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Review Article

Propagation of acid lime, *Citrus aurantifolia* (Christm.) swingle and lemon, *Citrus limon* L. through stem cuttings - A review

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Abstract
Cultivation of acid lime (*Citrus aurantifolia* (Christm.) swingle) and lemon (*Citrus limon* L.) holds significant importance in India. Despite its economic importance, propagation faces challenges, primarily due to the recalcitrant nature of seeds, leading to loss of viability and lack of uniformity in seedlings. This necessitates the adoption of vegetative propagation method for producing true-to-type planting materials. Stem cuttings, budding and air layering are the primary methods employed, with stem cuttings being the most suitable for their simplicity and ability to induce precocity in fruit crops. The successful rooting of stem cuttings depends on the maturity of the cuttings, auxins, growing media, nutrition, irrigation water, endogenous food materials, season and microclimatic conditions. The maturity of cuttings influences rooting and uniformity, with virus-free hard, semi-hard, and softwood cuttings being preferred for propagation. The application of Indole Butyric Acid (IBA) significantly influences rooting. Cool season is generally favored for better rooting. Pre-conditioning treatments such as ringing, girdling and wounding influenced rooting. Microclimatic conditions, including temperature, relative humidity and light intensity, are critical for successful rooting. This review is a compilation of up-to-date research and development in stem cuttings of lime and lemon to understand better physiology, the role of auxins, growing media and microclimatic factors.

Keywords: Acid lime, auxins, Girdling, Growing media, Indole Butyric Acid (IBA), Lemon, Stem cutting, Rooting

INTRODUCTION

Lime, *Citrus aurantifolia* (Christm.) Swingle and Lemon, *Citrus limon* (L.) Burm. are important citrus fruit crops grown in India. It belongs to the family Rutaceae. It is indigenous to tropical and subtropical Southeast Asia. Acid lime is also known as Kagzi lime, Mexican lime, or Pati lime. The greater genetic variability in lime and lemon (Dubey *et al*., 2016; Kumari *et al*., 2021) and adaptability in citrus species (Lalramhluna and Prasad, 2016) have been recorded. India is the largest producer of lemons in the world. In India, acid lime and lemon are cultivated in 2.96 lakh hectares, producing 33.97 lakh tonnes (National Horticulture Board Database, 2019-20). Lime is largely cultivated in Gujarat, Andhra Pradesh, Karnataka, Orissa, and Maharashtra, India. Lime and lemon fruits are a rich source of vitamin C used to treat scurvy and contain vitamin B, pectin and minerals like magnesium, calcium and potassium. They are used as fresh and processed products such as juice, squash, lemonade, marmalade, pickles, citric acid, essential oil and preserved peel. Lemon juice with common salt is recommended to remedy dysentery and heat strokes during summer. Lime juice has a laxative effect on the digestive system. The bioactive compounds of essential oils, flavonoids, terpenoids,

phenolic, limonoids and alkaloids of lime have antibacterial, antioxidant, anticancer, hypolipidaemic and anti-inflammatory (Indriyani *et al*., 2023) and good industrial substrate (Kaur *et al*., 2023) which could be utilized in the preparation of cosmetic products, health benefiting herbal formulation and aromatherapy. Peel is a source of essential oil and pectin.

Generally, lime and lemon are generally propagated through seeds. Due to the recalcitrant nature of seeds, desiccation and chilling sensitivity, seeds lose viability and cannot be stored for long before sowing (Chin and Roberts, 1980). The seedlings produced from seeds are not uniform in quality and transfer viral disease from seeds (Babu, 2001). It is advisable to avoid seedlings for commercial cultivation. Vegetative propagation is vital in this situation to produce true-to-type, uniform-quality planting materials. The vegetative propagation methods such as stem cuttings (Bhatt and Tomar, 2010; Singh *et al*., 2015b), budding (Singh *et al*., 2019) and air layering (Seran and Umadevi, 2011) are being employed in lime and lemon. Air layering and budding require trained labours and suitable virus-free rootstocks for good rooting and budding union success. Stem cuttings are an easy, rapid, simple and suitable propagation method, resulting in precocity, a desirable quality trait in fruit crops. The successful rooting of stem cuttings depends on physiological stage of cuttings, maturity of cuttings (Singh, 2018), presence of leaves in cuttings, time of collection, endogenous/exogenous growth regulators (auxins, cytokinins, anti-gibberellins, ethylene), natural plant extracts (Handayani *et al*., 2023), growth retardants, inhibitors, rooting media, season, irrigation water, condition of stock plants (age, wounding, girdling, stock plant etiolation), endogenous food materials (carbohydrates, nitrogen) and environmental factors (temperature, relative humidity, shade, light intensity, photoperiod, air circulation). This review discusses the maturity of cuttings, growth regulators, growing media, season and microclimatic factors influencing the rooting and sprouting of stem cuttings in lime and lemon.

