Effect of IBA on rooting of Acid lime (Citrus aurantifolia Swingle) stem cuttings cv. PKM1

J Rajangam, C Sankar and M Kavino

Abstract
Effect of various concentrations of IBA and semi hard wood cuttings on the performance of rooting in acid lime (Citrus aurantifolia Swingle) cv. PKM1 under mist was studied in the Horticulture College and Research Institute, Tamil Nadu Agricultural University, Periyakulam. The treatments of five IBA concentrations (500, 1000, 1500, 2000 and 2500 ppm) along with a control were prepared and treated with semi hardwood cuttings as quick dip method. The study was laid out in a Completely Randomized Block Design (CRD) with six treatments and five replications. The results revealed that cuttings treated with IBA of 2000 ppm (T 5) recorded the highest values of all the traits viz., success per cent (80.72%), least 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), number of leaves (43.52), root length (26.15 cm), fresh root weight (4.50 g) and dry root weight (1.80 g) followed by T4 (1500 ppm of IBA). Control (T1) (without IBA treatment) observed the lowest values of the traits like success per cent of cuttings (26.14%), longest days for sprouting (13.50 days), number of sprouts per cuttings (1.50), plant height (25.48 cm), shoot length (13.00 cm), number of leaves (22.15), root length (15.80 cm), fresh root weight (1.95 g) and dry root weight (0.85 g). It is suggested that semi-hardwood cutting treated with 2000 ppm (T 5) concentration of IBA gives the overall best performance for mass multiplication of acid lime.

Keywords: Acid lime, PKM 1, semi hard wood cutting, IBA, propagation

Introduction
The acid lime [Citrus aurantifolia (Christm.) Swingle] is the most important fruit of India, belongs to the family Rutaceae. It is originating in tropical and subtropical Southeast Asia, these plants are among the oldest fruit crops to be domesticated. Important species of citrus group is acid lime (Citrus aurantifolia) which is also known as kagzi lime or maxican lime or pati lime. Citrus fruits are one of the major choicest fruit crops of the world having high consumer’s preference both as fresh and as well as its processed juice. Citrus possess greater genetic variability and adaptability compare to other fruit crops (Lalramhluna and Prasad, 2016) [9]. India ranks sixth in the production of citrus fruit in the world. It is the rich source of vitamin C. It also contains vitamin B, pectin, minerals and other nutritive substance which are required for human health. Lime juice is used for scurvy diseases. They also have laxative effect on the digestive system. In India, citrus fruits are primarily grown in Maharashtra, Andhra Pradesh, Punjab, Karnataka, Uttaranchal, Bihar, Orissa, Assam and Gujarat. Acid lime is grown in 230 thousand hectares with the production of 2273 thousand metric tonnes having productivity 10.8 tonnes/ha (Horticultural data base, 2018-19).

Generally, acid lime is regenerated through seeds, but acid lime seeds are recalcitrant in nature. The recalcitrant seeds impose serious storage problems due to their desiccation and chilling sensitivity (Chin and Roberts, 1980) [4]. These seeds undergo no maturation and drying during final phase of seed development and are thus shed in moist condition. Storage above critical level of time leads to loss of viability. Seed propagation methods also problems of non-uniformity of progeny and high chance of viral disease contamination by this method (Babu, 2001) [2]. Also, it is not advisable to use seeds for commercial planting because these seeds do not produce true fruits. Therefore, it is advisable to avoid seedlings for commercial plantation. For overcoming this problem, the vegetative propagation is vital to produce citrus plants having desirable characters as mother plant and they are propagated true-to-type from cuttings, budding, grafting, layering, etc., (Seran and Umadevi, 2011) [19]. However, vegetative multiplication through cutting is only cheap, practicable and widely used option for augmenting natural regeneration and for large scale cultivation programmes.

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Vegetative propagation is preferred because it ensures true to type plants, uniform quality and regular bearing. The purpose of treating cuttings with auxin type growth regulators is to increase the percentage of rooting, to hasten root initiation, to increase the number and quantity of roots produced per cuttings and to produce uniformity of rooting. Trees on these rootstocks are vigorous, precocious, less thorny, highly drought resistant and heavy bearer (Singh and Singh, 2016) [25].

Owing to high intensity of polyembryony (90-100%) and least chance of contamination of viral diseases (Babu, 2001) [2] in acid lime, the stem cutting appears as most suitable method for regeneration of the species. Beside plant multiplication through seed is based on the availability of the fruits. Further, it is reported that juvenile stage of plants can be decreased through stem cutting technique and the time of nursery development can also be reduced. It is inexpensive, mass rapid production and for perpetuating the characteristics of the parent pant move over simple and does not require the special techniques as required in other vegetative methods (Sebah et al., 1991) [18].

