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The use of auxin and media for rooting of citrus stem cuttings: A review

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Abstract
Citrus is a genus consisting of a larger number of species, varieties, cultivars and hybrids. The citrus (*Citrus* spp.) can be propagated by both the ways seeds as well as vegetative means, majorly budding is a very usual practice for propagation but the cutting method of propagation is more common among all, as this method is the easiest and simplest way for multiplication along with proper rooting by the treatment with different auxins and use of different types of media. Hormone treatment and rooting media play a major significant role in the rooting behavior of cuttings. Higher the concentration of auxin based rooting hormones, especially Indole butyric acid (IBA) resulted in a greater significant rooting effect on the citrus cuttings and the use of rooting media like cocopeat, vermicompost, sand along with a proper combination of other substrates like sawdust, soil, perlite, compost in a suitable concentration ensured a better-rooting success and qualitative root production of Citrus cuttings. Despite these auxins and different types of media, other components like time & season of cuttings taken, the portion of the cuttings taken, planting time, environmental conditions and mechanical treatments which can affect the rooting success are also discussed in this review which would help to provide the knowledge for further research.

Keywords: IAA, IBA, NAA, compost, cutting type, juvenility, season, vegetative

1. Introduction
Citrus is a genus belonging to the family Rutaceae. South-East Asia is believed to be its origin (Roger, 2001) [49]. Over the world, Citrus production ranks 7th and India occupies 3rd place. Citrus spp. includes different types of varieties and cultivars with charming colour, use and flavour. Also, it is a fruit known worldwide (Raja, 2012) [43]. Acid lime (*Citrus aurantifolia*), Rough lemon (*Citrus jambhiri*), Orange (*Citrus sinensis*), Lemon (*Citrus limon*), Citron (Citrus medica), Rough lemon (*Citrus jambhiri*), Rangpur lime (*Citrus limonia*), Volkamer lemon (*Citrus volkameriana*), Sweet lime (*Citrus limettioides*), Kagzi lime (*Citrus aurantifolia*) etc are some of the citrus sps. Usually, Citrus fruit comprises of richest vitamin C content, along with that it has special properties like Antioxidant, anticancer and anti-inflammatory, these are due to the presence of various constituents of nutrients and vitamins present in them (Okwu, 2008) [40] and are essential for human health. Because of the essential nutrients, efficient production and the large food commodities made from citrus spp, the citrus is considered as the world's topmost fruit (Kour & Singh 2012) [30]. The total area under citrus cultivation is 1003 thousand hectares with the production of 12546 thousand hectares, Lime / Lemon consists area of 286 thousand hectares with the production of 3148 thousand hectares production, the total area covered by Mandarin is 428 thousand hectares with the production of 5101 thousand hectares, Sweet orange has 185 thousand hectares of area with the production of 3266 thousand hectares, other Citrus species with an area of 103 thousand hectares with the production of 1030 thousand hectares during the season of 2017-2018 (NHB 2017-2018) [39].
Both sexual and vegetative methods are used in citrus propagation, but the vegetative method is more preferred when compared to the sexual propagation method because it has many advantages like, it provides true to type, uniform quality of fruits in the early bearing plants, the spread of diseases and infections are rare (Babu, 2001) [8] and from a few stock plants, numerous new plants can be produced within a restricted space. Typically, raisers who face the deficiencies of seed-source accessible for the propagation can easily adopt the spread of rootstocks through cuttings to create enormous quantities of hereditarily indistinguishable plants. The rootstocks propagated by means of vegetative reproduction attains a greater length and number of roots (Albrecht *et al*., 2017) [4]. Even without the distinct practices, cuttings can be easily propagated with less expense, faster & in a simpler manner (Dawa *et al*., 2017) [17]

without the practice of special procedures or skills as it is necessary in other methods of propagation. For the vegetative propagation of citrus this strategy is fundamental, not exclusively to keep up attractive qualities of the mother plant (Hartmann & Kester 1975) [23], yet in addition to giving them true-to-type rootstocks with a desirable character. The complexities of meeting increased demand for crop production have sparked a surge in interest in root biology (De dorlodot *et al*., 2007) [18] because Roots are considered as a critical organ system of plants that determine the difference in the success rate of plant multiplication through cuttings. Roots can retain supplements to the cuttings to attain maximum development hence the use of growth regulators and rooting media are the most effective and convenient means to maintain the optimum development of roots and nutrient supplies according to the specific requirement of cuttings.