Effect of type, maturity and age of stem cuttings

Disease-free, especially virus-free stem portions, are used for rooting purposes because they usually have undifferentiated tissues, which may permit the initiation of primordial root (Gurumurthy *et al*., 1984). The genotype, species, cultivars, maturity, and age of stem cuttings play major roles in plants' successful rooting, establishment, and survival. The presence of leaves in stem cuttings influences induction of roots. Leaf is considered an important functional unit of the plant which contributes to the formation of photoassimilates. The number of leaves per cutting significantly affects plant photosynthetic efficiency. The carbohydrates moved from leaves to stem for better root development at the base of the cuttings. Auxins produced in the leaves and buds move from the apical end to the basal end and thus enhance rooting at the base of the stem portions (Muhammad *et al*., 2021). The maturity of cuttings influences the rooting percentage, number of roots (Rajangam *et al*., 2022) and uniformity in rooting. A significant variation is usually observed with maturity of stem cuttings. The hard, semi-hard (Rajangam *et al*., 2022), softwood cutting (Kumar and Kumar, 2022), and single leaf bud cutting (Maurya *et al*., 2022) of uniform size, 10-25 cm with three nodes and internodes are used for propagation.

Stem cuttings of 22-24 cm long with 6-8 nodes with 0.6 - 1.2 cm diameter were prepared from the central and basal parts of the branch and performed well in rooting during June (Bhatt and Tomar, 2010). About 25 cm long cuttings of lemon cv. Eureka with 4-5 leaves was successful in rooting in media containing sand and peatmoss (1:1) under mist condition (El-Shazly *et al*.,1994). About 20 cm long cuttings of lemon cv. Pant lemon 1 with three nodes taken from the middle portion of one-year-old shoots performed better in rooting (Singh *et al*., 2015a). The mature shoots of 20-25 cm long containing 4-5 buds were used for propagation (Patel *et al*., 2021). While preparing cuttings, a slanting cut should be given at the distal end, whereas at the proximal end, a smooth cut should be given just below the lower node.

Greenwood cuttings of lemon performed better in rooting (Dimitar, 2014). About 9-10 month-old shoots of Pant Lemon 1 performed better in rooting, sprouting and survival of plants (Patel *et al*., 2018). The semi-hardwood cuttings of acid lime resulted in better rooting (Prati *et al*., 1999). Satpal *et al*. (2014) reported that the semi-hardwood cutting of 15 cm collected from 4-6-year-old lemon trees cv. Pant lemon 1 produced significantly better rooting (64.44%), number of primary roots (5.89), length of roots (7.58 cm), fresh weight of roots (0.75g), dry weight of roots (0.13 g), number of sprouts (4.11), length of sprout (6.63 cm), number of leaves (17.89), fresh weight of shoot (0.99 g), dry weight of shoot (0.18 g) and survival of plants (64.44%) in a media containing sandy soil and farm yard manure (1:1) compared to softwood and hardwood cuttings. The hardwood cuttings were found to be better for induced rooting in lemon cv. Pant lemon 1 (Satpal *et al*., 2014). The hardwood cuttings of Eureka seedless lemon produced earlier sprouting (42.8 days), maximum sprouting (58.09%), number of shoots (2.86), length of the shoot (7.09 cm), number of roots (10.85) and length of longest root (7.55 cm) compared to semi-hardwood cuttings (Gnawali *et al*., 2022).

Effect of growth regulators

Plant growth regulators are organic compounds synthesised in plant species. They are known as

phytohormones and include auxins, cytokinins, gibberellins, ethylene, and abscisic acid. Auxins such as Indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA) are used growth regulators to induce rooting on stem cuttings. Auxin controls cytokinin synthesis in the stem's nodal region (Tanaka *et al*., 2006). Auxin is synthesized in young leaves and then transported in a polar fashion. The polar movement of auxins is mediated by a membrane transport carrier (Lomax *et al*., 1995). Cytokinin, synthesized in the root tips, regulates apical dominance (Tanaka et al., 2006), root proliferation, and shoot growth. Cytokinins are indirectly involved in rooting through the accumulation of carbohydrates at the stem end. A high auxin: cytokinin ratio supports callus development (Hartmann and Kester, 2014) and rooting. A low auxin: cytokinin ratio supports shoot formation. Gibberellins may suppress root initiation and be involved in regulating protein and nucleic acid synthesis.

The regulation of endogenous auxin is mediated by IAA oxidase-peroxidase enzyme complex. Peroxidase activity is used as a marker of ease in rooting, while IAA oxidase metabolizes auxin. The activity of IAA oxidase is controlled by phenolic compounds. The application of auxins triggers the amylase and invertase enzymes which converts starch into sugars, which is required for the production of new tissues during rooting. The response of auxins varies with physiological state of stem portion and microclimatic factors. Type of application, concentration and kind of auxins are considered in the successful rooting of stem cuttings. IBA and NAA were used auxins for rooting of stem cuttings of lime and lemon (Sabah *et al*., 1991).

