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Effect of growth regulators and rooting media on the regeneration of Kagzi lime (*Citrus aurantifolia* Swingle) through air layering

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Abstract
The present investigation was undertaken to study the "Effect of growth regulators and rooting media on the regeneration of kagzi lime (*Citrus aurantifolia* Swingle) through air layering" at the Horticulture Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology Kanpur (U.P.) during the rainy season of 2018-19 and 2019-20. The experiment was laid out in a Factorial C.R.D. with replication trice. Twenty treatments two levels each of IBA and NAA (2000 ppm and 4000 ppm) and four rooting media i.e. soil, F.Y.M., vermicompost and moss grass were taken and these rooting media were also taken as unit of control. Data on all root parameters i.e. callus formation, days required for root initiation, number of primary roots, length of primary roots, diameter of primary roots, fresh weight of primary roots, dry weight of roots and percent of rooting were taken. IBA 4000 ppm was found significantly more effective and all above root characters revealing subsequently 0.59, 0.61cm; 22.87, 22.46 days; 19.35, 20.41; 4.58, 4.66 cm; 1.62, 1.66 mm; 4.39 and 4.53 g; 0.52, 0.53 g and 80.59, 82.55% values respectively. Rooting media moss grass proved superior among all the media maximizing all the above traits and recording 0.63, 0.64 cm; 22.17 and 21.71 days; 21.38, 22.47; 4.70, 4.79 cm; 1.67 and 1.72 mm; 4.83, 5.05 g; 0.57, 0.49 g and 86.01, 88.03; values respectively during corresponding years. Combined effect of growth regulators and rooting media did not prove significant on all the root parameters barring percent of rooting. In this regard interactive treatment IBA 4000 ppm × moss grass enhanced rooting (90.33 and 92.02%). Control versus treatment varied significantly in all rooting characters during both the years of study.

Keywords: IBA, NAA, vermicompost, moss grass, Kagzi lime and root parameters

Introduction
The kagzi lime a native of India is an important citrus crop grown on commercial scale for multipurpose in India. It belongs to family Rutaceae and has very wide distribution in all the parts of India. It has very good refreshing value to eat and gives us very fair amount of vitamin-C, minerals and other substances for human health. It is also used for pickles, preparation of refreshing drinks, seasoning foods, citrate of lime and cosmetics, etc. In vegetative propagation air layering is used commercially and seems to be rather an easy method of propagation. Its vegetative propagation by air layering ensures true type plants, regular bearing and uniform quality. According to Loach (1988) [14], the rooting media is an integral part of propagation system, percentage rooting and the quality of root produced are directly influenced by the medium (Hartmann *et al*. 2002) [11]. Auxins play a vital role in plant propagation, particularly IBA and NAA have been tried to induce rooting in various forms with varied success. These growth regulators than others were found better for inducing rooting in cutting and air layering due to their stable and non-toxic nature, the information of rooting media and growth substances on Kagzi lime air layering is yet scanty. The present study in view of the above was planned to find out the concrete role of growth regulators their optimal concentration and rooting media on the rooting attributes of Kagzi lime.

Method and Materials
The experiment was conducted during rainy season of 2018-19 and 2019-20 at the Horticulture Garden of Department of Fruit Science, C.S.A. University of Agriculture and Technology Kanpur- 208002 (U.P.). The experiment was conducted in Factorial C.R.D. with twenty treatments under three replications.

The growth regulators i.e. IBA and NAA each in two concentrations i.e. 2000 ppm and 4000 ppm and four rooting media i.e. soil, F.Y.M., vermicompost and moss grass were tried. Plot size comprised twenty layers for each treatment. Layering operation was done on one year old branches having pencil thickness and the bark was removed 4 cm around the shoots. It was ensured that cambium was removed fully. IBA and NAA hormones as above respective concentration were prepared in talc powder. All the treatments were applied evenly around the ring out portion with the help of hand and wrapped with polythene 100 gauge and tied with the help of rope. The layering was done on 10 July 2018 and 2019 and separated and planted on 10 September of respective years. Pre-planting observations i.e. callus formation, days required for root initiation, number of primary roots, length of primary roots, diameter of primary roots, fresh weight of primary roots, dry weight of primary roots and per cent of rooting were recorded by routine methods.

