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Influence of plant bio-regulators and growing media on vegetative propagation of Citrus species: A review

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Abstract
Citrus (*Citrus* spp.) is grown commercially via asexual methods with the exception of India. Cutting, grafting and layering are the most common forms of asexual propagation in citrus. Cuttings and layering treated with a high concentration of IBA had the maximum rooting and survival rates, but the mist house growth environment was shown to be efficient in enhancing the success rate of cuttings. Rooting media, with regards to the success rate of vegetative multiplication in citrus species, play a vital role in the multiplication of roots and subsequently boost growth in plants grown vegetatively. As per this review based on various research studies, the rooting medium, field soil + sand + coco-peat (1:1:1) enhanced rooting percentage, field soil + sand + farm yard manure (1:1:1) improved survival percentage and the field soil + sand + vermicompost (1:1:1) treatment demonstrated the maximum percentage of survival and roots.

Keywords: *Citrus* spp., cuttings, grafting, air layering, rooting, PGR, growing media

Introduction
Citrus belongs to the Rutaceae family, and the commercial species are found in the genera Citrus (oranges, mandarins, grapefruit, pomelos, limes, lemons, and citrons), Poncirus and Fortunella (Swingle and Reese, 1967) [42]. Citrus is a generic phrase that refers to a variety of fruit species and types that are well-known around the world for their distinct flavors and vibrant colors. Citrus trees have an added aesthetic value due to their gorgeous evergreen foliage and blossoms, as well as their exceptional aroma (Rathour, 2022) [34]. Citrus fruits are becoming increasingly popular in international trade. Citrus fruits are mostly cultivated in India's subtropical areas. Vitamin C is abundant in citrus fruits. As a result, citrus fruit has a significant socioeconomic impact on fruit producers in terms of improving their living standards. Vitamin C deficiency is fairly frequent among humans in impoverished nations like India. As a result, lemon is an excellent source of vitamin C, which can help to compensate for vitamin C deficiency symptoms by increasing the availability of this vitamin in fruits like lemon and incorporating it into a daily diet (Kumar *et al.*, 2015) [23]. The total area under citrus cultivation in India during the year 2020-21 was 1097 Ha with a total production of 14245 MT yielding (NHB, 2022) [30].

The output of authentic and high-quality planting material is a pre-requisite for maximizing production and bearing in mind the high demand. Fruit farmers must constantly have the highest quality planting material. As a result, many experiments have been carried out with the purpose of producing high-quality planting material that could be distributed to fruit growers. To achieve the aforementioned goal, a fast process for multiplying planting material is required in order to get high-quality plants (Frey *et al*., 2006) [18]. Ahmad, *et al.*, (2018) [1] investigated that the Citrus trees are grown vegetatively and by seeds. Vegetative propagation produces true-to-type plants with consistent quality and regular bear. auxin-type growth regulators increase rooting proportion, speed up root initiation, and achieve rooting uniformity by increasing the rooting number per cutting. Stem cutting is an appropriate means of regeneration in *citrus* species. It is low-cost, rapid, and simple to implement, and it does not need the employment of any specific techniques, as do other vegetative alternatives. Other, more complex, or expensive ways may exist, although they are rarely selected. The parent plant or stock plant, as well as the propagating circumstances and cutting propagation processes and equipment, are all important considerations.

The site from whence this cutting was obtained might be used to refer to it. Cutting rooting is one of the ways for vegetative propagation, and cuttings have been shown to be crucial in the rooting of major fruit species and clonal rootstocks. Auxins have long been used to stimulate the growth of adventitious roots in stem cuttings. One of the most often utilized ways of propagation is exogenous plant growth regulators. However, several endogenous and external elements, like growth chemicals, have an impact on cutting rooting (Al-Zebari, and Al-Brrifkani, 2015) [3, 4]. Cuttings (softwood, semi-hardwood, hardwood, and single-leaf node cuttings) are frequently used among the different vegetative propagation methods since they are considered to be one of the most straightforward. Rhizogenesis or the formation of roots, is an essential requirement that occurs only when various contributing elements are taken into account. Plant growth regulators have been demonstrated to stimulate cutting roots (Audus, 1965) [7]. Auxins like IBA (Indole-3-butyric acid) have been found to be superior overall to a variety of synthetic plant growth agents employed so far for roots (Hartman and Kester, 1983) [20]. IBA is the most extensively used auxin for commercial rooting. The purpose of administering auxin to cuttings is to enhance the proportion of cuttings that root, speed up the root initial stage, increase the number of roots per cutting, and achieve root uniformity. As may be seen from the above description, auxin has a variety of effects on plant physiological processes. Nucleic acid-driven protein synthesis, enzyme activity, and membrane permeability are affected at the molecular level through metabolites. One or more of these impacts express themselves in all physiological outcomes (Srivastava, 2012) [41].