Plant growth regulators are substances that are natural or manufactured that can alter the plant's development and enhancement by affecting their physical forms and through expanding the efficiency of the crops (Kakimoto, 2003) [25]. Auxin plays a basic part in most major development reaction (Guilfoyle, 2015) [22]. It appears to be the foremost issue acting on all the events of root development (Dinneny & Benfey 2008) [19]. Recently IAA and IBA are the topmost auxins that are in use for rooting enhancement. The naturally presenting auxin in plants is Indole acetic acid (IAA) and Indole butyric acid (IBA) is well known as a synthetic rooting hormone (Bartel *et al*.,2001) [9], which helps to enhance root growth and development.

The diverse sort of variables can influence the development and advancement of plants among the distinctive components, but for the production of qualitative roots of cuttings, the rooting media or rooting mixture is foremost important. The medium in which a cultivated plant's root grows is considered as "growing media" (Kampf, 2000) [26] and its primary purpose is to aid the growth of the plant (Robert, 2000) [48]. The distinctive potting mixture or media like Cocopeat, Vermicompost, peat moss, sand, soil *etc*. are used but the selection of growing media should be done based on the study of physical and chemical properties of growing media (Raviv, 2005) [44]. The details regarding the influence of auxin treatment and media on rooting aspects of various Citrus spp. cuttings are discussed further.

2. Effect of Auxins

Auxin is a fundamental part of the reconciliation of assorted biotic and abiotic natural signs to plant root improvement. As per the study conducted by many researchers, various rooting behavior of citrus cuttings that are affected by auxin treatments is discussed.

According to the research conducted by Chahal and Adesh (2018) [16], the Cutting method of propagation resulted in a larger number of primary roots followed by seedlings in – Carrizo citrange which is a cross between *Citrus sinensis* Osb. and *Poncirus trifoliate* L. Raf. This shows the success of the cutting method of propagation meanwhile the concentration of auxin and its effects differ accordingly. In some of the citrus sps, the IBA treatment affected greatly (100%) in rooting. The cuttings of Citrumelo which is cross between *Citrus paradisi* Macf and *Poncirus trifoliata* L. Raf treated with the concentration of IBA @ 100 ppm recorded 100% rooting success and survivalism (Fadli *et al.*,2017) [20]. For the development of roots of Kagzi-lime (*Citrus aurantifolia*) cuttings, treatment of IBA @ 500 ppm proved most effective (Bhatt & Tomar 2010) [12]. Also, Corsian cultivar, Citron (*Citrus medica* Linnaeus) stem cuttings with the concentration of 500 and 1000 ppm of IBA has a significant effect on rooting percentage (100%) (Al-Zebari & Al-Brifkany 2015) [5].

In a few researches, it is proved that developmental characteristics of rooting are increased, as the concentration of IAA rises in stem cuttings of a few cultivars followed by lesser concentration. Seran and Uma devi (2011) [52], reported that cuttings taken from Lemon *(Citrus limon* L.*)* treated with 2500 ppm of IBA proved superior with establishing the rate of rooting about 73.33%, compared with a lesser dose of IBA. the sweet lime (*Citrus limettioides* Tanaka) treated with IBA @ 2000 ppm benefited with a maximum number of rooting followed by lesser concentration of IBA @ 1500 and 1000 ppm (Kumar *et al*., 2004) [33].

In other cases, IBA treatment proved to enhance the overall rooting parameters of citrus cuttings and are discussed here. Singh *et al*., (2013) [54] proved the use of IBA along with 2000 ppm improved the rooting parameters like length, diameter and the number of roots in hardwood cuttings of Pant Lemon (*Citrus limon*). Also, the study conducted by Uzun and Seday in 2011 [59], recommends IBA at 4000 ppm to obtain a greater length and higher number of roots by the hardwood cuttings of Volkamer lemon (*Citrus volkameriana*) and Rough-lemon (*Citrus jambhiri).* Sweet Orange (*Citrus sinensis* L. Osbeck) cv. Malta treated with the dose of 5000 ppm IBA provided significant effects with a percentage of rooting (71.67%), root lengths (13.63 cm), total primary roots (5.33), (Singh & Singh 2016) [56].

