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Propagation of citrus species through cutting: A review

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Abstract
Citrus (*Citrus spp*.) is commercial propagated through vegetative propagation. Stem cutting is the most important method of vegetative propagation of the world, excluding India. The cuttings treated with high concentration of IBA, performed the best in rooting and survival percentage, whereas, the mist house growing condition was found effective in increasing the success rate of the cuttings. Semi-hardwood cuttings gave the best result in terms of rooting and survival performance. In case of growing condition, the stem cuttings can give good rooting and survival, when cuttings are taken in July planting time.

Keywords: *Citrus* spp., cuttings, bio regulators, planting time, rooting media, cutting type

Introduction
Citrus belong to Rutaceae family, the genera *Citrus* (oranges, mandarins, pomelos, grapefruit, lemons, limes and citrons), *Fortunella* (kumquats) and *Poncirus* (trifoliate oranges) contain the principal commercial species (Swingle and Reese, 1967) [70]. It is originating in tropical and subtropical Southeast Asia, these plants are among the oldest fruit crops to be domesticated. India ranks sixth in the production of citrus fruit in the world. It is of particular interest because of its high content of vitamin C (Katz and Weaver, 2003) [34]. In India, citrus fruits are primarily grown in Maharashtra, Andhra Pradesh, Punjab, Karnataka, Uttaranchal, Bihar, Orissa, Assam and Gujarat. At present, in India total area under citrus fruit production is 1042.0 thousand hectare (13.3% of total area under fruits) with a production of 10090.0 thousand MT (12.4% of total production under fruits) and productivity of 9.87 MT/HA and total area under Sweet orange cultivation in India is 323.2 thousand hectare (4.6% of total area under fruits) with a production of 3520.0 thousand MT (4.3% of total production under fruits) and productivity of 10.9 MT/hectare during the season 2012-13.(NHB 2012-13) [40].
Vegetative propagation of plants by stem cuttings is the most commonly used method for producing herbaceous and woody plant in many part of the world. A cutting is a piece of the part of plants used to propagate which regenerate there missing part is called cutting. Stem cutting can be classified as follows: hardwood cuttings, semi hardwood cuttings, softwood cutting and herbaceous cuttings. Stem cuttings have been used for the vegetative propagation of several fruit trees including citrus (Platt and Opitz, 1973; Debnath *et al*., 1986; Singh *et al*. 2013) [45, 20, 66].
There are several factors that can affect the rooting potential of stem cuttings including species and specific cultivar needs; the source, position, and type of cutting taken; juvenility and condition of stock plant; wounding or leaf removal; stock plant etiolation and girdling; cutting date; or is influenced by growing 9 conditions such as media, mist, bottom heat, use of hormones, fertilizer, and supplemental lighting (Hartmann *et al*., 2002) [31]. Ferguson and Young (1985) [23] showed that the Indole butyric acid and naphthalene acetic acid, both at 3000ppm, stimulated the greatest root production in juvenile and mature cuttings of citrumelo (*Citrus paradise* Macf. X *Poneirus trifoliata* L.). Ozcan *et al*. (1993) [41] studied the best results of growth regulator were obtained with 4000ppm concentration of IBA for common sour orange (57.77% rooting). IBA at 2000ppm gave the highest rooting percentage of lemon (cv. Baramasi) stem cutting Kumar *et al*., 1995) [35, 48]. Experiments have been conducted by various workers on the effect of different concentrations of IBA, NAA treated with different combinations in cuttings on the rooting percentage, number of roots, promoting better shoot characters using different media and observed better performance on the root as well as shoot characteristics (Cooper,1940; Shafriz *et al*. 1968; Gabricidze 1970; Mendilcioglu, K. 1968;

Bhusa *et al*. 2001; Daoud *et al*. 1995; Bajwa *et al.* 1977; Yamashita and Astsuda 1977; Singh, R.P. 1959; Sabah *et al.* 1991; Deb *et al*. 2009; Abdullah and Al-Khateeb, 2004; Raj, 1990; Singh *et al*., 2011) [14, 63, 26, 38, 12, 17, 8, 79 68, 51, 19, 3, 47, 82].

1. Effect of bio-regulators on the rooting of citrus species

Today, IBA and NAA are still the most widely used auxins for rooting stem cuttings. It has been repeatedly confirmed by several workers that auxin is required for initiation of adventitious roots on stems and indeed it has been shown that divisions of the first root initian cells are dependent upon either applied or endogenous auxins (Tanabe, 1982) [71]. IBA at 2000ppm gave maximum rooting and success with Sweet lime and Lemon cuttings (Singh and Singh, 1964) [67].

