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Effect of indole-3-butyric acid (IBA) and different rooting media on air- layering of acid lime (*Citrus aurantifolia* Swingle)

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Abstract

Acid lime belongs to the Rutaceae family, extensively grown in tropical and subtropical climates in India for its highly nutritive and medicinal properties. Air-layering is one of the most used propagation methods in acid lime to overcome great genetic variation and poor plant performance, but rooting success in air-layering depends significantly on external factors like rooting media and growth regulator application. Thus, the present investigation was conducted to identify the best combination of rooting media and IBA (Indole-3-butyric acid) concentration to enhance rooting efficiency in acid lime. The experiment was laid out in a factorial randomized block design (FRBD) with 15 treatment combinations and three replications of each treatment. Treatments included five different rooting media (soil + FYM, soil + vermicompost, peat moss, coir peat, and sawdust) and three indole-3-butyric acid (IBA) concentrations (0 ppm, 2500 ppm, and 5000 ppm). The observations were taken at 50 days and 100 days after layering. The interaction effects between rooting media and IBA concentration were statistically significant for most of the parameters. Among all the rooting media, peat moss produced the best results, including the highest number of primary roots (25.56) and secondary roots (7.66), the longest length of primary root branches (6.11 cm), the largest root diameter (1.51 mm), and the maximum fresh weight (3.15 gm) and dry weight (1.58 gm) after 100 days of air-layering. Similarly, from all the different concentrations of IBA, the 5000ppm concentration gave higher results, i.e., the maximum number of primary roots (24.67) and secondary roots (6.50), the longest length of primary root branches (5.96 cm) and the maximum fresh weight (2.98 gm) and dry weight (1.41 gm) after 100 days of air-layering. Hence, from the study it is concluded that peat moss combined with 5000 ppm IBA is the most effective treatment for air layering in acid lime.

Keywords: Acid lime, propagation, air-layering, IBA (Indole-3-butyric acid), peat moss

Introduction

Acid lime (*Citrus aurantifolia* Swingle) is also known as 'Kagzi lime', and it is one of the world's most economically significant and widely cultivated citrus fruits, belonging to the Rutaceae family (Swingle and Reece, 1967) [19]. It originated in India. The fruits are extensively cultivated in tropical and subtropical climates throughout the world. It is well-known for its high vitamin C content, antioxidant properties, refreshing flavour and presence of secondary metabolites like flavonoids, limonoids, alkaloids, phenolics and coumarins, making them an important part of people's daily diets around the world (Bhowal *et al.*, 2022) [3]. Acid lime is primarily propagated through vegetative methods to ensure genetic uniformity and early bearing (Hartmann *et al.*, 2011) [5]. Although seed propagation is possible, it results in variability and delayed fruiting. The most common propagation techniques are budding (T-budding) on rootstocks like Rangpur lime (*Citrus limonia*) or rough lemon (*Citrus jambhiri*) and air layering in some areas. Air layering is the most reliable and efficient vegetative propagation technique widely employed in acid lime to ensure genetic uniformity, rapid establishment, and early fruiting.

Among different rooting hormones, generally auxins like IBA and NAA are used for air-layering in several fruit species. Indole-3-butyric acid is the most commonly used, with concentrations ranging from 500 ppm to 3000 ppm (Khan *et al.*, 2006) [9]. Studies also show that 2000 ppm IBA generally results in the highest rooting percentage and the best root development

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in acid lime (Hossain, 2006)^[6]. The choice of rooting media for air-layering also plays a significantly important role in rooting success. Most commonly used rooting media include moist sphagnum moss, coco peat, vermiculite, and sand. The cocopeat mixed with vermicompost or sphagnum moss alone provides optimal moisture and aeration, promoting faster root initiation (Hartmann *et al.*, 2011)^[5].

Numerous studies have explored different IBA concentrations and rooting media for various citrus species. Most researchers found IBA effective, particularly when combined with sphagnum moss, vermicompost, sand, or FYM (farmyard manure). However, results varied depending on species, varieties, physiological factors, and environmental conditions (Khan *et al.*, 2006; Hossain, 2006)^[9, 6]. However, the best combination of IBA along with a rooting medium for better survival of air layers in acid lime is yet to be unveiled. Therefore, the present investigation was conducted to identify the best combination of rooting media and IBA (Indole- 3-butyric acid) concentration to enhance rooting efficiency in acid lime.