The study conducted by Malakar *et al.,* (2019) [36] made a way to generate a better-quality planting material in acid lime cuttings, also in his study, he found the maximum establishment of rooting characteristics by the number of primary roots (11.10), lengthy roots (15.47 cm) and percentage of established roots of cuttings (44.44%), in Hardwood cuttings of Cv. Kagzi lime treated with 500 ppm IBA. An increasing rooting percentage of sweet lime (*Citrus limetta* L.) was observed when treated with IBA 1000-2000mg/l (Aboutalebi & Tafazoli 2006) [2].

Many works carried out by researchers proved that IBA treatment was found to be superior over the other hormonal treatments in obtaining a successive rooting effect in citrus cuttings. During the comparison among the IBA with other plant growth regulators like GA3, it is observed that Rangpur lime (*Citrus limonia* Osbeck) treated with IBA @ 1000 ppm responded to a greater number of roots in cuttings (3.93), size of the thickest root (1.38 mm), measurement of lengthy root (4.47 cm), percentage of rooting/cutting (45.37), survival percentage of rooted cuttings (60.00) (Ahmad *et al*., 2018) [3]. Even the cuttings of cv. Pant lemon-1(*Citrus limon* Burm) when compared with other concentrations of different auxins, it proves IBA at 500 ppm resulted in maximum rooting characteristics (Singh *et al*.,2015). Among the different combinations, treatment with IBA @ 600 ppm resulted in significant rooting effects on cuttings of lemon (*Citrus limon* Burm.) cv. pant lemon (Satpal *et al*., 2014) [50].

The research conducted at Horticultural Research Centre U.P by Kumar *et al*., (2015) [34] suggested the optimum dose of IBA for better rooting on stem cutting of *Citrus limon* Burm. cv. Pant Lemon-1. His study utilized IBA at different levels of doses like 400ppm, 800 ppm and 1200 ppm but IBA took @800 ppm resulting in maximum rooting effects. In other

cases, the auxin like IBA when mixed with other ingredients gives a better rooting effect (Reddy *et al*., 2008) [45, 46]. Stem cutting of lemon (*Citrus limon* Burm) cv. Pant lemon-1 treated with the IBA @ 4000 ppm gave better results, whereas the cuttings not treated with the indole butyric acid (IBA) are not much effective, this suggests the presence of IBA plays a significant effect in rooting (Patel *et al*., 2018) [41]. The solution of IBA @ 2500 ppm with other mixture, is recommended to the farmers propagating the cuttings of Kagzi lime (*Citrus aurantifolia* Swingle) by Kumar and Singh (2020) [31].

NAA treatment also showed positive rooting effects in cuttings (Reddy & Chandrasekar (2008) [45, 46]. Mendes, (2011) [37] reported that root differentiation was carried by NAA and root elongation is carried by its mixture with the help of IAA. Cuttings of 'Nam Roi' pummelo (*Citrus grandis* (L.) Osbeck.) showed a significant rooting effect treated with 1000 ppm of NAA (Le *et al*., 2002) [35]. When the research has been conducted to find out the effect of different auxin over the cuttings of Orange (*Citrus sinensis* L.) Local Cv. IBA and NAA are found to be most effective in enhancing rooting behavior at 200ppm and 100 ppm levels (Khursheed & Salih 2007) [29]. In some studies, NAA is found to be most supportive of IBA in rooting. The treatment is done with a mixture of NAA @ 500 ppm and IBA @ 500 ppm has resulted in significant effects with a maximum number of primary (7.30) and secondary roots (15.30) of lemon (*Citrus limon* L.) cuttings (Singh *et al*., 2015).