Bhatt and Tomar (2011) [3] 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) [18]. Sweet orange tree, maximum rooting and shoot growth characters were recorded under 5000 ppm concentration of IBA (Singh and Singh, 2016) [25]. For overcoming such types of problems at nursery stage, the present experiment on effect of IBA on rooting of Acid lime (Citrus aurantifolia) propagation through semi hardwood cutting was undertaken at Department of Fruit Science, Horticultural College and Research Institute, Periyakulam during 2019-2020.

Materials and Methods
The experiment site was conducted in month of July (2019) under mist chamber growing condition at Horticulture college and Research Institute, Periyakulam. The study was laid out in a Completely Randomized Block Design (CRD) with six treatments and five replications. Stem cuttings of acid lime cv. PKM1 was collected from five to six year old plants and prepared in 15-20 cm long cuttings whereas diameter 0.8-0.9 cm for semi hard wood cutting. Six level of IBA viz; 500, 1000, 1500, 2000, 2500 ppm and 0 (control) concentrations were prepared and treated with semi hard wood cutting as quick dip method. Sandy soil and farm yard manure (FYM) in ratio of 2:1 by v/v were mixed properly for preparing the rooting medium, cleaned for stones, grasses, then the mixture was filled in root trainers (Singh and Tomar, 2015) [23]. The basal ends of the cuttings were dipped in dilute solutions of IBA by quick dip method for 10 seconds before planting in the rooting medium (Oni, 1987 and Singh et al., 2011) [11, 22]. The treated cuttings were planted carefully in the root trainer. The experiment was replicated thrice with 30 cuttings in each treatment and a total of 240 cuttings were planted in mist chamber. The mist chamber has the arrangement for intermittent misting to 60 seconds at every 30 minutes interval between 8 am and 8 pm (Rawat et al., 2014) [15]. Observations on success per cent (%), number of sprouts, days taken for sprouting (days), number of leaves, plant height (cm), shoot length (cm), root length (cm), fresh root weight (g) and dry root weight (g) were recorded at six month after sowing and planting of cutting and analysed statistically as per the method was suggested by Panse and Sukhatme (1967)[12].

Results and Discussion
The data (Table 1) regarding plant survival percentage showed that there is a significant effect of different IBA concentrations on the plant survival percentage of semi hard wood cutting. The highest plant survival (80.72%) was found in cuttings treated with 2000 ppm of IBA, followed by cuttings treated with 1500 ppm of IBA, whereas the minimum number of plants survived in control (26.14%). The survival of the cuttings treated with high concentrations of IBA may be directly linked with the capacity of the growth regulator to stimulate the generation of adventitious roots system and increase in number and length of roots per cutting as influenced by the uptake of mineral nutrients and water from the soil, which helps in the survival of the cuttings (Reddy et al., 2008) [10]. The reason might be that application of IBA has been found to stimulate cambial activity thereby resulted the mobilization of reserve food material to the site of root initiation as stated by Gurumurthy et al. (1984) [7]. The enhanced hydrolytic activity in presence of applied IBA might be responsible for the increased percentage of rooted cuttings. High carbohydrate and low nitrogen have been reported to favour root formation reported by Singh and Tomar, 2015 [23]. The application of IBA might had an indirect influence by enhancing the speed of transformation of rooting primordia and movement of sugars to the base of cuttings and consequently formation of young and active roots (Rani et al., 2018) [14].

In the case of days taken for sprouting, T5 (IBA 2000 ppm) recorded the minimum days taken for sprouting (6.85 days) followed by T4 (IBA 1500 ppm) of 9.00 days. The longest days taken for sprouting (13.50 days) were observed in control (T1). It might be due to wood maturity of cutting which probably reserves high starch and sugar (Singh, 2013) [21]. This might be due to better utilization of stored carbohydrates, nitrogen in the semi hardwood cuttings treated with IBA application. Enhanced IBA concentration in the cell and increased the cell division which resulted on quick callus formation in the cuttings (Patil et al., 2000) [13].