Materials and Methods

The present investigation was conducted during 2024 (from August to November) in the Horticultural Farm and Laboratory of Department of Horticulture and Post-harvest Technology, Palli-Siksha Bhavana, Visva-Bharati, Sriniketan, West Bengal. The experimental site is situated at 23.67° N latitude and 87.68° E longitude, with an average elevation of 59 meters above mean sea level. The experimental site falls under a sub-humid semi-arid zone, and the area experiences three distinct seasons: summer, monsoon, and winter. The average annual rainfall ranges between 1200 mm and 1400 mm, with the majority received during the southwest monsoon period. The maximum temperature during summer may rise to around 40°C, while the minimum temperature during winter can fall to 10°C or lower. The experiment was conducted in a factorial randomized block design for which five different rooting media, i.e., soil + FYM (M1), soil + vermicompost (M2), coco peat (M3), peat moss (M4), and sawdust (M5), along with 3 different dosages of IBA concentration (0 ppm, 2500 ppm, and 5000 ppm), were taken. For the experiment, fifteen well-matured, healthy acid lime plants (3-4 years old) of uniform vigour and size were selected. On each plant, six air layers were performed, with three replications designated for observation on the 50th and 100th days, respectively. Additionally, 2-3 extra layers were prepared on each plant to avoid any potential damage or loss during the course of the experiment.

Approximately one-year-old shoots of pencil thickness were chosen for layering. All thorns and leaves within the immediate vicinity of the bud were removed with clean secateurs. A 2-3 cm bark ring was then carefully girdled just below the bud on each selected branch, taking care not to injure the underlying xylem. The growth regulator, i.e., IBA paste at different concentrations, was applied uniformly to the upper margin of the girdle, and the girdled section was immediately covered up with different rooting medium and tightly wrapped with 200-gauge transparent polyethylene film, secured with jute thread.

After 50 days of air layering, a set of the treated layers was detached by making a clean cut just below the lower end of the girdled area using a sharp pair of secateurs. The polyethylene wraps were carefully removed, and the rooting media were gently washed off under running water to expose the root system

without causing any damage to the newly formed roots. Following this, various experimental parameters were recorded. A similar set of observations was also recorded from the remaining air layers 100 days after air layering. Parameters studied included number of primary root branches per layer, length of primary root branches (cm), number of secondary root branches per layer, diameter of primary root branches (mm), dry weight and fresh weight of roots per layer (mg). The data collected were subjected to statistical analysis using the analysis of variance technique suggested by Pansey and Sukhatme (1985)^[14]. Analysis of variance (ANOVA) was prepared for each parameter separately to get the best of the treatment combinations.

Results and Discussion

Number of primary root branches per air layer

Under the influence of rooting media, 50th days after air-layering, the maximum number of primary root branches was observed in M4 (peat moss) with 14.78 branches, followed by M3 (cocopeat) with 9.56. By 100 days, M4 again exhibited superior performance with a significantly higher number of primary root branches (25.56), followed by M3 (18.44).

Superiority of peat moss in both cases reflects that it has great ability for better aeration and water retention, which promote better root development. Similar results were reported by Rymbai and Reddy (2010)^[16], who reported significantly higher rooting success and root quality in guava air layers using sphagnum moss as the rooting medium.

Among the IBA concentrations, C3 (5000 ppm) recorded the highest number of primary root branches at both 50 days (9.60) and 100 days (24.67), followed by C2 (2500 ppm). The application of a higher concentration of hormone could be an accumulation of certain chemical substances (carbohydrates, rooting co-factors, etc.) at the base of the cut which might have stimulated the meristem to divide quickly and produce roots (Kumar and Syamal, 2005)^[10]. The mobilisation and utilisation of carbohydrate and nitrogen fractions in the presence of cofactors at the wounding portion might have helped in better root initiation, root number and root length (Pandey *et al.*, 2003)^[13]. Naithani *et al.* (2018) also observed that the application of 4500 ppm IBA significantly improved root formation in Pant Prabhat guava air layers.