Despite stem cuttings, IBA treatments to the nodule and leaf cuttings also shows significant rooting effects. Single nodule and leaf cuttings of citrus spp. Kuharske has quick dipped in a 7500-ppm solution for 0.5 seconds resulted in most root generation (Beeson & Silva 2017) [10]. During the nodule cutting treatment of propagation, the number of nodules taken also influences the rooting parameter (Bhagya *et al*., 2014) [11]. The higher rooting percentage (98.5%) and survival (100%) were recorded for those cuttings treated with 3000 IBA ppm, on leaf bud cuttings of lemon (*Citrus limon*) cv. (Nath, 2000) [38].

IBA treatment also helped to increase the root-hair growth of trifoliate orange, which is a widely used rootstock for citrus. Also, it induces a signalling pathway for root hair growth (Zhang *et al*.,2018) [60] and controls root interaction (Kazan, 2013) [27]. The most generally utilized auxin is artificial Indole-3- butyric acid (IBA), which ends up being the best chemical advancing the creation of adventitious roots followed by the natural Indole -3-acetic acid (IAA) (Pop *et al*., 2011) [42].

Table 1: Recommended dose of auxin to enhance the rooting behavior of Citrus spp

Sl. No	Citrus spp.	Recommended Concentration	References
1	Acid lime, (*Citrus aurantifolia* (Christm.))	IBA @ 500 ppm	Malakar *et al*. (2019) [36]
2	Rangpur lime (*Citrus limonia* Osbeck)	IBA @ 1000 ppm	Ahmad *et al*. (2018) [3]
3	cv. Pant lemon, Lemon (*Citrus limon* Burm)	IBA @ 4000 ppm	Patel *et al*., (2018) [41]
4	Citrus sp. Kuharske	IBA @ 7500 ppm	Beeson & Silva (2017) [10]
5	Citrumelo (*Citrus paradisi* Macf. *x Poncirus trifoliata* L. Raf)	IBA @ 100 ppm	Fadli *et al*., (2017) [20]
6	Sweet orange (*Citrus sinensis* L. Osbeck) cv. Malta	IBA @ 5000 ppm	Singh & Singh (2016) [56]
7	Citron (*Citrus medica* Linnaeus)	IBA @ 1000 ppm	Al-Zebari & Al-Brifkany (2015) [5]
8	Rough lemon (*C. jambhiri*)	IBA @ 4000 ppm	Uzun & Seday (2011) [59]
9	Volkamer lemon (C. *volkamerianana*)	IBA@ 4000 ppm	Uzun & Seday (2011) [59]
10	Lemon (*Citrus limon* L.)	IAA @ 2500 ppm	Seran & Umadevi (2011) [52]
11	Kagzi-lime (*Citrus aurantifolia*)	IBA @ 500 ppm	Bhatt & Tomar (2010) [12]
12	Orange (*Citrus sinensis* L.) Local cv.	IBA @ 200 ppm NAA@ 100 ppm	Khursheed & Salih (2007) [29]
13	Sweet lime (*Citrus limetta* L.)	IBA @ 1000-2000 ppm	Aboutalebi & Tafazoli (2006) [2]
14	Sweet lime (*Citrus limettioides*)	IBA @ 2000	Kumar *et al*., (2004) [33]
15	Lemon (*Citrus limon*) cv. Assam	3000 IBA ppm	Nath (2000) [38]

3. Effect of rooting media

Rooting medium has a vital role in rooting percentage, ample aeration and drainage to make certain quicker and higher exceptional of root development. Even for the advancement of plant growth, the main rudiments such as nitrogen (N), phosphorus (P) and potassium (K) media are most effective. While selecting the appropriate media, the sort of establishing and their characteristics is the most extreme significance for the production of the quality of rooted cuttings (Khayyat *et al*., 2007) [28].

Cocopeat is one of the best components of growth substrate and has suitable chemical properties required for growth like high phosphorous & potassium content, adequate EC, pH etc (Abad *et al*., 2002) [1]. Due to its special characteristics, cocopeat showed better rooting effects in acid lime, (*Citrus aurantifolia*) Swingle Cv. Kagzi lime (Malakar *et al*., 2019) [36]. Also, the cocopeat can blend easily with other substrates (Bhattacharjee, 2006) [13], this has been proved in case of lemon (*Citrus limon* Burm) cuttings, by the study conducted at Horticulture Research Centre of G. B. Pant Agriculture university & Technology (Pantnagar) during September - April by showing the significant rooting effect when the cocopeat is used along with other substrates like Soil, Sand and Cocopeat enhanced rooting of cutting at (Singh *et al*., 2015).

