	1.34	0.10	0.18	0.08	0.04	0.03	0.09	0.04	1.19
CD ( $p=0.05$ )	0.91	4.03	0.30	0.53	0.23	0.11	0.08	0.27	0.13	3.56
T <sub>1</sub> : Shade net	23.38	45.06	4.62	13.07	6.03	3.84	2.36	4.28	9.07	42.59
T <sub>2</sub> : Poly house	26.17	10.49	3.11	4.57	4.01	3.91	2.81	2.63	7.74	10.49
T <sub>3</sub> : Mist house	23.23	33.33	3.27	13.08	6.33	4.36	3.17	4.55	8.68	31.48
SEm±	0.30	1.34	0.10	0.18	0.08	0.04	0.03	0.09	0.04	1.19
CD ( $p=0.05$ )	0.91	4.03	0.30	0.53	0.23	0.11	0.08	0.27	0.13	3.56

Treatment	Treatment details	Days taken for sprout initiation	Sprouting percentage	Shoot length (in cm)			No. of primary roots	Length of the longest root (in cm)	Rooting percentage
				30 DAP	60 DAP	90 DAP			
T <sub>1</sub>	C <sub>1</sub> +T <sub>1</sub>	22.94	16.66	0.64	2.68	5.42	4.17	7.62	14.81
T <sub>2</sub>	C <sub>2</sub> +T <sub>1</sub>	23.73	51.85	2.58	3.47	5.14	3.53	8.21	48.15
T <sub>3</sub>	C <sub>3</sub> +T <sub>1</sub>	23.47	66.66	3.42	5.29	7.53	5.13	11.38	64.81
T <sub>4</sub>	C <sub>1</sub> +T <sub>2</sub>	24.33	5.55	1.10	2.63	3.50	2.33	5.67	5.55
T <sub>5</sub>	C <sub>2</sub> +T <sub>2</sub>	28.06	12.96	0.93	2.16	4.39	3.83	8.30	12.96
T <sub>6</sub>	C <sub>3</sub> +T <sub>2</sub>	26.11	12.96	1.66	3.05	4.14	1.72	9.24	12.96
T <sub>7</sub>	C <sub>1</sub> +T <sub>3</sub>	23.17	11.11	1.94	2.66	4.85	4.17	5.36	11.11
T <sub>8</sub>	C <sub>2</sub> +T <sub>3</sub>	23.40	38.88	3.25	3.47	4.98	4.29	10.26	37.03
T <sub>9</sub>	C <sub>3</sub> +T <sub>3</sub>	23.13	50.00	3.23	5.46	9.15	5.18	10.41	46.29
SEm±		0.53	2.33	0.18	0.15	0.14	0.16	0.08	2.06
CD ( $p=0.05$ )		1.58	6.99	0.53	0.45	0.39	0.47	0.23	6.17

C<sub>1</sub>: Shoot-tip cuttings; C<sub>2</sub>: Semi-hardwood cutting; C<sub>3</sub>: Hardwood cutting; T<sub>1</sub>: Shade net; T<sub>2</sub>: Poly house; T<sub>3</sub>: Mist house

relative humidity. Similarly, Bhatt and Tomar (2011) recorded highest per cent of sprouted bud (68.50%) in *Citrus aurantifolia* (Swingle) cuttings treated with IBA at 500 ppm. Beeson and Silva (2017) obtained maximum number of viable cuttings of about 100% success rate in Single node-single leaf cuttings of *Citrus sp* Kuharske treated with 7500 ppm solution of dip and grow with a quick dip (0.5 s) method.

