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Impact of Auxins on Rooting and Establishment of Seedless Lemon (*Citrus limon* L. Burm.) Air-layers for Successful Propagation

Shubham Singh Rathour[1*], K. S. Tomar[1], Poonam[1], Shubham Bhadoriya[2] and R. S. Katoriya[1]

[1]Department of Horticulture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, 474002, India.
[2]Department of Agricultural Extension, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, 474002, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SSR investigated, conceptualized, data curation, performed analysis, original drafting, visualization. Author KST conceptualized, methodology, supervised and validation. Authors Poonam and SB managed resources, reviews and literature. Author RSK visualize and editing. All authors collaborated for this experiment and finally approved the manuscript.

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ABSTRACT

Study Design: Randomized Block Design.
Place and Duration of Study: Department of Horticulture, College of Agriculture, RVSKVV, Gwalior, MP between 2018 - 2019.
Methodology: In 2018-19 the study conducted amidst rainy season and Gird agro-climatic context of Madhya Pradesh to evaluate the effect of phytohormones on rooting and establishment of seedless lemon air layers. Experiment consisting nine treatments replicated three times under Randomized Block Design. Treatments comprising of two plant hormones namely IBA and NAA each with four concentration levels i.e. 1250, 2500, 3750 and 5000 ppm including control. Plants age were seven years.

**Corresponding author: E-mail: shubhamrathourag@gmail.com;*

Results: The results revealed maximal rooting percentage (92.00), number of primary roots (22.72) and secondary roots (49.97), length of primary roots (5.05 cm) and secondary roots (2.25 cm), diameter of primary roots (1.68 mm) and secondary roots (0.84 mm) and dry weight of roots (2.32 g) and planted air-layers (89.21 %) were recorded with application of IBA @ 5000 ppm.
Conclusion: Indole-3-butyric acid performed better than naphthalene acetic acid for promoting rooting and establishment in seedless lemon air-layers. Application of IBA @ 5000 ppm significantly increased rooting characters followed by NAA @5000 ppm for better establishment of Lemon air layers for its propagation.

Keywords: Air-layering; auxins; rooting; propagation; seedless lemon.

ABBREVIATIONS

IBA : Indole-3-butyric acid
NAA : Naphthalene acetic acid
FYM : Farmyard Manure
PGR : Plant growth regulator
DAT : Days after Transplanting

1. INTRODUCTION

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 [1]. In India, Lemon (*Citrus limon* L. Burm.) is cultivated in all parts of the country mostly in the four southern states of Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. On the whole, India accounts for 27.57% of world's Lemon production, the four southern states alone occupy more than 70% of the total area among the Citrus species found in central India. Lemon comprises adequate amount of minerals, citric acid, ascorbic acid, antioxidants and other beneficial compounds [2].

Citrus limon cv. seedless lemon is the principal lemon cultivar of the North to Central region of India. Lemon fruit is medium large, long elliptic to oval, base rounded, nipple broad but low, rind medium thick, firm, surface very smooth, shinning, yellow colour, segments 9-12, axis hollow, pulp vesicle crystal white, juice abundant, acidic, fruit weight (g) 81.33, peel thickness (mm) 1.73, number of segments 10.67, seeds/ fruit 0 or 1, TSS 7.33, acidity (%) 7.90, flavor good, usually seedless, occasionally contains a few seeds [1]. In India the "Seedless" lemon is profuse and among the prime lemon cultivar.

Being a seedless cultivar, commercial sexual propagation in lemon is a challenge, therefore mainly two methods of vegetative propagation i.e. air layering and stem cutting are common. However, the success percentage of stem cuttings is low in North-central part of India for lemon propagation on a commercial scale. Cutting is more favourably done in spring season as compared to the rainy season because the incidence of the pathogenic attacks on cuttings is more in humid rainy days which hampers its overall success. But spring season is dry and less humid in North-central India, so adopting cutting for mass lemon propagation is quite difficult. Practice of air layering in lemon is common in North-central India and fairly more feasible when done in rainy season. Humid conditions of rainy season promote plant vegetative growth and carbohydrate accumulation which favours better development of air-layers. Beside this air layering escapes the pathogenic incidence in a better way during rainy days because it is attached to the plant wrapped with polythene till the development of roots. Therefore, air layering in lemon is more beneficial for commercial nursery growers. Among several factors, the use of phytohormones contributes indeed towards advantageous multiplication of citrus plants.