In case of interaction, at 50 days the highest number was observed in M1C3 (soil + FYM with 5000 ppm IBA), recording 17.67 branches. At 100 days, M4C3 (peat moss + 5000 ppm) (31.67) recorded the highest values, closely followed by M4C2 (29.67). The high concentrations of IBA and various rooting media gave an advantageous response in rooting and growth of air layers in lime. Such types of observation might be due to activation of auxin in vegetative parts by using auxins like IBA. The optimum level of IBA accelerated cell elongation, cell division and proliferation of callus, resulting in high rooting and growth (Jan *et al.*, 2003)^[7]. Similarly, media like peat moss provide good congenial conditions for the penetration of roots in rooting media, helping early rooting as well as excellent growth of roots in air layers. Similar findings were reported by Naithani *et al.* (2018), who concluded that air-layering treated with high IBA concentration and the use of sphagnum moss as growing media have been found significantly superior to all other treatments in the Pant Prabhat guava cultivar.

Length of primary root branches per air layer

The beneficial effect on root growth parameters is due to improved media porosity, water-holding capacity, activity of

useful soil microflora and fauna, maintained soil temperature and improved soil health and nutrient status of the medium (Bhojvaid and Negi, 2003) [2]. In the case of rooting media, on the 50th day M4 (peat moss) had a maximum root length of 4.90 cm, followed by M3 (cocopeat) at 4.05 cm. After 100 days of air-layering, again, M4 had the longest root length (6.11 cm). The findings are consistent with those of Kadman and Slor (1974) [8], who found that peat moss was the optimum rooting medium for air layering in litchi.

Among the IBA concentrations, on the 50th day, C3 (5000 ppm) had the longest root length (3.49 cm), followed closely by C2 (2500 ppm) with 3.46 cm. Similar patterns were observed on the 100th day; C3 (5000 ppm) remained the most effective, with 5.96 cm. The increase in length of root at higher concentrations of IBA might be due to hormonal effects and accumulation of other internal substances and their basipetal (downward) movement (Singh 2001) [18].

On the 50th day, the maximum root length was 6.33 cm in the combination M4C3 (peat moss + 5000 ppm), demonstrating a high synergistic effect between the medium and the chemical treatment. On the 100th day, the interaction M4C3 continued to outperform, reaching a maximum root branch length of 7.71 cm. Similar results were reported by D.M. Patel *et al.* (2010), i.e., maximum root length was observed in sphagnum moss + IBA 5000 ppm in different pomegranate cultivars.

Diameter of primary root branches

A critical examination of data on root and growth characters (Table 3) revealed that different doses of IBA and rooting media significantly affect the rooting and growth parameters of air layers of acid lime. In the case of rooting media, on the 50th day, M4 (peat moss) showed the highest root diameter (1.28 mm), followed by M3 (cocopeat) at 1.21 mm. By the 100th day, M4 again recorded the highest root diameter (1.58 mm), followed by M1 (soil + FYM) at 1.51 mm. These results align with studies by Maindad (2022), who reported that sphagnum moss with a maximum diameter of primary roots (1.72 mm) outperformed coco peat and sawdust in improving the rooting characteristics and survival of air layers in guava cv. L-49.

Among the IBA concentrations, on the 50th day, C3 (5000 ppm) showed the highest root thickness (1.32 mm). By the 100th day, although C3 continued to show the highest root thickness (1.45 mm), the differences among treatments were statistically non-significant (NS). In case of interaction, M4C3 (peat moss + 5000 ppm) also showed best results (1.31 mm).

Number of secondary root branches per air layer

In the case of rooting media, 50 days after air-layering, the maximum number of secondary root branches was observed in M4 (peat moss) with a mean value of 5.14. By 100 days, M4 again exhibited superior performance with a significantly higher number of secondary root branches (7.66). Likewise, Maindad (2022) found that sphagnum moss outperformed coco peat and sawdust in improving the rooting characteristics and survival of air layers in guava. Although peat moss and sphagnum moss are different materials, both share similar properties, which may explain their comparable effectiveness in promoting root branching.

Among the IBA concentrations, C3 (5000 ppm) recorded the highest number of secondary root branches at both 50 days (4.12) and 100 days (6.5), followed by C2 (2500 ppm). The treatment (C1, 0 ppm) consistently recorded the lowest values at

both stages. Similar results were reported by Baghel *et al.* (2016) [1], stating maximum primary and secondary roots were achieved at higher IBA concentrations in guava cv. L-49.