### 3.3. Number of sprouts

There were significant differences in number of sprouts cutting<sup>-1</sup> at 30, 60 and 90 DAP among the treatments due to individual and the interaction of type of cutting and propagation condition (Figure 1). Maximum number of sprouts of 4.43 (Shoot tip cutting) and 4.62 (Shade net) were recorded at 90 DAP. The highest sprouts cutting<sup>-1</sup> were obtained in the interaction of shoot-tip cuttings+shade

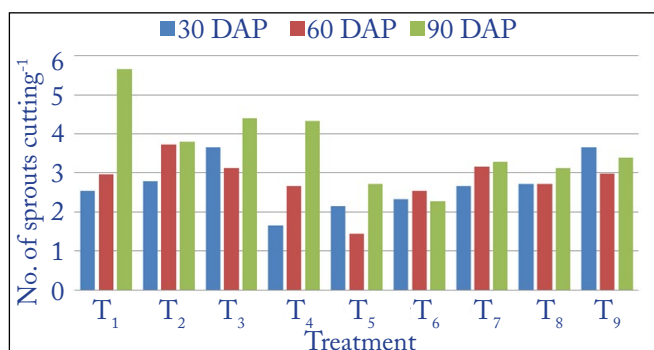


Figure 1: Number of sprouts influenced by interaction of strength of tissue and propagation conditions

net with 5.67 sprouts. Overall, 90 DAP, the shoot tip cuttings under shade net were more responsive than other types of cuttings and propagation conditions which may be due to better translocation of photosynthates in shoot tips for vegetative growth and maintenance of optimum temperature and relative humidity throughout the period under shade net under the highly hot climate at the place of experimentation. Similarly, cuttings treated with IBA showed 100% rooting with 2.06 sprouts in *C. swingle* and *C. sacaton* (Fadli et al., 2017) in hardwood cuttings. These results explain that the sprouts cuttings<sup>-1</sup> depends on the type of cuttings used, crop and PGR treatments. However, Das et al., (2021) demonstrated NAA, another form of auxin, has a great role in growing leaves of a plant along with growing shoots. Further, Cao et al. (2023) has opined that bud break is correlated to changes in phytohormones.

### 3.4. No. of leaves cutting<sup>-1</sup>

There were significant differences recorded in number of leaves cutting<sup>-1</sup> at 30, 60 and 90 DAP due to both individual and the interaction effect of cuttings and propagation conditions (Figure 2). Maximum number of leaves of 11.40 (Hard wood cuttings) and 13.08 (Mist chamber) were recorded at 90 DAP. The highest number of leaves cutting<sup>-1</sup> was recorded in the interaction of hardwood cutting+mist house with 14.53 leaves, which was on par with hardwood cutting+shade net and semi-hardwood

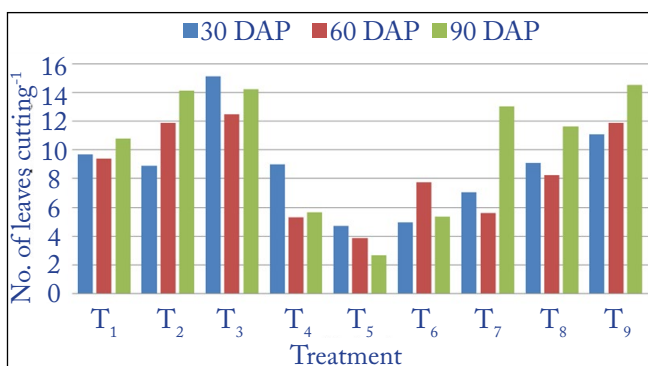


Figure 2: Number of leaves influenced by interaction of strength of tissue and propagation conditions

cutting+shade net with 14.27 and 14.15 leaves, respectively. There was consistency in type of cuttings and conditions showed best response with respect to number of leaves, which is very important, as leaves are important for further growth, development and response of cuttings. Overall, the interaction of hardwood cuttings+mist house shade<sup>-1</sup> net house performed better to show highest number of leaves cutting<sup>-1</sup> which may be due to activation of auxin content in vegetative part and growth favoured by the optimum temperature and relative humidity. Singh et al. (2019) stressed that the number of leaves is considered a fundamental parameter in assessing plants as it reflects the overall productivity and health of the plant. Al-Zebari et al. (2013) recorded highest number of leaves (16.44 leaves plant<sup>-1</sup>) by treating citron cuttings with IBA 500 IBA ppm.