The success of air layering in lemon can be achieved by exogenous application of plant hormones like auxin which enhance rooting and root characters [3]. Several researchers have studied the use of these growth substances for vegetative propagation among which auxins like IBA and NAA have been found to be the most effective in rooting with varied success [4]. Thus, this study evaluates the influence of auxins on rooting and establishment of seedless lemon air layers.

2. MATERIALS AND METHODS

2.1 Study Site and Location

Experiment was performed at orchard of Horticulture Department, RVS Agricultural University, Gwalior amidst monsoons of 2018-19 under Gird agro-climatic and soil context of MP at 26° 13' 44.7492" N latitude and 78° 14' 50.1072" E longitude, elevation 211.50 m above the mean sea level. Monsoons period from June

to September with mild showers in winters and winters are chilly where temperature can go down below to 2°-1°C during December-January and frost may occur in start of February too. Summers are hot and dry.

2.2 Experimental Details

The experiment constituted nine treatments, three replications under Randomized Block Design comprising two plant hormones *viz,* indole-3-butyric acid and naphthalene acetic acid each with four concentration levels i.e. 1250, 2500, 3750 and 5000ppm including control. Air layering was performed during rainy season. In the orchard seven years old 27 plants of lemon cv. seedless lemon possessing consistent vigor and size were picked, as nine plants comprise each replication and 20 developed robust branches of pencil's thickness were picked on each plant for layering. PGR mixture in powder form was applied. Same rooting media consisting (2:1:1) soil + FYM + leaf mould were utilized for all treatments. Only talcum powder was used for treating air layers under control and utilizing same wrapping material and medium. Air-layers detachment was done after 50 days of operation and dipped in 0.1% solution of Carbendazim then transplanted in poly-bags hold in media composed of soil + FYM + leaf mould (2:1:1). Randomly five air-layers per treatment were selected for observations and rooting characters recorded just after detaching of air-layers.

2.2.1 Intercultural operations

Weeding after 15 days and light irrigation were applied at 15 days interval from planting of air-layers. For protection from insects and pests, spray of dimethoate solution @ 0.2% was done twice at twenty days intervals.

2.3 Parameters Measured

2.3.1 Success in rooting of air-layers

It was observed while detachment with the help of following formula.

Success of rooting (%) = (Number of rooted air layers / Total number of air layers) X 100

2.3.2 Average number of adventitious primary and secondary roots per air layer

Primary roots were removed by means of sharp blade from air-layers and secondary roots were separated from primary roots and counted.

2.3.3 Average length of adventitious primary and secondary roots per air layer (cm)

Length of primary and secondary roots was scaled by placing on graph paper and the data was recorded.

2.3.4 Average diameter of adventitious primary and secondary roots per air layer (mm)

Ten roots per air-layer were taken randomly and their diameters were recorded by means of screw gauge and mean computed.

2.3.5 Average dry weight of adventitious roots per air layer (g)

Excised roots were put in butter paper packet and kept in an oven maintained at 80°C±1°C for twenty-four hours. After drying, the roots were kept in desiccators for cooling. Proper dried roots' weight was noted by means of electronic weighing machine for statistical analysis.

2.3.6 Established air layers (%)

Observations were taken at 90 days after planting. It was computed as follows-

Established air-layers (%) = (Total number of established layered plants / Total number of layered plants planted) X100

2.4 Statistical Analysis

The observational data were noted and analyzed as per standard Randomized Block Design (RBD) technique suggested by Panse and Sukhatme [5]. "F" test for significant treatment differences and critical differences were worked out at 5% significance level.

3. RESULTS

3.1 Success in Rooting Percentage of Air-layers

The highest rooting percentage (92.00%) was noted with application of IBA @ 5000ppm followed by application of IBA @ 3750ppm (87.33%) and NAA @ 5000ppm (87.00%) (Table 1). However, application of IBA @ 3750ppm and NAA @ 5000ppm, NAA @ 3750ppm and IBA @ 2500ppm was found at par with each other. Air layers from control treatment had the lowest rooting percentage (62.33%).

3.2 Number of Primary Roots

The concentration levels of both phytohormones notably increased primary roots number per air-layer and maximum count was achieved at the highest hormone concentration (5000ppm) (Table 1). IBA treated (5000ppm) air layers had the highest number (22.72) primary roots followed by NAA (5000ppm) and IBA (3750ppm) treated air layers which had 20.36 and 19.50 primary roots, respectively, which was at par altogether. Control treated air-layers had the lowest (10.67) number of primary roots.