Bhatt and Tomar (2011) [11] has reported the maximum root formation, length of root, diameter of root, sprout in shoot was recorded under 500ppm concentration of IBA. Application of these hormones- Indole-butyric acid (IBA) and Naphthalene-acetic acid (NAA), to induce rooting in stem cuttings of citrus trees has gained importance over time (Sabah *et al*., 1991) [51]. Maximum rooting and shoot growth characters were recorded under 500ppm concentration of IBA (Singh *et al*., 2015) [64, 74]. The stimulation of adventitious root formation in stem cuttings treated with auxins is well known (Blazich, 1988) [13]. Thangji and Wunnachit (1994) [72] observed that the maximum rooting (50%), average root length (3.09cm) and shooting (33.33%) in pummelo was recorded under 6000ppm concentration IBA. Sandhu and Singh (1986) [58, 65] showed that the percentage of call used stem cuttings of sweet lime (*Citrus Limettioides* Tanaka) was highest in IBA 200ppm and the amount of callus per cutting was maximum in NAA 100ppm treatment. The proportion of rooted cuttings was higher with IBA 100ppm. However, number of roots per cutting and root length was more with 200ppm NAA they also found that there is no correlation between callusing and rooting was found. Bhusal (2001) [12] showed that the rooting abilities varied among species and varieties (0-100%). Rough lemon and lemon had 100% rooting while Tengu and Kuno Satsuma mandarin had 0%. In common trifoliate orange, the maximum rooting (100%) was found in June cutting followed by July cutting (90%) and the minimum rooting (30%) was recorded in September.

Table 1: Rooting in Citrus spp. stem cutting with PGRs with other chemicals formulation

Citrus Spp. Cultivars	Type of Stem cutting	Method of treatment	Optimum concentration mg/l	References
Citrus medica L. (Corsian)	Semi hardwood	Quick dip	IBA 500 and 1000ppm	Kako Al-Zebari *et al*. (2014)
Citrus limon B. (Pant Lemon=1)	Hardwood	Quick dip	IBA 2000ppm	Singh *et al*. (2013) [66]
Citrus aurantifolia L. (Mexican)	Hardwood	Quick dip	IBA 6000ppm	Elsheikh (1999) [69]
Citrus limon B (Baramasi)	Hardwood	Quick dip	IBA 2000ppm	Kumar *et al*. (1995) [35, 48]
Citrus aurantium L. (Sour orange)	Softwood	Quick dip	IBA 1000ppm	Malik and Harnard (1983) [37]
Citrus. limon B.	Semi-hardwood	Quick dip	IBA 2000ppm and pHBA 2000ppm	Rakesh-Kumar *et al*. (1995) [35, 48]
Citrus aurantifolia S. (Kagzi lime)	Hardwood	Quick dip	IBA 500ppm	Bhatt and Bhatt (2014) [10]
Citrus limon B.	Hardwood	Quick dip	Frolic acid 200 ppm NAA 5,000 ppm	Debnath *et al*. (1986) [20]
Citrus maxima (Hom Hat Yai)	Hardwood	Quick dip	IBA 6000 ppm	Thangjit, S. and Wunnachit, W. (1994) [72]
Citrus latifolia Tan. Lima acida 'Tahiti	Semi-hardwood	Quick dip	IBA 2000ppm	Prati *et al.* (1999) [46]
Citrus limetioides T. (Sweet lime)	Hardwood	Socking method	IBA 100ppm	Bajwa *et al*. (1978) [7]

Similar experiments have been carried out by different workers in *Citrus species* on various parameters of cuttings and root formations with minimum time with respect to the various concentrations of growth subastances (Kumar *et al*. 2004; Kako Al-Zebari *et al*. 2014; Singh *et al*. 2013; Elsheikh, 1999; Kumar *et al*. 1995; Malik and Harnard, 1983; Rakesh-Kumar *et al*. 1995; Prati *et al*. 1999; Bhatt and Bhatt 2014; Jelev, 2014; Danos *et al*. 1994; Pandey *et al*. 2003; Roberto *et al*. 2001; Singh *et al*. 1986) [33, 37, 46, 35, 48, 66, 10, 21, 16, 42, 49 58, 65].