Number of sprouts per cutting recorded the highest (4.30) in T5 (IBA 2000 ppm) treatment followed by T4 (IBA 1500 ppm) of 4.10 and the lowest number of sprouts was found in control (T1) of 1.50. This might be influenced by the number of branches per shoot. It is a fact that IBA directly affects the number of root and root growth and indirectly affects shoot length, which may result in a high number of branches per shoot (Stefancic et al., 2005) [27]. The more number of sprout formation with the growth regulators might be due to the vigorous root system which increased nutrient uptake under the combined influence of IBA application. It affected the cell division in the vascular cambium, cell expansion and control of differentiation into different types of cambial resulting in increase in number of sprouts (Devi et al., 2016) [6].

Plant height recorded the highest (47.76 cm) in T5 (IBA of 2000 ppm) followed by T4 (44.37 cm). The lowest plant height was noticed in control (T1) of 25.48 cm. Singh and Singh (2016) [25] reported that Indole Butyric Acid (IBA) is the active inhibiting axillary bud break on developing shoots.
and it stimulates the shoot initiation. IBA treatment enhanced rooting, plant growth and produced taller and healthy plants (Umrao, 1999) [28].

The data regarding shoot length showed that the highest shoot length (23.65 cm) was recorded in cutting treated with 2000 ppm of IBA while minimum shoot length (13.00 cm) was recorded in control (Table 1). This significant effect of IBA concentration on shoot length is in accordance with that of Naghmouchi et al. (2008) [16]. As a growth promoter IBA promotes cell division, which results in early rooting leading towards efficient absorption of mineral nutrient and hence maximizes shoot length. This might be due to increased concentrations and activity of IBA which be caused hydrolysis and translocation of carbohydrates and nitrogenous substances in the cellular level at the base of cuttings which resulted in accelerated cell elongation and cell division under favorable environmental condition (Singh and Singh, 2011) [24].

The number of leaves per plant was a significant effect of IBA concentration on cutting. The maximum number of leaves per plant (43.52) was recorded in IBA concentration was used at the rate of 2000 ppm, whereas the minimum numbers of leaves per plant (22.15) were recorded in control (Table 1). Maximum number of leaves was produced in cuttings treated with IBA 2000 ppm which might be due to activation of shoot growth leading to an increased number of nodes that leads to development of more number of leaves. The increase in number of leaves per cutting might be due to the reason that the plant might diverted maximum assimilate quantities to the leaf buds, since the leaves are one of the production sites of natural auxins in them besides being very important for vital processes like photosynthesis and respiration (Wahab et al., 2001) [29].

A significant effect of IBA concentration on root length, the highest root length (26.15 cm) was recorded in cutting where IBA concentration was used at the rate of 2000 ppm, followed by 23.66 cm where IBA concentration was used at the rate of 1500 ppm, whereas the minimum root length (12.80 cm) was recorded in control T1 (Table 2). The increase in root length may be due to the maximum number of branches per shoot whose tips produces more auxin which results in root elongation, and the effect of metabolites translocation and carbohydrates metabolism. Bhatt and Tomar (2010) [3] also found increase in root length in Citrus aurantifolia with increasing IBA concentration. An increase in number of roots due to IBA application may necessitate the increased activity of photosynthesis, transpiration and respiration in leaves and thus may result in the increase in number of leaves per cutting (Singh et al., 2015) [23]. Increase in leaf number might be due to the vigorous rooting induced by the growth regulator enabling the cuttings to absorb more nutrients and thereby producing more leaves as reported by Stancato et al. (2003) [26].

Regarding fresh root weight, IBA 2000 ppm (T5) recorded the maximum fresh weight of roots (4.50 g) due to production of more number of roots as seen with the result on number of roots in the present study. The lowest fresh root weight (1.95 g) was observed in control (T1). Maximum root weight was attributed to the fact that auxins naturally occurring or exogenously applied are for initiation and growth of roots. Low auxin activity and its slow degradation by auxin destroying enzyme lead to the growth and vigour of roots. This might also be due to the reserved food in the cuttings (Singh et al. 2013) [22]. The fresh weight of the root is directly proportional to number of roots in each cutting. The results are in line with Wahab et al. (2001) [29] in guava and Riaz et al. (2007) [17] in kiwi, Arumugam et al. (1996) [1] in pomegranate and Singh and Tomar (2015) [23].