Vermicompost is enriched soil produced by the activities of earthworms also it is a major part of organic farming. Along with the compost it has wastewater which contains beneficial nutrients required for plants which are termed as 'Teas' (Arancon *et al*., 2020) [7], this helps to aid the rooting in cuttings as it contains readily available nutrients along with some growth hormones. For the cuttings of lemon (*Citrus limon* Burm) cv. Pant Lemon-1 the vermicompost along with a combination of other substrates like garden soil and sand in the ratio of 1:1:1 played a most beneficial role when compared with the other combinations of media (Kumar *et al*., 2015) [34]. Also, this has been repeatedly proved in the year 2018 by Patel *et al*., in case of lemon (*Citrus limon* Burm) cv. Pant Lemon-1 cuttings, but here the vermicompost is utilized along with combination of other substrates like soil and sawdust. Through the observation of these researches, vermicompost can be suggested as suitable media necessary for the combination and rooting in case of lemon cuttings.

The study conducted by Nath, (2000) [38] proved sand as one

of the best media for the higher root induction up to 98.5% in case of the lemon *(C. limon)* leaf bud cuttings. also, the sand along with the combination of compost showed significant effects (Singh & Singh 1973) [55]. Other media like peat is also one of the major substrates used as a potting media, due to its higher capacity of water holding capacity when mixed with other media (Kumar *et al*., 2019) [32]. Citron (*Citrus medica* Linnaeus) stem cuttings treated with the peat moss and sand in a 1:2 ratio was succeeded in higher rooting percentage (Al-Zebari & Al-Brifkany 2015) [5]. Also, peat and perlite in 1:1 ratio were suggested as best media in rooting of rough lemons (Ford, 1957) [21].

Table 2: The recommended combination of media to enhance the rooting behavior of Citrus spp

Sl. No	Citrus spp. Cultivars	Recommended Rooting media	References
1	Acid lime (*Citrus aurantifolia*)	Cocopeat + soil (1:1)	Malakar *et al*., (2019) [36]
2	Pant lemon (*Citrus limon* Burm)	Soil+ Sawdust+ Vermi-compost (1:1:1)	Patel *et al*., (2018) [41]
3	Lemon (*Citrus limon* Burm) cv. Pant lemon	Garden soil + sand + vermicompost (1:1:1)	Kumar *et al*., (2015) [34]
4	Citron (*Citrus medica* L.)	Peatmoss + Sand (1:2)	Al-Zebari & Al-Brifkany (2015) [5]
6	Lemon (*Citrus limon* L.)	Soil+ Sand+ Cocopeat (1:1:1)	Singh *et al*., (2015) [57, 58]
7	Lemon (*C. limon*)	Sand	Nath (2000) [38]

4. Other Aspects

Despite auxins and media, for the successive rooting of the cuttings, other aspects like the use of quality planting material (Ambebe *et al*., 2017) [6], the season of planting, juvenility, time of planting material collection, origin and condition of mother plants (Sarmiento *et al*., 2016) [51], mechanical treatments like girdling, ringing and can also play a significant effect. Even some times the physical characteristics of the potting mixture such as structure, texture and pH (Riaz *et al*., 2008) [47], even pH which alters the auxin uptakes (Shinohara *et al*., 2006) [53] also need to be considered while media and hormone selection.

The study conducted by Singh *et al*., in 2015 [57, 58] at Patharchatta suggested, propagating the cuttings of Lemon (*Citrus limon* Burm.) cv. Pant lemon-1 in the rainy season to ensure better-rooting effects. Time of cutting's Collection also influenced the rooting parameters in sweet lime (*Citrus limetta* L.) Cuttings, hence early spring propagation is suggested by the researcher for the best rooting in lime (Aboutalebi & Tafazoli, 2006) [2] but in case of 'Sunki' Mandarin hybrids, the cuttings gathered during the late spring resulted in approximately about 100% rooting (Sarmiento *et al*., 2016) [51].