IBA stimulates better elongation of roots, which could be associated with enzyme synthesis (Wada *et al*., 1968). IBA is the most commonly used auxin for better lime and lemon rooting, establishment, and survival. Application of IBA resulted in increased rooting percentage and number of roots. As the concentration of IBA increased the root length was also increased in acid lime (Bhatt and Tomar, 2010). The applied IBA has an influence on endogenous hormonal balance, differential auxin mobilization, hydrolysis of reserve food materials, enzyme activity, protein synthesis, and membrane permeability metabolism at the rooting zone for root regeneration. IBA increases the synthesis of food materials, including carbohydrates, which encourages quick healing and better callusing of plants. The applied IBA-enhanced hydrolytic activity and the rooting medium are responsible for the increased percentage of rooted cuttings of lemon cv. Pant lemon 1 (Kumar and Kumar, 2022). The application of IBA indirectly enhances the transformation of rooting primordia and movement of sugars to the base of cuttings and consequently formation of roots. High concentrations of IBA inhibit the survival rate of plants. The response of stem cuttings to auxins in lime and lemon with differential success rate is given in Table 1.

Effect of natural root inducing substances

Aloe vera gel is a natural source of auxins and gibberellic acid, salicylic acid (Surjushe *et al*., 2008), carbohydrates and proteins (Ramachandra and Rao, 2008), which induce rooting in stem cuttings. Aloe gel contains IAA and could be utilized as an alternative hormone. It also contains enzymes that stimulate root growth, making it a great alternative to commercial rooting hormones. Aloe gel contains root inducing substance in the rooting of semi-hardwood cuttings of lime with root length of 5.02 cm and 1.72 roots compared to control with root lengths of 2.26 cm and 1.61 roots (Mirihagalla and Fernando, 2020). Shallot extract (75%) taken from the middle portion of the stem significantly affected shoots' growth (Handayani *et al*., 2023). Shallot contains auxins and allithiamin compounds which facilitate metabolism in plant tissues.

Effect of endogenous food materials in stem portions

The presence of reserved food materials in stem cuttings enhances the rooting. Carbohydrates are positively correlated with the rooting ability of cuttings. The carbohydrate to nitrogen (C:N) ratio plays a crucial role in maintaining the source-sink relationship in the stem cutting. The presence of high carbohydrates, high C:N ratio and low phenols in stem cuttings serves as food materials and congenial for rooting and new growth of shoots. Hydrolysis of polysaccharides occurs in matured stem cuttings to improve root formation. Invertase activity and changes in leaf expansion rate was not correlated positively with changes in endogenous free IAA level (Schaffer *et al*., 1987). The presence of high phenol content in stem portions adversely affected the rooting ability. Movement of IAA in citrus leaf midrib sections was shown to be polar and essentially basipetal. Endogenous cytokinins were maximum at anthesis of citrus spp. The concentration of gibberellins varied considerably with the development of citrus spp. Endogenous polyamines were at their maximum level at early flower development, whereas ABA peaked at petal fall in citrus *spp*.

Effect of growing media and nutrients

The selection of suitable growing media is essential to supply adequate nutrients, organic matter, good drainage, porosity, and better water holding capacity, as well as to allow optimum gaseous exchange for successful growth of roots and shoots. The growing medium should be easy to wet, well decomposed, firm enough to hold the cuttings, porous enough for

Table 1. Effect of growth regulators on rooting of stem cuttings in lime and lemon