In case of interaction, on the 50th day, the highest number was observed in M1C3 (soil + FYM with 5000 ppm IBA), recording 5.38 branches, closely followed by M4C2 (peat moss with 2500 ppm IBA) at 5.23. At 100 days, M4C3 (31.67) recorded the highest value. These findings are similar to reports of Sharma *et al.* (2022) [17], who stated IBA concentration @1500 ppm and growing media, soil + sphagnum moss, recorded a significantly higher number of secondary roots. Similar results were reported by Patel *et al.* (2012) [15], i.e., the maximum number of secondary roots at 45 days in sphagnum moss + IBA 5000 ppm in different pomegranate cultivars.

Fresh weight of roots per air layer

In the case of rooting media, on the 50th day M4 (peat moss) had a maximum fresh weight of 1.92 gm at par with M1 (soil + FYM) with 1.80 gm. On the 100th day, M4 had the maximum weight with 3.15 gm, while M2 (soil + vermicompost) had the least with 1.20 gm. These findings coincide with those of Rymbai and Reddy (2010) [16], who found considerably greater rooting success and root quality in guava air layers utilising sphagnum moss as the rooting media.

Among the IBA concentrations, on the 50th day, C3 (5000 PPM) had the maximum fresh weight with 1.49 gm. Similarly, on the 100th day, C3 (5000 PPM) remained the most effective with 2.98 gm. The greater growth at higher concentrations could be attributable to increased nutrient intake and hormonal stimulation that promotes root development. Similar results were reported by Baghel *et al.* (2016) [1], stating maximum fresh weight of roots is achieved at higher IBA concentrations in Guava cv. L-49.

In case of interaction, on the 50th day, the maximum weight was 3.94 gm in M4C3 (peat moss + 5000 ppm). On the 100th day, the interaction M4C3 continued to outperform with a maximum fresh weight of 4.02 gm, which is at par with M1C3 (Soil + FYM + 5000 ppm) at 3.99 gm. Similar results were reported by Patel *et al.* (2012) [15], i.e., maximum fresh weight of roots in sphagnum moss + IBA 5000 ppm in different pomegranate cultivars.

Dry weight of roots per air layer

In the case of rooting media, M4 (peat moss) recorded the highest dry weight on both the 50th day (0.86 gm) and the 100th day (1.58 gm). Likewise, Maindad (2022) found that sphagnum moss with 0.60 gm outperformed coco peat and sawdust in improving the rooting characteristics and survival of air layers in guava cv. L-49.

Among the IBA concentrations, IBA at 5000 ppm (C3) yielded the highest dry weights (0.71 gm on the 50th day and 1.41 gm on the 100th day). This indicates a dose-dependent stimulation of root development and biomass accumulation with IBA, consistent with the role of auxins in promoting adventitious root formation. Das *et al.* (2014) [4] reported that the dry weight of roots was found to be maximum (1.11 gm) in high concentrations of auxin-treated air layers in the litchi cv. Purbi.

In case of interaction, the combination of M4C3 (peat moss + IBA 5000 ppm) resulted in the highest dry weight (1.7 gm) by the 100th day. Naithani *et al.* (2018) also reported that air-layering treated with high IBA concentration and use of sphagnum moss as growing media have been found significantly superior to all other treatments in guava cv. Pant Prabhat.

Table 1: Effect of IBA and rooting media on number of primary root branches per air layer

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	5.67	6.33	17.67	9.89	15.00	13.67	26.00	18.22
SOIL+VERMI	1.33	2.67	2.00	2.00	7.00	15.33	22.67	15.00
COCOPEAT	16.33	7.00	5.33	9.56	15.67	21.00	18.67	18.44
PEAT MOSS	15.33	16.33	12.67	14.78	21.67	23.33	31.67	25.56
SAWDUST	4.33	9.00	10.33	7.89	13.33	19.67	24.67	19.22
MEAN	8.60	8.27	9.60		14.53	18.60	24.67	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.6198		1.795		0.8782		2.544	
B (IBA conc.)	0.4801		1.391		0.6803		1.971	
AB (Interaction)	1.0735		3.110		1.5211		4.406	

Table 2: Effect of IBA and rooting media on Length of primary root branches per air layer

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	3.55	3.93	3.01	3.49	5.11	4.82	4.82	4.92
SOIL+VERMI	0.44	0.97	1.15	0.85	4.09	4.76	4.23	4.36
COCOPEAT	4.18	4.47	3.50	4.05	5.43	6.09	5.58	5.70
PEAT MOSS	4.46	3.93	6.33	4.90	5.23	5.60	7.71	6.18
SAWDUST	1.52	4.03	3.49	3.01	3.87	5.12	7.43	5.47
MEAN	2.83	3.46	3.49		4.74	5.28	5.96	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.1569		0.455		0.2558		0.741	
B (IBA conc.)	0.1215		0.352		0.1981		0.574	
AB (Interaction)	0.2718		0.787		0.4430		1.283	