### 3.5. Shoot length (cm)

Significant differences were found in shoot length due both individual and interaction effect of cuttings and propagation conditions at 30, 60 and 90 DAP (Table 2 and 3). Maximum shoot length of 6.90 (HWC) and 6.33 (Mist) were recorded at 90 DAP. The longest length of shoot at 90 DAP had been observed in the interaction of hardwood cutting, mist house with 9.15 cm and the shortest length of shoot was recorded in the interaction of shoot-tip cuttings+poly house with 3.50 cm. At 90 days DAP, hardwood cuttings under mist house might had ability to feed sprouted shoots both from stored food and fresh nutrition prepared from leaves. Nevertheless, it also might be due to humid conditions prevailed in mist house. Our results were also in confirmation with Awan et al. (2012) in cuttings of various olive cultivars.

### 3.6. Fresh weight (g)

Significant differences were found in fresh weight of roots at 90 DAP among various treatments and their interaction (Figure 3). Maximum fresh weight of roots was recorded in hardwood cuttings (6.2 g) and mist house (4.36 g). The highest fresh weight was recorded in the interaction of

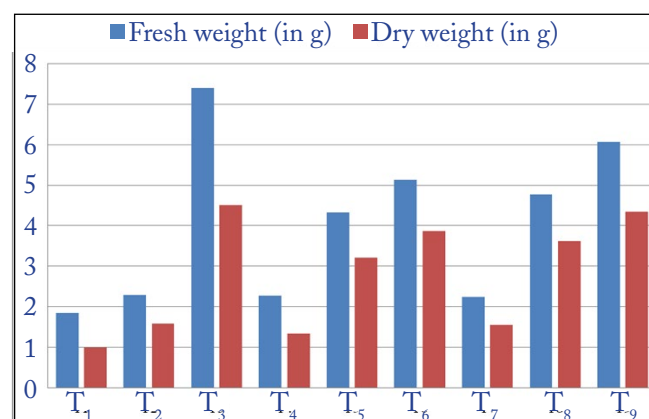


Figure 3: Fresh weight and dry weight at 90 DAP as influenced interaction of strength of tissue and propagation conditions



hardwood cutting+shade net with 7.40 g and the lowest fresh weight was recorded the interaction of shoot-tip cuttings+shade net with 1.839 g. Overall, hardwood cuttings had more fresh weight as compared to other types of cutting as the diameter was more and the cuttings kept in the mist house condition were having more fresh weight compared to other propagation conditions as it provided efficient condition for vegetative growth. Muttaleb et al. (2017) reported highest fresh weight of the roots in the semi

hardwood cuttings of *Piper beetle* L. Treated with 2000 mg l<sup>-1</sup> IBA to be 3.94 g. These findings are in confirmation with Sardoei (2014) in guava (*Psidium guajava* L.).

### 3.7. Dry weight (g)

Significant differences in dry weight were found among the treatments due to cutting and growing conditions (Figure 3). Maximum dry weight of roots was recorded in hardwood cuttings (4.29 g) and mist house (3.17 g). The



A: C<sub>1</sub> Shoot-tip cuttings+T<sub>1</sub> Shade net



D: C<sub>1</sub> Shoot-tip cuttings+T<sub>1</sub> Shade net



B: C<sub>2</sub> Semi-hardwood cutting+T<sub>1</sub> Shade net



E: C<sub>2</sub> Semi-hardwood cutting+T<sub>3</sub> Mist house



C: C<sub>3</sub> Hardwood cutting+T<sub>3</sub> Mist house



F: C<sub>3</sub> Hardwood cutting+T<sub>1</sub> Shade net

Figure 4: Best treatments with respect to shoot growth (A-C) and root growth (D-F) in the study of effect of strength of tissue and propagation conditions on rooting ability of cuttings

highest and lowest dry weight of cuttings at 90 DAP was recorded in the interaction of hardwood cutting+shade net with 4.52 g and shoot-tip cuttings+shade net with 0.983 g, respectively. Overall, more dry weight had been recorded in the hardwood cuttings as the diameter of cuttings were more and the cuttings planted in the mist house incubated cuttings were recorded dry weight. Similarly, Singh et al. (2015) reported dry weight of cutting of about 8.05 g in lemon (*Citrus limon* Burm.) cv. pant lemon-1 planted in Soil+Sand+FYM media.