Sl. No.	Growth regulators	Results	Reference
	Auxins: IBA **Single leaf bud cutting**		
1	Single leaf bud cutting *Citrus lemon* L. Lime cv. Nepali Oblong	IBA @ 2000 ppm performed better with respect to maximum survival percentage (47.50%), minimum days taken to first sprouting (20.38 days), maximum number of leaves/cutting (14.41), shoot length (18.02 cm), shoot diameter (2.04 mm), sprouting (55.33%), maximum number of roots (27.02), diameter of primary root (1.25 mm), length of longest root (6.10 cm), average fresh weight (3.72 g) and dry weight of cuttings (1.41 g).	Maurya *et al*. (2022)
2	Leaf bud cuttings *C. limon* L. Lemon cv. Assam	The higher rooting (98.5%) and survival (100%) of plants were recorded in leaf bud cuttings (leaf blade, petiole and a short piece of stem bearing axillary buds), treated with 3000 ppm IBA and planted in sand medium compared to 1000 and 2000 ppm IBA. Number of primary roots, root length, dry weight of roots, number of leaves and shoot length per cutting were significantly superior to 1000 and 2000 ppm IBA. 4000 ppm IBA showed an inhibitory effect on rooting and rooting characters	Nath (2000)
3	Mature shoots *Citrus aurantifolia* (Christm.) Swingle Kagzi lime	3000 ppm IBA + 3000 ppm NAA showed early days of sprouting (22.00 days), more number of sprouts per cuttings (8.33), sprout length (14.56 cm), number of leaves per cutting (20.97), length of leaves (5.16 cm) and sprouted cuttings (73.09%).	Patel *et al*. (2021)
4	Stem cuttings from middle portion of the shoots *Citrus limon* L. Lemon cv. Pant lemon 1	500 ppm IBA was effective in producing more number of sprouts (2.42), average length of sprout (7.06 cm), number of leaves (15.10), average fresh weight of cutting (11.92 g) and survival of plants (81.68%) followed by 500 ppm IBA + 500 ppm NAA with significantly higher number of primary roots (7.74) and secondary roots (16.19) per cutting	Singh *et al*. (2015a)
5	Stem cuttings *Citrus limon* L. Lemon cv. Eureka	1000 ppm IBA was found better in rooting and number of roots/cutting (70.22-76.20). 4000 ppm IBA was found better in rooting (95.50-98.00%), root length/cutting (42.51-44.60 cm), shoot length/cutting (22.60-23.50 cm) and number of leaves/cutting (16.33-18.24) in media containing sand and peatmoss (1:1) under mist condition	El-Shazly *et al*. (1994)
6	Stem cuttings *Citrus limon* L. Lemon cv. Baramasi	Treatment with 2000 ppm IBA in combination with 2000 ppm p-hydroxybenzoic acid was effective in rooting than 1000, 1500 ppm IBA in combination with 1000 or 2000 ppm p-hydroxybenzoic acid.	Kumar et al. (1995)
7	Juvenile branches *Citrus aurantifolia* (Christm.) Swingle Kagzi lime	500 ppm IBA produced more sprouted cuttings (68.5%) at 130 days of planting followed by 1000 ppm IBA with 51.83% sprouting. 1000 ppm IBA produced more number of primary roots (8.76) followed by 500 ppm IBA with 7.54.	Bhatt and Tomar (2010)
8	Greenwood cuttings *Citrus limon* L. Lemon	2000 ppm IBA was effective in rooting than 2000 ppm NAA	Dimitar (2014)
9	Stem cuttings from 10 months old shoots *Citrus limon* L. Pant Lemon-1	Treatment with 800 ppm IBA showed significant results on rooting and survival of rooted cuttings planted in a media containing garden soil, sand and vermicompost (1:1:1) under net house condition	Kumar and Kumar (2022)

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	Semi-hardwood cuttings		
10	Semi-hardwood cuttings *C.limon* L. Lemon	2000 ppm IBA and 2000 ppm pHBA showed better rooting	Kumar *et al*. (1995)
11	Semi-hardwood cuttings *Citrus aurantifolia* (Christm.) Swingle Lime cv. Loomi	4000 ppm IBA produced better rooting (92.5%) during autumn compared to spring (72.5%) in media containing vermiculite and peat moss	Abdullah and Al-Khateeb (2004)
12	Semi-hardwood cutting *Citrus limon* L. Lemon	Treatment with 2500 ppm IBA for 30 minutes with chalk powder resulted in significantly higher survival (89.83%), number of branches (5.08), leaves (34.30), roots (35.66), leaf chlorophyll content (45.41 mg/100 g), stem diameter (10.64 mm), root diameter (2.68 mm), average length of roots (15.83 cm), root dry weight (1.48 g) and shoot dry weight (22.73 g)	Deb *et al*. (2009)
13	Semi-hardwood cuttings *Citrus aurantifolia* (Christm.) Swingle Kagzi-lime	Treatment of stem cuttings with 500 ppm IBA produced higher sprouted buds (68.50%) followed by 1000 ppm IBA with 53.67% sprouting	Bhatt and Tomar (2011)
14	Semi-hardwood cuttings *Citrus limon* L. Lime cv. Pant Lemon 1	Treatment of stem cuttings with 600 IBA was effective in rooting (72.59%), number of primary roots (6.63), length of root (8.50 cm), fresh weight of roots (0.82 g) number of sprouts (4.48), length of sprouts (6.75 cm), number of leaves (18.44) and fresh weight of shoot (1.01 g) compared to 400 ppm IBA (rooting: 65.19%), number of primary roots (6.07), length of root (7.88 cm), fresh weight of roots (0.65 g) number of sprouts (4.33), length of sprouts (6.61 cm), number of leaves (17.26) and fresh weight of shoot (0.88 g)	Satpal *et al*. (2014)
15	Semi-hardwood cuttings *Citrus aurantifolia* (Christm.) Swingle Acid lime cv. PKM 1	Treatment with 2000 ppm IBA recorded the better results in terms of number of days for sprouting (6.85 days), number of sprouts per cuttings (4.30), plant height (47.76 cm), shoot length (23.65 cm), root length (26.15 cm), fresh root weight (4.50 g) and success (80.72%)	Rajangam *et al*. (2022)
	Hardwood cuttings		
16	Hardwood cutting *Citrus limon* L. Lemon cv. Baramasi	2000 ppm IBA resulted in higher rooting percentage of lemon	Kumar *et al*. (1995)
17	Hardwood cutting *Citrus aurantifolia* (Christm.) Swingle Mexi-can lemon	Treatment with 6000 ppm IBA recorded better rooting	Elsheikh (1999)
18	Hardwood cutting *Citrus aurantifolia* (Christm.) Swingle Lime cv. Pant lemon	Cuttings treated with 2000 ppm IBA and planted in media containing soil, sand and farm yard manure (1:1:1) produced more number of sprouted cuttings (6.29), length of sprout (23.77 cm), diameter of sprout (1.52 cm), number of sprouts (17.77), number of leaves (23.00), number of roots/cutting (52.42), average length of roots (26.33 cm) and diameter of roots (1.33 cm) compared to 1000 and 1500 ppm IBA in Garhwal region	Singh *et al*. (2013)
19	Hardwood cuttings *Citrus aurantifolia* (Christm.) Swingle cv. Kagzi lime	Cuttings of 22 cm long (4-6 buds) without leaves and thorns treated with 2000 ppm IBA + 1000 ppm PHB was effective in sprouting (24.33%), number of roots per cutting (7.67) and survival (77.00%) of plants	Diwaker and Katiyar (2013)
20	Hardwood cuttings *Citrus aurantifolia* (Christm.) Swingle Kagzi lime	Treatment with IBA 500 ppm recorded better rooting	Bhatt and Bhatt (2014)