3.3 Length of Primary Roots (cm)

IBA and NAA with incremental concentration levels notably increased the primary roots length than control. IBA @ 5000ppm showed longest length (5.05) followed by NAA @ 5000ppm (4.84 cm) and IBA @ 3750ppm (4.42 cm). All IBA concentrations were significantly superior compared with concentrations of NAA except concentration of 1250ppm. Lowest root length was observed from control (2.28 cm) treated air-layers. Results also indicated that IBA was found superior over NAA (Table 1).

3.4 Diameter of Primary Roots (mm)

The similar concentrations of IBA and NAA were not significantly different from each other. Longest primary roots' diameter (1.68 mm) was noted with application of IBA @ 5000ppm followed by NAA @ 5000ppm (1.60 mm), IBA @ 3750ppm (1.58 mm) and NAA @ 3750ppm (1.50 mm) and lowest (1.11 mm) with control (Table 1).

3.5 Number of Secondary Roots

The incremental concentration of both auxins increased the number of secondary roots significantly. Application of IBA @ 5000ppm recorded highest number (49.97) of secondary roots followed by NAA @ 5000ppm (45.82), IBA @ 3750ppm (44.30) and NAA @ 3750ppm (41.30). However, no significant difference was found between NAA @ 3750ppm and IBA @ 2500ppm. Control treated air-layers had the lowest number (24.17) of secondary roots. Results also indicate IBA performance was better than NAA (Table 2).

3.6 Length of Secondary Roots (cm)

Layers treated with 5000ppm IBA had produced the longest (2.25cm) secondary roots followed by application of IBA @ 3750ppm (2.10cm) and NAA @ 5000ppm (2.09cm) (Table 2). However, no significant differences were recorded with application of NAA @ 3750ppm (1.96cm), IBA @ 2500ppm (1.95cm) and NAA @ 2500ppm (1.86cm) and lowest length (1.55cm) of secondary roots was noted with the control.

3.7 Diameter of Secondary Roots (mm)

All concentrations of IBA and NAA notably improved the diameter against control. Significantly longest diameter (0.84mm) was noted with IBA @ 5000ppm, secondary roots' diameter increased with rise in the concentration of both hormones (Table 2). However, there was no significant difference found between application of NAA @ 5000ppm (0.73mm), IBA @ 3750ppm (0.71mm) and NAA 3750ppm (0.68mm) as well as between NAA @ 3750ppm (0.68mm), IBA @ 2500ppm (0.66mm) and NAA @ 2500ppm (0.64mm).

3.8 Dry Weight of Roots (g)

IBA and NAA notably boost the dry weight of roots per air-layer with each incremental concentration up to highest (5000ppm) concentration. Application of IBA @ 5000ppm recorded maximum dry weight of roots (2.32g) followed by application of NAA @ 5000ppm (1.86g), which was at par with the application of IBA @ 3750ppm (1.81g). A similar concentration of IBA and NAA up to 2500ppm was not significantly different from each other. Minimum dry weight was noted in control (0.59g). Results also indicated IBA treated air-layers performed better compared with air-layers treated with similar concentrations of NAA (Table 2).

3.9 Established Air-layers (%)

Results indicated better performance of IBA than NAA (Table 2). The established air-layers percent (89.21) was reported significantly highest with IBA @ 5000ppm followed by NAA @ 5000ppm (83.00%), which was not significantly different when compared with IBA @ 3750ppm (82.46%), NAA @ 3750ppm (80.22%) and IBA 2500ppm (78.92%). A similar concentration of IBA and NAA up to 3750ppm was found at par with each other and lowest percent (58.67) was noted with control.

Table 1. Effect of auxins on success rooting percent, number, length (cm) and diameter (mm) of primary roots per air layer

Auxin concentration	Success rooting percentage	Number of primary roots per air layer	Length of primary roots per air layer (cm)	Diameter of primary roots per air layer (mm)
Control	62.33	10.67	2.28	1.11
IBA 1250 ppm	73.67	14.93	3.11	1.29
IBA 2500 ppm	80.33	15.66	3.98	1.49
IBA 3750 ppm	87.33	19.50	4.42	1.58
IBA 5000 ppm	92.00	22.72	5.05	1.68
NAA 1250 ppm	72.67	12.74	3.00	1.28
NAA 2500 ppm	79.00	14.99	3.78	1.41
NAA 3750 ppm	84.33	17.88	4.11	1.50
NAA 5000 ppm	87.00	20.36	4.84	1.60
S.E.(m) ±	**1.46**	**0.54**	**0.05**	**0.03**
C.D.* (at 5%)	**4.41**	**1.64**	**0.16**	**0.09**

**Critical Difference*

Table 2. Effect of auxins on number, length (cm), diameter of secondary roots (mm) per air layer, dry weight of roots (g) per air layer and established air-layers (%)