Results and Discussion

IBA 4000 ppm concentration proved significantly more effective recorded maximum 0.59 cm and 0.61 cm callusing against minimum 0.47 and 0.48 cm under 2000 ppm NAA during 2018 and 2019 respectively (Table-1). In size of callusing was observed by 25.53 and 27.08% due to IBA 4000 ppm over 2000 ppm NAA. The superiority of IBA may be attributed to enhance of synthesized food materials including carbohydrate accumulation in the plants which encourages quick healing and better callusing. These finding are in line with the reports of Sanchezurdaneta (2009) [25] in guava. Moss grass caused significantly longest callus measuring 0.63 and 0.64 cm which revealed 46.51 and 45.45% increase than soil during respective years. Moss grass could have promoted higher content of air, moisture and suitable pH of media which led callusing. The findings are in line with the reports of Hartmann and Kester (1986) [10]. Interactive effect of growth regulators and rooting media did not touch level of significance (Table-2). Similarly, influence within controls was found to be non-significant during respective years. Control versus treatment showed significant variation during both the years of study (Table-1). The former recorded 0.32 and 0.34 cm against the later (0.52 and 0.53 cm) during respective years of study being 62.50 and 55.88% more callusing due to treatment over control during corresponding years.

IBA 4000 ppm significantly induced earliest root initiation taking 22.87 and 22.46 days against the delayed initiation noted 25.53 and 25.12 days under 2000 ppm NAA during respective years. Thus, it was 10.41 and 10.59% earlier respectively during respective years. Haising (1971) [9] reported that root initials in stem are dependent upon native auxin and its synergism. Results talied with the reports of Baghel *et al.* (2016) [3] and Naithini *et al.* (2018) in guava and Chawla *et al.* (2012) [4] in litchi. Moss grass proved most effective exhibiting earliest (22.17 and 22.71 days) root initiation against the most delayed (26.53 and 26.11 days) noted under soil. In this respect moss grass caused 4.36 and 4.40 days earlier root initiation than soil during respective years. It have moss grass contain large amount of water in its cell and small amount of minerals and pH about 3.5 to 4.00, therefore, it could have played effective role in rooting. These findings are in line with the reports of Reddy *et al*. (2014) [22] in fig and Naik *et al*. (2016) [17] in guava. Effect within control did not touch level of significance and interaction between growth regulators and rooting media was also non-significant. Control versus treatment revealed significant influence showing 28.90, 28.42 days and 24.47, 24.02 days requirement in this regard.

Production of primary roots (19.35 and 20.41) was significantly higher with IBA 4000 ppm against the lowest 15.81 and 16.91 exhibited with NAA 2000 ppm. In this parameter an improvement was recorded by 22.39 and 20.70% with IBA 4000 ppm. The findings are similar with the reports of Dubey and Yadav (2003) [8] in orange, Lalramhluna and Prasad (2016) [13] in lemon and Vyas *et al*. (2017) [30] in red jamun. When efficacy of rooting media was judged moss grass excelled others during both the years recording 21.38 and 22.47% primary roots against the minimum of 14.16 and 15.24 under soil. Thus, there was an increase of 50.99 and 47.44% due to moss grass over soil. In addition to the above desirable abilities of moss grass its light weight gives water holding capacity and low pH. These findings are in with the reports of Shajh *et al*. (2013) [25] in olive, Naik *et al*. (2016) [17] and Das *et al*. (2016) [7] in litchi. Interactive treatments did not cause significant variation in this contest (Table-2). Similarly, all the four unit of control did not bring significant variation. Control versus treatment varied significantly during both years producing 11.73, 12.78 and 17.28, 18.35 numbers of primary roots respectively.