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21	Hardwood cutting *Citrus aurantifolia* (Christm.) Swingle Lime cv. Kagzi	Treatment with 500 ppm IBA and planted in cocopeat resulted in better sprouting (47.22 per cent), number of sprouts (7.40) at 90 DAP, length of shoots (14.59 cm) at 90 DAP, average fresh weight (13.52 g) and dry weight (10.19 g), number of primary roots (11.10), length of longest root (15.47 cm) and rooting (44.44%)	Malakar *et al*. (2019)
22	Hardwood cuttings Eureka seedless lemon	Treatment with 4000 ppm IBA produced earlier sprouting (30.67 days), maximum sprouting (93.33%), number of shoots (4.67), length of the shoot (7.86 cm), number of the roots(18.00)	Gnawali *et al*. (2022)
	Auxins: IAA		
23	Stem cuttings *Citrus limon* L. Lemon	At 8th week of planting, shoot length (5.73 cm), rooting (73.33%) and survival (90.0%) of plants were significantly higher in cuttings dipped in 2500 ppm IAA than 500-2000 ppm IBA	Seran and Umadevi (2011)
	Auxins: NAA		
24	Semi-hardwood cuttings *Citrus limon* L. Lemon	The rooting was higher (95%) in cuttings pre-treated semi-hardwood cuttings of lemon with 200 ppm ferulic acid and then treated with 5000 ppm NAA	Debnath *et al*. (1986)
25	Stem cuttings (One year old) *Citrus limon* L. Lemon	Days taken to first bud sprout (8.53 days), sprouting (83.33%), plant height (15.55 cm), number of shoots per cutting at 90 days after planting (4.88), number of leaves per cutting (14.28) and survival (83.33%) of cuttings were significantly higher in cuttings dipped in 2000 ppm IBA+ 1000 ppm NAA	Gautam et al. (2022)
	PHB		
26	Mature shoots *Citrus aurantifolia* (Christm.) Swingle Kagzi lime	Minimum days to sprouting (20.64), maximum number of sprouts (5.88), sprouting (17.68%), maximum length of sprouts (14.88 cm), maximum diameter of sprouts (3.28 cm), maximum number of roots (42.02), longest root (2.24 cm), rooting (70.12%) and maximum survival of plants (82.66%) were recorded with 2500 ppm IBA + 1000 ppm PHB	Kumar and Singh (2020)

movement of oxygen and drain excess water and free from weeds, pests and pathogens. Appropriate water holding capacity of media, physical properties, low salinity, aeration in the root zone and nutrient availability to release of enough nutrients are responsible for better survival percentage of rooted cuttings. The C:N media ratio should be optimum, preferably 20:1 to prevent nitrogen immobilization. A well-drained rooting medium helps in better root development by penetration of roots.