Auxin concentration	Number of secondary roots per air layer	Length of secondary roots per air layer (cm)	Diameter of secondary roots per air layer (mm)	Dry weight of roots per air layer (g)	Established air-layers at 90 DAT (%)
Control	24.17	1.55	0.39	0.59	58.67
IBA 1250 ppm	32.77	1.76	0.56	0.91	70.33
IBA 2500 ppm	39.67	1.95	0.66	1.18	78.92
IBA 3750 ppm	44.30	2.10	0.71	1.81	82.46
IBA 5000 ppm	49.97	2.25	0.84	2.32	89.21
NAA 1250 ppm	29.20	1.69	0.52	0.88	68.92
NAA 2500 ppm	36.70	1.86	0.64	1.18	74.33
NAA 3750 ppm	41.30	1.96	0.68	1.63	80.22
NAA 5000 ppm	45.82	2.09	0.73	1.86	83.00
S.E.(m) ±	**0.90**	**0.04**	**0.02**	**0.02**	**1.75**
C.D. (at 5%)	**2.72**	**0.12**	**0.07**	**0.06**	**5.29**

**Critical Difference*

4. DISCUSSION

Both Indole-3-butyric acid and Naphthalene acetic acid employed at 3750 and 5000ppm significantly increased the rooting traits as well as establishment of air-layers of lemon over control. More developed roots and establishment increased in treated air-layers with the incremental concentration levels of both plant hormones which was due to the increased accumulation of auxin at the base [6]. It seems that endogenous auxin content of the branches was insufficient to differentiate the rooting primordia and then the exogenous application of growth regulators enhanced the development of callus cells and thereafter root initiation which ultimately helped in better root development as well as growth, theory supported by others [3,7,8].

An analytical scrutiny of data on rooting characters showed IBA @ 5000ppm recorded maximum rooting (92.00), count of primary (22.72) and secondary roots (49.97), length of primary (5.05 cm) and secondary roots (2.25 cm), diameter of primary (1.68 mm) and secondary roots (0.84 mm), roots' dry weight (2.32g) and percent established air-layers (89.21) followed by NAA @ 5000ppm for all parameters except diameter of primary roots, which were at par for both the growth regulators. Similar results were reported in previous research studies in lime [9,10,11,12]; litchi [13,14] and Karonda [15]. However, application of NAA @ 5000ppm was found at par with the application of IBA @ 3750ppm for all the parameters except the length of primary roots. Control showed minimal response for all the roots characters.

During the process of rooting, callusing occurs first and root primordial forms after wards. For prompt callusing, proper concentration of carbohydrates in the branches used for air layering is essential. Layering is usually done during rainy season because synthesized food material including carbohydrates gets accumulated in the plants and it encourage quick healing and better callusing [16]. Application of exogenous auxins enhanced rooting especially at higher concentrations which performed better than lower ones, this might be at higher concentration auxins reaching the cambial activity may be adequate for initiating root primordia, so the highest performance was seen at higher concentrations (3750 and 5000ppm) of IBA and NAA [17]. The highest roots' count, length might be due to the hormonal effect leading to accumulation of internal substances and their downward movement, cell division as stimulated by higher concentration of IBA, theory supported by others in guava [18] and karonda [15]. The highest roots' weight may be attributed to the fact that external application of auxin generally stimulates the movement of natural auxin in downward direction from the leaves and shoot tips, which accumulate at the incision made on the shoot resulting in the formation of roots with higher root dry weight. Better rooting leads to better plant establishment considerably [14,19].

Air layers responded notably to both phytohormones for all root parameters. IBA positively ifluenced the rooting parameters of air layers better than NAA. Similar phenomenon is observed by other authors [14,17,20]. Other researchers [16,21] have also reported that higher concentrations such as 5000ppm of IBA and NAA gave better results on rooting characters. In our study, among all concentrations of IBA and NAA, 5000ppm performed best for rooting and establishment of lemon air layers. However, the effect of auxins on lemon air layers with concentrations higher than 5000ppm is yet to be explored and is subjected to the future studies. The effect of different growing media along with varied auxin concentrations may be explored in lemon air layers which provides scope for the future studies.