Auxin application has been found to enhance the histological features like formation of callus and tissue and differentiation of vascular tissue (Mitra and Bose 1954, Satpal *et. al*., 2014) [2, 59]. Adding a small percentage of certain phenoxy compounds to either IBA or NAA increased rooting and produced root systems better than those obtained when phenoxy compounds are used alone (Davis and Haissig, 1990). Sandhu and Singh (1986) [18, 58, 65] observed the percentage of call used Stem Cuttings of Sweet Lime (*Citrus Limettioides* Tanaka) was highest in IBA 200ppm and the amount of callus per cutting was maximum in NAA 100ppm treatment. The proportion of rooted cuttings was higher with IBA 100ppm. However, number of roots per cutting and root length was more with 200ppm NAA. Sadhu (1997[a]) [57] noted that IBA was effective in promoting rooting in both lemon and lime. He also reported successful rooting on leaf cutting of Elachi lemon, Seedless Lemon and Kagzi lime. Popovic *et al*., (1998) reported that Statistically very significant greater influence of IBA to the percentage of rooting of mature cuttings of the variety Meyer (86.66). The highest rooting percentage and survival were recorded for leaf-bud cuttings of Assam lemon (*Citrus limon* Burm.) treated with 3000ppm IBA under sand medium (Nath, 2000) [39]. Synergistic improvement in rooting of cuttings is due to treatment with IAA or IBA + CCC and IAA + ascorbic acid as well in lemon. Anatomical studies of rooting lemon cuttings revealed that roots can originate from secondary phloem, pericycle, cambium and medullary rays (Sadhu, 1997[b]) [52]. Seran and Umadevi (2011) [61, 62, 73] noted that the shoot length (5.73 cm), rooting percentage (73.33%) and survival rate (90.0%) of stem cuttings in lemon (*Citrus limon* l.) were significantly higher in cuttings dipped in 2500ppm IAA than other treatments. Bester and Rabe (1996) [9] reported that the translocation of IAA in citrus rootstock stem cuttings did not correlate with plant vigor. Thayamini *et al.,* (2011) [73] showed that shoot length (5.73 cm), rooting percentage (73.33%) and survival rate (90.0%) of *Citrus limon* cuttings were significantly higher in cuttings dipped in 2500ppm

concentration of IAA than other treatments. Alhossein, Tafazoli. (2006-2007) [5]. observed that IBA 1000-2000mg/1 with 2% ascorbic acid was the best treatment for increasing rooting cuttings of sweet lime.

2. Effect of planting time on the rooting of citrus species

The time of preparation of cuttings in *C. limonia* greatly affected the extent and success of root formation, the optimum time of cuttings preparation and planting is related to the physiological condition of the plant and environmental conditions. Both time of cutting collection and rooting success varies with the climatic condition and prevailing outdoor temperature (Abou and Rawash *et al.* (1998) [4]. This may be affected by season and several factors such as temperature, light and nutrient availability to the rooting percentage of cuttings. The low rooting percentage during winter may be attributed to temperature level at the time of planting. The stem cuttings can give good rooting and survival, when cuttings are taken in July (Ozcan *et al*., 1993, Singh *et. al*., 2017) [41, 83, 84]. Singh *et al*., (2015) [64, 74] showed that the maximum survival percentage (77.37%) was observed under rainy season planting time.

Kumar *et al*. (1995) [35, 48] showed that cuttings taken in July had maximum rooting success, required fewer days for sprouting and showed better shoot and root growth than those taken in February. The vegetative propagation of citrus junos by softwood stem cuttings under mist chamber. They found the best percentage of rooting in cuttings taken during July and August (Kim *et al*. 1992). In case of crop response, lemon significantly response to cuttings made from lower end portion of ringed branch followed by pomegranate subjected to shoot and root characteristics (Pandey and Bisen., 2010) [43]. July was far better than September for making Sweet lime cuttings and IBA excelled over NAA (Gangwar and Singh, 1965) [27]. Alhossein, Tafazoli. (2006-2007) [5]. reported that the best time for cutting was early spring was the best treatment for increasing rooting of sweetlime cuttings.

3. Effect of growing condition on the rooting of citrus species

In plant propagation, the different environment viz., glass house, net house, poly-house and mist chamber have been widely used for rooting of different types of cutting. Development of mist chamber is a major breakthrough in propagation of plants. Creating humid atmosphere by means of artificial mist around the planted cuttings either in concealed pot culture house or in open conditions has proved to enhance the process of rooting (Prolings and Therios, 1976) [81]. Intermittent mist is often used on cuttings because it reduces the temperature of the leaves, lowers respiration, and increases relative humidity around the leaf surface (Langhans, 1955) [80]. Mist house condition is often used on cuttings because it reduces the leaves temperature, increases relative humidity and lowers respiration around the leaf surface (Dev *et. al*., 2017) [83]. Softwood cuttings of Meyer lemon planted in the open and covered with polythene and muslin cloth rooted 100 percent as compared with those in a greenhouse (79.1%) or those in the open without cover (65%) (Sadhu, 1997[c]) [53].

Table 2: Growing condition, Type of stem cutting and rooting percentage in Citrus spp.