Stem cuttings planted in pure sand produce unbranched, long, coarse, and brittle roots and branched roots in a media containing sand, soil, and peat. Stem cuttings of lemon cv. Pant Lemon 1 planted in a media containing garden soil, sand and vermicompost (1:1:1) resulted in better survival of plants (88.7%) with better-rooting characteristics under shade net conditions (Kumar *et al*., 2015). Singh *et al*. (2015b) recorded that media containing soil, sand and farm yard manure was found suitable for producing higher survival (82.33%) of plants and average dry weight of lemon cv. Pant lemon 1 cuttings (8.05 g) compared to media containing soil, sand and cocopeat with better rooting (64.26%), number of primary roots (9.03), secondary roots (16.67) and length of sprout (7.10 cm). The easy penetration of roots in cocopeat could be due to better texture and porosity. Stem cuttings of lemon cv. Pant lemon 1 collected from the middle portion of one-year-old shoots produced 2.58 sprouts per cutting planted in media containing soil, sand and vermicompost (Singh *et al*., 2015b). Sabari Priya *et al.* (2020) reported that the microbial strain II of *Bacillus* spp. @ 2% resulted in better rooting, survival of plants (93.33%), shoot length (37.00 cm), number of shoots (9.00), root length (27.29 cm) and number of roots (13.67) in medium cuttings planted in media containing red soil sand and organic manure (2:1:1).

Kumar and Kumar (2022) found that media containing garden soil, sand and vermicompost was found suitable to produce more number of shoots (8.22), length of primary roots (7.95 cm), length of longest root (9.59 cm) and survival (89.90%) of stem cuttings of lemon cv. Pant lemon 1 was treated with 800 ppm IBA followed by stem cutting treated with 800 ppm IBA and planted

in a media containing garden soil, sand and farm yard manure with 82.22% survival of plants under shade net condition. Maurya *et al*. (2022) reported that lime cuttings cv. Nepali Oblong resulted higher survival of plants (47.92%), minimum days taken to first sprouting (20.04 days), maximum number of leaves/cutting (14.27), shoot length (17.79 cm), shoot diameter (2.11 mm), sprouting (54.14%), the highest number of roots (28.17), diameter of primary root (1.27 mm), length of longest root (5.99 cm), average fresh weight (3.55 g) and dry weight of (1.46) in media containing soil, peat moss and sand. Abdullah and Al-Khateeb (2004) found that semi-hardwood cuttings of lime cv. Loomi produced significantly higher rooting in a media containing vermiculite and peat moss (72%) during autumn (September) compared to spring (March), with a rooting percentage of 55.76.

Vermicompost is rich in nutrients *viz*., 2-3% nitrogen, 1.85-2.25% potassium and 1.55-2.25% phosphorus, micronutrients, beneficial soil microbes, plant growth hormones and enzymes. Rooting medium containing soil, sawdust and vermicompost was found significantly superior in sprouting (6.92 days), sprouting (79.38%), survival (76.38%) of plants, plant height (19.80 cm), number of shoots/cutting (7.34), number of leaves/ cutting (12.88), stem girth (2.15 cm) than medium containing soil, sawdust and farm yard manure in lemon cv. Pant Lemon 1 was wounded and treated with 4000 ppm IBA (Patel *et al*., 2018). Vermicompost containing rooting medium supplied quick and rapid availability of nutrients to the cuttings and increased the survival percentage (Sinha *et al*., 2009). Sawdust and vermicompost in the growing medium create favorable conditions by providing better moisture holding capacity, better aeration, drainage and high porosity from sawdust and high nutritive and growth-promoting nutrients from vermicompost. The enhanced microbial activity due to the presence of vermicompost helped the plants better uptake of nutrients. Vermicompost retains nutrients for a longer time. Humic substances in vermicompost stimulate plant growth (Canellas *et al*., 2002; Canellas *et al*., 2010).

The emergence of more number of leaves was recorded in stem cuttings when treated with 4000 ppm IBA + wounding and planted in a media containing soil, sawdust and vermicompost. Singh *et al*. (2015b) reported stem cuttings taken from the middle portion of lemon cv shoots. Pant lemon 1 planted in media containing soil, sand and cocopeat produced more number of primary roots per cutting (9.03) and the number of secondary roots per cutting (16.67), whereas media containing soil, sand and vermicompost was found effective in producing more number of sprouts per cutting (2.58). Survival of rooted stem cuttings was better (82.23%) in media containing soil, sand and farm yard manure. Lalhruaitluanga *et al*. (2022) found that dipping of stem cutting cv. Assam lemon in 800 ppm IBA and planted in media containing soil, sand, vermicompost and cocopeat (1:1:1:1)) was found better treatment in first new leaf initiation (16.33), number of leaves per plant (13.50), plant height (21.43 cm), shoot length (6.59 cm), number of branches (6.08), length of longest root (7.92 cm), fresh weight of root (0.54 g), and survival percentage of cuttings (1.00). Stock plants should contain optimum nutrients, especially carbohydrates, nitrogen, zinc, manganese and boron. The application of nitrogen should be reduced to allow for carbohydrate accumulation. Nitrogen is related to the quantity of carbohydrates, C:N ratio and hormones.