Rooting media significantly contributes to better development and roots. A good rooting medium should be loose, porous, and able to hold a lot of water. Rooting media is essential for retaining cuttings in place, supplying moisture for newly produced roots, encouraging respiration, and maintaining the proper temperature for root initiation. Pure sand generates long, coarse, unbranched, and brittle roots, whereas a mixture of soil, sand, and peat produces fine, healthy branches.

1. Impact of PGRs on rooting of C*itrus* species

Nowadays, the most often used auxins for rooting stem cuttings are IBA and NAA. Several studies have shown that auxin is required for the formation of adventitious roots on stems and that divisions of the first root cells are dependent on either administered or endogenous auxins (Tanabe, 1982) [43]. Auxins have been shown to stimulate adventitious root formation in stem cuttings (Blazich, 1988) [11]. Bhusal (2001) [10] discovered that rooting ability varied between species and cultivars (0-100 percent). Tengu and Kuno Satsuma mandarin had no roots, although rough lemon and lemon did. The highest rooting was discovered in June, followed by 90% in July and 30% in September. IAA or IBA + CCC plus ascorbic acid treatment enhances rooting synergistically. Secondary phloem, pericycle, cambium, and medullary rays can all give rise to roots (Sadhu, 1997) [36]. Phenoxy compounds in IBA or NAA improved rooting and root systems compared to phenoxy compounds alone (Davis and Haissig, 1990) [14]. Sandhu and Singh (1986) [37] discovered that the IBA 200 ppm treatment resulted in the greatest callus percentage on sweet lime stem cuttings, whereas the NAA 100 ppm treatment resulted in the most callus per cutting. With IBA 100 ppm, the proportion of rooted cuttings rose, whereas NAA 200 ppm boosted root quantity and length. There was no connection discovered between calluses and roots. Rooting and root systems were enhanced when a little amount of specific phenoxy compounds were introduced to either IBA or NAA (Davis and Haissig, 1990) [14]. Maximum root formation, root diameter, root length and shoot sprouting were all seen at 500 ppm IBA concentration (Bhatt and Tomar, 2011) [9]. Under 500 ppm IBA concentration, maximum rooting and shoot growth features were observed (Singh *et al*., 2015) [39]. Malakar *et al*., (2019) [25] investigated that the Bagasse media containing IBA @750 ppm as a growth regulator had the greatest root length, root volume, dry weight, and fresh weight among the other growing media tested. With IBA @ 1000 ppm, the largest number of root/cutting and the highest percentage of root/cutting, girth of the thickest root and length of the longest root, were reported by Ahmad *et al.* (2018) [1]. IBA 1000-2000mg/L with 2% ascorbic acid was shown to be the most effective therapy for enhancing sweet lime-rooted cuttings. An improved root system may be attributed to a superior response of IBA @ 2000 ppm to all shoot parameters. Higher survival rates of IBA @ 2000 ppm-treated cuttings could be attributed to a greater number of main roots, branches, and leaves, which resulted in increased water intake (Angami and Das, 2011; Kher, 1987) [5, 21]. Assam lemon single-leaf-bud cuttings 3000 ppm IBA treatment in sand medium showed the highest rooting percentage and survival. (Nath, 2000). According to Rathour, *et al*., (2018-19) [33] IBA at 5000 ppm boosted roots substantially, followed by NAA @ 5000 ppm for better establishment of Lemon air layering for propagation. Pummelo showed the highest root length (3.09cm), rooting (50%), and shooting (33.33%) under 6000 ppm IBA concentration (Thangji and Wunnachit, 1994) [44]. Indole-butyric acid (IBA) and Naphthalene-acetic acid (NAA) are two hormones that are increasingly being used to stimulate roots in citrus tree stem cuttings.