5. CONCLUSION

Indole-3-butyric acid treated layers performed better for most of the rooting attributes. Rooting and establishment enhanced with the rise in concentration levels of both hormones. Application of IBA @ 5000ppm significantly increased rooting followed by NAA @ 5000ppm for better establishment of Lemon air layers for its propagation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

ANALYSIS OF VARIANCE FOR DIFFERENT CHARACTERS IN LEMON AIR LAYERS (MEAN SUM OF SQUARES)

Appendix – a

Source of variance	D.F.	Success rooting (%)	Dry weight of roots (g)	Established air layers (%)
Replications	2	1.925	0.001	84.965
Auxin concentration	8	252.18**	0.950**	253.790**
Error	16	6.380	0.001	9.185

***Significant at 5%*

Appendix – b

Source of variance	D.F.	Number of primary roots	Length of primary roots (cm)	Diameter of primary roots (mm)
Replications	2	0.915	0.001	0.001
Auxin concentration	8	44.240**	2.590**	0.100**
Error	16	0.880	0.010	0.001

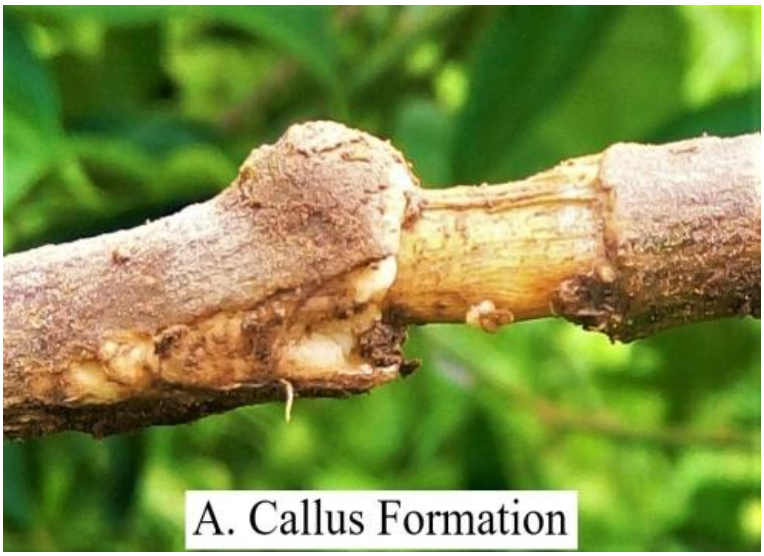
***Significant at 5%*

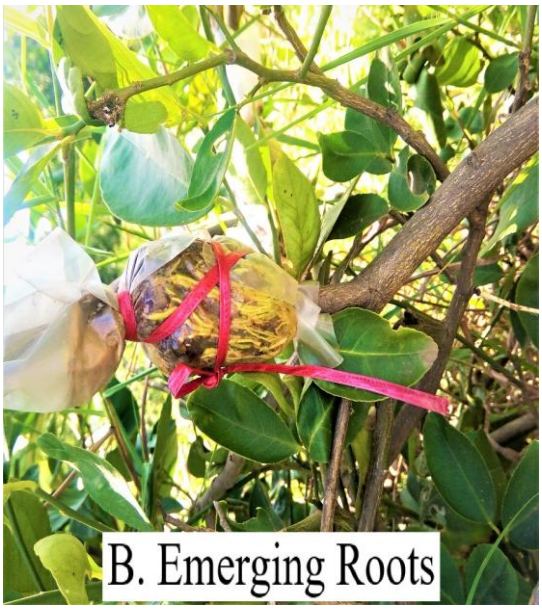
Appendix – c

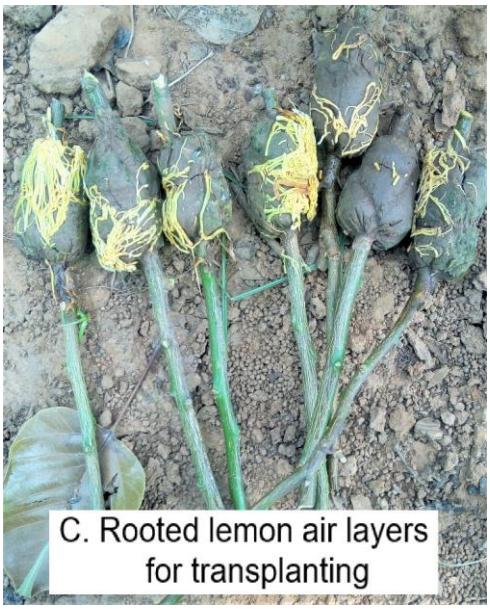
Source of variance	D.F.	Number of secondary roots	Length of secondary roots (cm)	Diameter of secondary roots(mm)
Replications	2	3.665	0.005	0.000
Auxin concentration	8	208.180**	0.140**	0.050**
Error	16	2.430	0.001	0.001

***Significant at 5%*

Pictures:







D. Lemon air layers root samples after detachment

E. Established plants

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