Effect of season

Rooting of stem cuttings is influenced by temperature, relative humidity, light intensity, and photoperiod, which prevails during planting season. Planting in cool season is preferred for propagation through stem cuttings. The higher rooting was recorded with semi-hardwood cuttings taken in July compared to October planting (Ozcan *et al*., 1993). Kumar *et al*. (1995) found that lemon cuttings cv. Baramasi planted in July (rainy season) was found to have better rooting, sprouting, root, and shoot growth than cuttings planted in 15th February (Spring season). Singh *et al*. (2015a) found that rainy season was found better in producing average length of sprout (7.48 cm), average diameter of sprout (2.02 mm), days taken to sprout (17.52) average fresh weight of cutting (11.78 g), average dry weight of cutting (7.93 g), rooting (61.85%), number of secondary roots (15.30), length of longest root (7.17 cm), average diameter of roots (1.20 mm) and survival (77.37%) compared to spring season with survival of cuttings (69.06%). Maurya *et al*. (2022) found that rainy season showed significantly higher survival of plants (51.46%), minimum days taken to first sprouting (19.75 days), maximum number of leaves/cutting (14.06), shoot length (17.29 cm), shoot diameter (1.96 mm), sprouting (54.75%), highest number of roots (33.28), length of longest root (5.81 cm), diameter of primary root (1.20 mm), average fresh weight (3.46 g) and dry weight of lime cuttings cv. Nepali Oblong (1.31 g). Excessive rainfall during the rainy season may adversely affect the growth and mobilization of metabolites in plants.

The rooting percentage was significantly higher in stem cuttings of lime cv. Baladi was planted more during autumn and winter than in summer (Swelith and Said, 2023). Abdullah and Al-Khateeb (2004) found that semi -hardwood cuttings of lime cv. Loomi produced significantly higher rooting (56.07%) during autumn (September) compared to spring (March) with rooting of 40.36%. December planting of stem cuttings of lemon cv. Pant lemon 1 was found superior in rooting (63.33%), number of primary roots (6.69), length of root (7.99 cm), fresh weight of roots (0.65 g), number of

leaves (18.22) and survival (63.33%) of rooted cuttings, compared to January planting with rooting of 59.44% and November with rooting of 52.22% under valley conditions of Garhwal Himalaya (Satpal *et al*., 2014). Singh *et al*. (2015b) reported that spring season was found suitable for producing more number of sprouts (2.29) and number of primary roots (7.30).

Effect of girdling, ringing and wounding

Pre-conditioning treatments such as girdling, ringing and wounding are known to give positive impact on rooting of stem cuttings. The down ward movement of growth hormones, carbohydrates and other root-promoting substances is interrupted by ringing and girdling, which promotes shoot initiation in ring and girdled areas. Removal of bark tissue in a ring shape in a branch is known as girdling. It stops phloem transport of photosynthates and accumulates carbohydrates above the girdle portion. It increases auxins and gibberellins and reduces the level of cytokinins. Wallerstein *et al*. (1974) reported that girdling triggered starch accumulation in roots in citrus species independent of carbohydrate transport from the leaves. And also, lemon responds well when cuttings were taken from the lower end portion of the ringed branch.

Semi-hardwood cuttings of lemon from ringed shoots generally rooted better than those from intact shoots (Debnath *et al*., 1986). Stem cuttings obtained from the lower end portion of the ringed branch promoted significantly better growth parameters, particularly the number of sprouted shoots (4.0), shoot length 120 days after planting (28.00 cm), number of leaves (45.66), length of leaves (8.03 cm), leaf area index (41.24 cm^2) and rooting (80%) in lemon (Pandey and Bisen 2010). The rooting percentage was significantly higher in girdled apical cuttings of lime cv. Baladi than girdled basal cuttings (Swelith and Said, 2023). The success rate varies with period between girdling and planting. There was a significant difference in the rooting of stem cuttings of lime cv. Baladi collected after 10 weeks of girdling compared to 2, 4, 6 and 8 weeks of girdling (Swelith and Said, 2023).

Wounding prevents the downward translocation of carbohydrate and accumulation of higher levels of endogenous auxins in the ringed, lower portion of treated cuttings during root initiation. Wounding also permits greater absorption of applied growth regulators by the tissues at the base of the cuttings. Wounding promotes root production in stem cuttings with older ones at the base. Upon wounding, callus production and root development are better along the wound's margins. Wounding stimulates cell division and the pri-mordial production of root. Wounding enhances the absorption of auxins. Wounding significantly increased the root growth of stem cuttings of lemon (Patel *et al*., 2021).