Significant variations were observed in dry weight of roots at two month old semi hardwood cutting sapling by the influence of IBA treatments were presented in Table 2. The result recorded a higher dry weight of roots (1.80 g) observed in IBA @ 2000 ppm whereas the lowest dry root weight was observed in control (T1) of 0.82 g. which might be due to auxins activated a higher number of primary and secondary roots through cell division and elongation of roots accounting for a higher dry weight of root (Debnath and Maui, 1990) [5]. The higher dry weight of the roots might be attributed to higher root length which accumulates more stored carbohydrates and more number of roots increased their volume per cutting in semi hardwood cuttings (Hartman et al. 1997) [4]. Similar results were also reported by Singh and Tomar (2015) [23] and Singh et al. (2015) [23].

**Conclusion and Recommendations**

Based upon the findings of this study, it is recommended that the semi hard wood cuttings of acid lime should be treated with IBA at 2000 ppm maximum plant survival percentage, shoot length, shoot diameter, number of leaves per shoot, number of branches per shoot, number of roots per plant, root length and root diameter are achieved. So increasing the production of citrus fruit cutting is one of the easy processes for developing the citrus plant as soon as possible.

**Fig 1:** Appearance of Acid lime seedling (one year old seedling) and semi hard wood cutting (two months old) of Acid lime cv. PKM 1
Table 1: Influence of IBA on success per cent and shoot characters of acid lime cuttings

<table>
<thead>
<tr>
<th>Treatment Details</th>
<th>Success per cent</th>
<th>Days taken for sprouting (days)</th>
<th>Number of sprouts per cutting</th>
<th>Plant height (cm)</th>
<th>Shoot length (cm)</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- Control</td>
<td>26.14</td>
<td>13.50</td>
<td>1.50</td>
<td>25.48</td>
<td>13.00</td>
<td>22.15</td>
</tr>
<tr>
<td>T2 - 500 ppm</td>
<td>41.86</td>
<td>11.80</td>
<td>3.50</td>
<td>30.60</td>
<td>16.50</td>
<td>28.49</td>
</tr>
<tr>
<td>T3 - 1000 ppm</td>
<td>64.00</td>
<td>11.24</td>
<td>3.80</td>
<td>37.21</td>
<td>17.13</td>
<td>28.71</td>
</tr>
<tr>
<td>T4 - 1500 ppm</td>
<td>73.00</td>
<td>9.00</td>
<td>4.10</td>
<td>44.37</td>
<td>19.08</td>
<td>35.80</td>
</tr>
<tr>
<td>T5 - 2000 ppm</td>
<td>80.72</td>
<td>6.85</td>
<td>4.30</td>
<td>47.76</td>
<td>23.65</td>
<td>43.52</td>
</tr>
<tr>
<td>T6 - 2500 ppm</td>
<td>68.94</td>
<td>7.50</td>
<td>4.00</td>
<td>40.80</td>
<td>15.40</td>
<td>30.91</td>
</tr>
<tr>
<td>SEd</td>
<td>1.95</td>
<td>0.32</td>
<td>0.11</td>
<td>1.18</td>
<td>0.53</td>
<td>0.95</td>
</tr>
<tr>
<td>CD (P=0.05%)</td>
<td>4.24</td>
<td>0.70</td>
<td>0.25</td>
<td>2.57</td>
<td>1.16</td>
<td>2.06</td>
</tr>
</tbody>
</table>

Table 2: Influence of IBA on root characters of acid lime cuttings

<table>
<thead>
<tr>
<th>Treatment Details</th>
<th>Root length (cm)</th>
<th>Fresh root weight (g)</th>
<th>Dry root weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- Control</td>
<td>12.80</td>
<td>1.95</td>
<td>0.82</td>
</tr>
<tr>
<td>T2 - 500 ppm</td>
<td>18.15</td>
<td>3.50</td>
<td>1.27</td>
</tr>
<tr>
<td>T3 - 1000 ppm</td>
<td>20.34</td>
<td>4.10</td>
<td>1.35</td>
</tr>
<tr>
<td>T4 - 1500 ppm</td>
<td>23.66</td>
<td>4.35</td>
<td>1.50</td>
</tr>
<tr>
<td>T5 - 2000 ppm</td>
<td>26.15</td>
<td>4.50</td>
<td>1.80</td>
</tr>
<tr>
<td>T6 - 2500 ppm</td>
<td>18.97</td>
<td>4.00</td>
<td>1.30</td>
</tr>
<tr>
<td>SEd</td>
<td>0.63</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>CD (P=0.05%)</td>
<td>1.38</td>
<td>0.27</td>
<td>0.09</td>
</tr>
</tbody>
</table>

References

21. Singh KK, Rawat JM, Tomar YK, Kumar P. Effect of IBA concentration of inducing rooting in stem in stem...
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