A study conducted by Saptal *et al*., 2014 proved that planting time (December) and type of cutting (semi-hardwood) affected the rooting behavior positively in lemon (*Citrus lime* Burm.) cv. pant lemon cuttings and even in cuttings of sweet orange (*Citrus sinensis* L. Osbeck) cv. Malta also the semi-hardwood type of cutting showed greater rooting (Singh and Singh, 2016) [56]. The cuttings taken from the basal part of the lateral shoot end resulted in a greater rooting percentage in Orange (*Citrus sinensis* L.) (Khursheed & Salih, 2007) [29]. Also, Al-Zebari and Al- Brifkany (2015) [5] reported that medial type of cuttings enhanced the rooting length (17.92 cm) in citron (*Citrus medica* Linnaeus) during the comparison between the tip, medial & base cuttings.

Hussain *et al*., (2016) [24] studied the different environmental conditions are provided to the cuttings by keeping them in different structures like a plastic tunnel, field, shade net, along with the different types of cuttings like semi hardwood, softwood and tip cuttings, among these he suggested that semi-hardwood and softwood cuttings of sweet lime (*Citrus limettioides*) performs well-rooting per cent under the condition of the plastic tunnel. Bhatt & Tomar (2010) [12] suggested the Kagzi-lime (*Citrus aurantifolia*) cuttings to be grown under polyhouse conditions to achieve a maximum success rate of rooting compared to open and shady areas.

Cuttings of trifoliate orange (*Poncirus trifoliata* [L.] Raf.) with a current age of one-two years resulted in a significant rooting effect rather than old aged cuttings (Bhusal *et al*., 2003) [14]. Bisen in 2010 [15], proved that Mechanical treatments like Girdling and ringing can also responded to higher rooting per cent (80%) in lemon cutting when compared to others.

Table 3: Other aspects affecting the rooting Success in Citrus spp.

Sl. No	Citrus spp. Cultivars	Other aspects	Major aspect	References
1	Sweet lime (*Citrus limettioides*)	Growing condition Cutting type	Plastic tunnel Semi hardwood & Softwood cutting	Hussain *et al*., (2016) [24]
2	'Sunki' Mandarin	Time of cutting collection	Late spring	Sarmiento *et al*., (2016) [51]
3	Sweet Orange (*Citrus sinensis* L. Osbeck) cv. Malta	Type of cutting	Semi-hardwood	Singh & Singh (2016) [56]
4	Citron (*Citrus medica* L.)	Type of cutting	Medial type	Al-Zebari & Al- Brifkany (2015) [5]
5	Lemon (*Citrus limon* Burm.) cv. pant lemon	Season	Rainy season	Singh *et al*., (2015) [57, 58]
6	Lemon (*Citrus lime* Burm.)	Planting time Cutting type	December Semi-hardwood	Satpal *et al*., (2014) [50]
7	Kagzi-lime (*Citrus aurantifolia*)	Growing condition	Polyhouse	Bhatt & Tomar (2010) [12]
8	Lemon	Ringing Girdling	Lower part Upper part	Bisen, (2010) [15]
9	Orange (*Citrus sinensis* L. Local Cv.)	Type of cutting	Basal part of lateral shoot end	Khursheed & Salih (2007) [29]
10	Sweet lime (*Citrus limetta* L.)	Time of cutting collection	Early spring	Aboutalebi & Tafazoli (2006) [2]
11	Trifoliate orange (*Poncirus trifoliata* L. Raf.)	Juvenility	The Current age of one - two years	Bhusal *et al*., (2003) [14]

5. Conclusion

A great deal of work has been done on factors affecting the rooting of Citrus cuttings. It seems that treatment with the auxin IBA at the optimum concentration and the selection of suitable rooting media are most effective in encouraging various rooting parameters. Furthermore, factors like age, growing condition, type of cutting, season are very important with respect to rooting behavior. This ultimately helps to advance citrus production by the means of cutting. Both the auxin type and rooting media at the optimum dosage and combination as required by different citrus species will ensure the acceptance by growers as well as consumers for the commercial Citrus multiplication to attain a maximum success rate with less effort.

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