Table 3: Effect of IBA and rooting media on diameter of primary root branches

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	0.99	1.17	1.3	1.15	1.72	1.41	1.41	1.51
SOIL+VERMI	0.69	1.19	1.18	1.02	1.37	1.26	1.35	1.33
COCOPEAT	1.12	1.03	1.5	1.21	0.89	1.17	1.46	1.17
PEAT MOSS	1.14	1.4	1.31	1.28	1.52	1.67	1.56	1.58
SAWDUST	0.92	0.98	1.33	1.07	1.29	1.57	1.48	1.45
MEAN	0.97	1.15	1.32		1.36	1.41	1.45	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.0504		0.146		0.0623		0.18	
B (IBA conc.)	0.0391		0.113		0.0483		0.14	
AB (Interaction)	0.0873		0.253		0.1079		0.313	

Table 4: Effect of IBA and rooting media on number of secondary root branches per air layer

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	2.00	4.64	5.38	4.01	7.53	7.77	4.17	6.49
SOIL+VERMI	0.00	1.23	0.87	0.69	6.33	5.43	5.67	5.81
COCOPEAT	1.84	5.00	4.84	3.89	5.77	6.30	7.80	6.62
PEAT MOSS	5.17	5.23	5.03	5.14	7.83	6.30	8.83	7.66
SAWDUST	1.36	3.81	4.50	3.23	3.37	4.27	6.07	4.57
MEAN	2.07	3.98	4.12		6.16	6.01	6.50	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.2259		0.667		0.1325		0.384	
B (IBA conc.)	0.1724		0.512		0.1026		0.297	
AB (Interaction)	0.3974		1.144		0.2294		0.665	

Table 5: Effect of IBA and rooting media on fresh weight of roots per air layer

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	1.84	1.60	1.96	1.80	1.46	1.61	3.99	2.37
SOIL+VERMI	0.02	0.04	0.10	0.06	0.83	1.13	1.63	1.20
COCOPEAT	1.76	0.81	0.21	0.93	1.51	2.56	1.79	1.95
PEAT MOSS	0.56	1.24	3.94	1.92	2.62	2.80	4.02	3.15
SAWDUST	0.53	0.78	1.23	0.85	0.64	1.66	3.47	1.92
MEAN	0.94	0.89	1.49		1.41	1.95	2.98	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.1264		0.366		0.1264		0.334	
B (IBA conc.)	0.0979		0.284		0.8931		0.254	
AB (Interaction)	0.2189		0.634		0.2824		0.578	

Table 6: Effect of IBA and rooting media on dry weight of roots per air layer

Treatments	50 days after air-layering				100 days after air-layering			
	OPP M	2500P M	5000P M	Mean	OPP M	2500P M	5000P M	Mean
SOIL +FYM	0.63	0.82	1.04	0.83	0.96	1.08	1.63	1.23
SOIL+VERMI	0.02	0.03	0.06	0.03	0.33	0.62	0.89	0.61
COCOPEAT	0.68	0.43	0.15	0.40	0.82	0.95	1.01	0.93
PEAT MOSS	0.34	0.54	1.71	0.86	1.41	1.44	1.89	1.58
SAWDUST	0.37	0.31	0.61	0.43	0.35	0.67	1.61	0.88
MEAN	0.41	0.43	0.71		0.77	0.95	1.41	
	SE(m)		CD 0.05		SE(m)		CD 0.05	
A (Rooting media)	0.0467		0.135		0.0926		0.268	
B (IBA conc.)	0.0362		0.105		0.0718		0.208	
AB (Interaction)	0.0809		0.234		0.1604		0.465	

Conclusion

The study concludes that both rooting mediums and high concentrations of IBA significantly affected the rooting success in air layering of acid lime. The peat moss is the most effective medium due to its moisture retention, aeration, and organic content. IBA at 5000 ppm significantly enhances root number, length, diameter, and biomass, outperforming lower concentrations. The combination of peat moss and 5000 ppm IBA results in superior root growth and supports of its use for efficient vegetative propagation of acid lime species; therefore, it can be suggested for the production of quality planting materials of acid lime species in commercial nursery production.

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