Effect of microclimatic conditions

After planting cuttings in the media, the prevalence of temperature, relative humidity, shade, light, moisture level of media, gases and aeration play a pivotal role in better rooting, shoot formation and survival percentage **(**Hartman and Kester, 2014). The congenial weather conditions result in early sprouting, rooting and shoot formation. Photoperiod, wavelength and irradiation play an essential role in maintaining stock plants and rooting of cuttings. The photoperiod influences photosynthesis and carbohydrate accumulation for better rooting. The irradiance of stock plants needs to be maintained to maintain sufficient endogenous auxin levels for inducing rooting in the stem cuttings. The high irradiance destroys auxin and affects water levels in the stock plants. The ideal temperature of 25-30℃ and relative humidity of 80-90% was essential to promote better rooting in acid lime cv. Kagzi lime (Malakar *et al*., 2019).

The prevalence of suitable weather inside propagation structures plays a significant role in realizing better rooting success. A controlled environment within the greenhouse, net house, plastic house, or mist chamber is necessary for better rooting. Shade intensity under shade net conditions influences the rooting of the stem cuttings. Misting and intermittent misting create a humid atmosphere around the planted cuttings in the net house or open conditions, enhancing the rooting process. Intermittent mist inside the mist house is often used on cuttings to reduce the temperature of the leaves, lower respiration, cool the atmosphere and leaf surface in the stem cuttings, increase relative humidity around the leaf surface, change the microclimate and reduce the heat load of the cuttings. Mist house growing condition was found effective in increasing the success rate of the cuttings. Water management is critical in propagation. The cuttings collected early in the morning are turgid and performs well in rooting. Since rehydration is very difficult without roots, the unrooted cuttings are susceptible to water stress. Leaf water potential influences the rooting of cuttings. A difference between transpiration loss and water absorption decides water level of cuttings. The stem base water content between 55 and 75% was found suitable for optimal rooting of lime (Swelith and Said, 2023). Table 2 shows the influence of growing conditions on propagation of lime and lemon through stem cuttings.

Conclusion

Vegetative propagation is practiced to produce true-to-type planting materials for fruit crops. Stem cutting is economic and efficient method of propagation to produce quality planting materials in acid lime (*Citrus aurantifolia* (Christm.) Swingle and lemon (*Citrus limon*

Table 2. Influence of growing conditions on propagation of lime and lemon through stem cuttings

Citrus species	Type of cuttings	Growing condition	Results	Reference
Lemon cv. Baramasi	Stem cuttings	Poly house	Primary roots (11.24) compared to open condition (7.08) and partial shade (4.06)	Kumar *et al*. (1995)
Meyer lemon	Softwood cuttings	Open condition Poly house	Cuttings planted in the open and covered with polythene and muslin cloth rooted 100% than planted in the greenhouse (79.1%) or in the open without cover (65%)	Sadhu (1997)
Citrus aurantifolia Swingle Kagzi-lime	Juvenile branches	Poly house, partial shade and open condition	Maximum mean sprouting (60.50%) was recorded in open area conditions closely followed by partial shade (48.54%). The lowest sprouting was recorded in polyhouse condition but diameter of thickest root (0.26 cm) was recorded maximum	Bhatt and Tomar (2010)
Citrus aurantifolia Swingle Kagzi-lime	Semi-hardwood cuttings	Open condition, partial shade, low-cost poly house	Success rate was higher in open condition. Vegetative growth of rooted cuttings was better in poly house condition	Bhatt and Tomar (2011)
Citrus limon (L.) Burm.	Hardwood cuttings	Net house	90% sprouting and 73.33% rooting	Seran and Umadevi (2011)
Citrus limon (L.) Burm. Pant Lemon 1	Semi-hardwood cuttings	Open condition	72.59% sprouting and 72.59% rooting	Satpal *et al*. (2014)

L.). The combination of the maturity of the stem portion, IBA, wounding, appropriate rooting media, season, and environmental conditions can significantly enhance the success of stem cutting. The semi-hard and softwood cuttings of lime and lemon gave a higher success rate. Depending upon the maturity of stem cuttings, wide range of IBA at the rate of 2,000-4,000 ppm was found successful in rooting, bud burst, shoot formation and survival of rooted cuttings. IBA coupled with wounding, further enhances rooting processes. The media *viz*., vermicompost, farm yard manure and cocopeat in combination with soil and sand were found suitable for rooting stem cuttings. Rainy seasons tend to exhibit better rooting, establishment and growth than those planted in spring, autumn or summer. Temperature of 25-30℃ and relative humidity of 80-90% were found ideal for better rooting. The mist and intermittent conditions within the poly house, mist chamber, or shade net house are necessary for better rooting. Research on rooting and sprouting of stem cuttings is lagging behind in terms of maintaining stock plants, endogenous auxins, endogenous food materials and carbohydrate: nitrogen ratio in the basal portion of cutting and microclimatic factors within propagation structures. Further research focusing on optimizing propagation techniques and elucidating the underlying physiological mechanisms can enhance productivity. Understanding the physiology of stem cuttings, auxins, media, endogenous food materials, growing conditions and their interaction is a greater possibility for producing disease-free, quality planting materials in acid lime and lemon.

Conflict of interest

The authors declare that they have no conflict of interest.

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