2. Impacts of growing media on rooting of *Citrus* species

Although research on this area in citrus spp. is limited, rooting medium plays an important role in root multiplication and subsequent growth in plants cultivated via stem cutting. In acid lime cuttings, hardwood cuttings, cocopeat and 500 ppm IBA were more effective in inducing greater shooting and rooting parameters (Malakar, *et al*., 2019) [25]. Al-Zebari and Al-Brifkany (2015) [3-4] observed that the peat moss and sand Media (1:2) were found to increase the percentage of stem cuttings rooting in the Corsian cultivar of (*Citrus medica* Linnaeus). The rooting medium Soil + Sand + Cocopeat enhanced the rooting percentage, but the rooting media soil + sand + FYM improved the survival percentage (Singh *et al.*, 2015) [39]. The field soil + sand + vermicompost (1:1:1) treatment demonstrated the maximum percentage of survival and roots (Kumar *et al*., 2015) [23]. To improve plant height and leaf quantity, Albouyeh (2007) [2] recommends using just peat or a mixture of peat, perlite, and cocopeat in a 2:2:1 ratio for citrus cuttings. In clay and loam rooting media, softwood sweet lime cuttings are rooted better than hardwood ones (Ghosh, 1990) [19]. For rooting of rough lemon cuttings, Ford (1957) [17] discovered that a 1:1 combination of peat and perlite worked best.

Table 2: *Citrus spp*. rooting and stem cutting with plant growth regulators and other chemicals formulation

Citrus species	Type of Stem cutting	Method of treatment	Optimum concentration mg/litre	Reference
Citrus medica	Semihardwood	A brief dip	IBA 500 and 1000 ppm	Al-Zebari *et al.* (2015) [4]
Citrus limetioides T. (Sweet lime)	Hardwood	Socking method	IBA@ 100 ppm	Bajwa *et al*., (1978) [8]
Citrus aurantifolia L. (Mexican)	Hardwood	A brief dip	IBA @ 6000 ppm	Elsheikh (2005) [16]
Citrus aurantium (Sour orange)	Softwood	A brief dip	IBA @ 1000 ppm	Malik and Harnard (1983) [26]
Citrus. limon B.	Semihardwood	A brief dip	PHBA @ 2000 ppm	Kumar *et al*., (1995) [22]
Citrus limon B (Baramasi)	Hardwood	A brief dip	IBA @ 2000 ppm	Kumar *et al*., (1995) [22]
Citrus latifolia	Semihardwood	A brief dip	IBA @ 2000 ppm	Prati *et al*., (1999) [32]
Pant Lemon-1	Hardwood	A brief dip	IBA @ 2000 ppm	Singh *et al*., (2013) [38]

Effects of PGRs and growing media on Air layering of citrus

The first callus and roots appeared in Marcotted pummelo species that had been treated with full, 3/4, and 1/2 strength commercial NAA. On root initiation, there was a substantial interaction between treatment combinations. The first roots were considerably started in Marcotted' Magallanes' treated with full, 3/4, and 1/2 concentrations of commercial NAA. More roots were generated by 'Siamese' and 'Red Chandler.' Significantly 'Siamese' (Libunao *et al.*, 2013) [24]. In seedless lemon air layers, IBA performed better than NAA at promoting roots. IBA at 5000 ppm considerably improved rooting properties, followed by naphthalene acetic acid @ 5000 ppm for better propagation of Lemon by air layers (Rathour *et al*., 2018-19) [33]. Many studies have discovered that PGRs such as GA3, auxins, and cytokinin play an important role in boosting plant survival and success after vegetative propagation procedures like grafting, budding and air layering (Anmol and Singh, 2018) [6]. Plant growth regulators are also said to help propagation by improving root formation in seedless lemon air layers when used in solutions containing 1 g-I" of either IBA or naphthalene acetic acid (Patil and Chakrawar,1979) [31].

Effects of plant bioregulators and growing media on the grafting of citrus species

PGRs were employed to improve bud grafting success, boost bud development, and minimize the interval between bud grafting and bud (Coggins and Hield, 1968; Nauer *et al.*, 1979) [13, 28]. Indole acetic acid and IBA enhanced the success of bud-grafting (Coggins and Hield, 1968) [13]. 6-benzyl adenine (BA) and 6-(benzylamine)-9-(2-tetra hydro pyranyl-) 9 H-purine promoted bud development (Nauer *et al.*, 1979) [28]. By shoot-tip grafting, virus-free citrus trees were created in vitro, growth regulators are also utilized in the medium (Navarro *et al.*, 1975) [29]. For citrus propagation and regeneration, growth-regulating compounds are commonly employed as a supplement to in vitro medium (E1-Otmani, *et al*., 2000) [15].

Conclusion

All of the research indicates that treatment of IBA is superior to other types of treatment for citrus cuttings, graftings, and air layering development and rooting media (soil + sand + cocopeat) helps in enhancing rooting percentage, soil + sand + FYM increases survival percentage and the vermicompost + sand + field soil (1:1:1) treatment demonstrated the maximum percentage of survival and roots. It can be concluded that the above-aforementioned plant growth regulator and rooting media could help in boosting citrus fruit- vegetative multiplication output which could as well be one of the quickest techniques for expanding the citrus plant as quickly as feasible with quality planting material.

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