Cultivar	Growing condition	Type of Stem cutting	*Sprouted, **Rooting	References
*Citrus auriantifolia (*Kagzi-lime*)*	Polyhouse	Juvenile branches	*68.50	Bhatt and Tomar (2011) [11]
Citrus limon L.	Net house	Hardwood	*90.00, **73.33	Seran and Umadevi (2011) [61, 62, 73]
C. paradisi x Ptrifoliata (Citrumelo)	Mist chamber	semi hard wood	**100.00	Sabah et al., (1991) [51]
Citrus limon B. Meyer lemon	Mist chamber	Softwood cutting	**98.00	Umarov (1983)
*Citrus limon B. (*Pant Lemon=1*)*	Open condition	Semi-hardwood	*72.59, **72.59	Satpal *et al*. (2014) [59]

4. Effect of rooting media on the rooting of citrus species

Rooting medium also play an very important role in the root proliferation and further growth in plants raised by stem cutting, although information on this aspect in very limited in citrus spp. Ford (1957) [24] found a 1:1 mixture of peat and perlite best for rooting rough lemon cuttings. Albouyeh (2007) [6] for citrus cuttings recommends only peat or mixture of peat, perlite and cocopeat with 2:2:1 ratio for plant height and leaf number increasing. Softwood cuttings of sweet lime rooted better than hardwood ones in clay and loam rooting media (Ghosh, 1990[b]) [28]. Singh *et al*., (2015) [64, 74] showed that the rooting media Soil + Sand + Cocopeat improved rooting percentage, while survival percentage was observed under Soil+Sand+FYM. Maximum percentage of survival and rooting was showed under garden soil + sand + vermi-compost (1:1:1) treatment (Kumar *et al*. 2015) [74]. Malik and Harnard (1983) [37] was reported that the maximum rooting percentage (85.6) was recorded under sand+peatmoss media. Kako Al-Zebari and Ali M. Al-Brifkany. (2015) [33]. Observed that the Media (1 part peat moss + 2 parts sand) to increase rooting percentage in the stem cuttings of (*Citrus medica* Linnaeus) Corsian cultivar.

5. Effect of girdling (ringing) on root formation

Girdling is one of the most important vegetative propagation method for root formation. This were applied for stems block the downward translocation of carbohydrates, hormone and other possible root promoting factors (Hartmann and Kester 1983) [30]. Girdling generally causes an accumulation of carbohydrates above the girdle and a diminution of carbohydrates in the root system (Wallerstein, *et al.*, 1974; Wallerstein *et al.*, 1978; Evert and Smittle 1990) [77, 78, 22]. Girdling also increases in the levels of auxin and gibberellin and reductions in the level of cytokinin above the girdle, and in each case, the opposite effect on the hormone level in tissues below the girdle (Wallerstein, *et al.*, 1973; Wallerstein *et al*, 1978; Grierson, *et al.*, 1982; Dan *et al.*, 1984; Schneider, 1954; Path *et al*., 1971) [77, 78, 29, 15, 60, 44]. Girdling on Citrus were applied by so many author, Johor and Rahman 1959; Wallerstei *et al*., 1972) [32, 75]. lemon significantly response to cuttings made from lower end portion of ringed branch followed by pomegranate subjected to shoot and root characteristics (Pandey *et al*., 2010) [43].

6. Effect of type of cutting on the rooting of citrus species

Fourrier. (1984) [25] reported that hardwood cuttings are those made of matured, dormant firm wood after leaves abscised. The use of hardwood cuttings is one of the least expensive and easiest method of vegetative propagation. Basal cuttings from the branch were the best in rooting and gave the highest percentage of rooting and survival compared to medium and terminal (Elsheikh, 1999) [33]. Semi-hardwood cuttings gave the best result in terms of citrus rooting percentage (Koltsov, 1988; Sabah *et al*. (1991) [51]; Rakesh-Kumar *et al*. (1995) [35, 48] and Satpal, *et al*. (2014) [59]. Transversal and longitudinal sections of the base and callus tissues of semi-hardwood cuttings of sweet lemon showed the presence of two types of roots. One type initiated from the callus cells and the other endogenously near the vascular bundles in the region between cambium and pericyclic fibers in the parenchymatous cells (Sadhu, 1997[d]) [54]. Sadhu (1997[e]) [55] noted that juvenile cuttings from one-year-old seedlings of sour orange and Cleopatra mandarin rooted easily than the mature cuttings from 15-year-old trees. Root production on mature cuttings was confined to the basal part around the cut end, whereas root formed on the whole of the earthed-up portion of juvenile cuttings. Sweet lime (*Citrus limettoides*) semi-hardwood cutting gave better rooting than hardwood (Gangwar and Singh, 1965) [27].

Conclusion

All the above investigation shows that IBA treatment is better than the other form of treatments for citrus cutting development. It is proved that the success of rooting depends on the phytohormone with which the cuttings were treated and the genetic characteristics of investigated varieties. So increasing the production of citrus fruit cutting is one of the easy processes for developing the citrus plant as soon as possible.

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