### 3.8. Number of primary root

The significant differences were found in number of primary root among the treatments and their interaction (Table 2). Maximum number of roots was recorded in hardwood cuttings (4.0) and mist house (4.55), respectively. The highest and lowest number of primary root cutting<sup>-1</sup> had been recorded in the interaction of hardwood cutting+mist house) with 5.18 roots and hardwood cutting+poly house with 1.72 roots, respectively. The number of primary roots was more in the hardwood cuttings as the dry matter of cuttings were more which got converted into root growth. Further, 500 ppm of IBA treated and mist house shade<sup>-1</sup> net incubated cuttings performed better for increasing the number of primary roots cutting<sup>-1</sup>. Dvin et al. (2011) had reported that the clonal apple rootstock M111 treated with IBA 2000 mg l<sup>-1</sup> gave the best results with respect to number of roots cutting<sup>-1</sup> (11.33).

### 3.9. Length of the longest root (cm)

There were significant differences in length of longest root at 90 DAP among the treatments and their interaction (Table 2). Maximum number of roots was recorded in hardwood cuttings (10.34 cm) and shade net (9.07 cm), respectively. The average longest and shortest roots cutting<sup>-1</sup> was recorded in the interaction of hardwood cutting+shade net with 11.38 cm and shoot-tip cuttings+mist house with 5.358 cm, respectively. The longest length of primary root was recorded in the hardwood cuttings due to the assimilation and translocation of auxins compound in rooted cutting and shade net incubation significantly favoured length of longest root. Hakim et al. (2018) recorded highest root length (27.60 and 25.80 cm) in pomegranate (*Punica granatum* L.) cuttings cvs. Bhagwa and Ruby treated with IBA 1500 ppm+NAA 1500 ppm+Biomix.

### 3.10. Rooting survival percentage

The rooting survival percentage<sup>-1</sup> of rooted cuttings at 90 DAP was significantly affected by treatments and their interaction (Table 2). Maximum number of roots was recorded in hardwood cuttings (441.3) and shade net (45.95), respectively. The highest and lowest rooting percentage was observed in the interaction of hardwood cutting+shade net with 64.81% and shoot-tip cuttings+poly

house with 5.55%, respectively. Highest percentage of rooting was recorded in the hardwood cuttings compared to other type of cuttings and shade net compared to other conditions. When growth regulators were used for boosting the rooting of cuttings that should be supported by the favourable conditions like optimum temperature (25–30°C) and high relative humidity (80–90%) which results in better survival of cuttings and show photosynthetic activity that promotes better rooting in cutting. Similar thing happened in this study. Pirlak and Cinar (2020) reported that semi hard wood and hardwood cuttings showed highest rooting rate (20%) than in soft wood cuttings in Carrizo citrange treated with 4000 ppm IBA but under continues fogging. Ullah (2020) found similar results in Kinnow mandarin. Mariano (2022) has found similar kind of rooting responses and survival in selected citrus species.

Overall, our observation in this study was, shade net was better to provide optimum condition in hot and dry regions. Further, initially, cuttings and their sprouts could not sustain in both the mist house and poly house due to high temperature irrespective of high humidity maintained which may be because of the very hot condition prevails in the region. However, a low percentage of cuttings that survived and acclimatized to the conditions, showed more growth in shoots, number of leaves and roots.

## 4. CONCLUSION

Overall, hardwood cuttings in combination with shade net were better to induce more shooting and rooting parameters in acid lime cv. Kagzi lime. Further, to increase planting material production with limited shoot material, shoot tips also may be employed in shade net house at places with hot and dry conditions.

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