As regards the length of root IBA 4000 ppm produced longest of it (4.58 and 4.66 cm) roots against the shortest (4.11 and 4.19 cm) recorded NAA 2000 ppm. The root length 11.44 and 11.22% greater was due to IBA 4000 ppm treatment during respective years. It may be due to the role of IBA in cell elongation as well as cell division which are apparently dependent on endogenous auxin and when applied exogenously it enhanced length of primary roots in present investigation. These findings are in close conformity with the reports of Tyagi and Patel (2004) [28], Manker *et al*. (2009) [15] and Naithani *et al.* (2018) [18] in guava. Moss grass media proved effective and promoted (4.70 and 4.79 cm) length of primary root to the maximum against the smallest (3.96 and 4.03 cm) noted under soil. An increase of 18.89 and 18.86% in this respect was observed under moss grass over soil media during respective years. The superiority of moss grass might be owing to its unique ability to enhance rooting along with root elongation. Similar result has been reported by Das *et al.* (2016) [7] in litchi and Naik *et al*. (2016) [17] in guava. Treatment versus control demonstrated significant differences recording 4.29, 4.37 cm and 3.62, 3.70 cm root length respectively.

Quite like root length the diameter was also boosted by treatment IBA 4000 ppm which showed significantly maximum (1.62 and 1.66 mm) against the thinnest diameter (1.42 and 1.47 mm) recorded under 2000 ppm NAA. The diameter was improved by 14.08 and 12.93% with IBA 4000 ppm over NAA 2000 ppm during respective years. The superiority brought about by higher concentration of IBA may be ascribed to its efficacy in cell division as well as elongation. These findings are in agreement with the reports of Jan *et al.* (2012) and Das and Prasad (2014) [6] in litchi, Tyagi and Patel (2004) [28] and Naithani *et al.* (2018) [18] in guava. When the effect of rooting media was examined thickest diameter 1.67 and 1.72 mm were produced under the moss grass and thinnest under soil (1.34 and 1.38 mm) during corresponding years of study. An enhancement in diameter by 24.63 and 24.65% was noted due to moss grass over soil. In the present investigation improvement in number of root and leaves ultimately led to carbohydrate accumulation whose response positively increased the diameter. These findings are in accordance with the reports of Rymbai and Reddy (2012) [24], Parmar *et al*. (2018) [19] in guava and Patel *et al*. (2012) [20] in pomegranate. Interactive effect of growth regulator and rooting media were found non-significant. Control versus treatment however brought about significant differences causing 1.50 and 1.54 mm diameter with treatment against 1.16 and 1.20 mm recorded under control respectively during corresponding years.

The fresh weight of roots with IBA 4000 ppm treatment to improved significantly maximum (4.39 and 4.53 g) against the minimum under 2000 ppm NAA (3.91 and 4.07 g) during both corresponding years. The former caused an increase of 12.28 and 11.30% over the later. In present study acceleration of root formation as well as strengthening them ultimately improved fresh weight of roots. These findings are in line with the reports of Ray *et al.* (2001) [21], Jan *et al.* (2003) [12] and Das and Prasad (2014) [6] in litchi, Chovatia and Singh (2000) [5] in sweet orange and Patel *et al*. (2012) [20] in pomegranate. Rooting media differed significantly in root production in term of their weight under moss grass gave the maximum 4.83 and 5.05 g fresh weight of root against the minimum in soil (3.51 and 3.63 g). A comparison of the above showed 37.61 and 38.84% improvement in this parameter was observed it is obviously due to high moisture retaining capacity, porocity and optimum pH of moss grass (Hartmann and Kester, 1986) [10]. The findings are in accordance with the reports of Singh and Pandey (2009) [26] in guava and Mishra (2014) [16] in Kagzi lime. Control versus treatment exhibited significant differences recording 3.04, 3.29 g and 4.12, 4.30 g fresh weight of root respectively.

The dry weight content of root was noted significantly highest under IBA 4000 ppm (0.52 and 0.53 g) against the lowest (0.46 and 0.46 g) under NAA 2000 ppm. The former treatment increased it by 10.63 and 10.20% over the later. The increase may be attributed to the increased size and number of roots caused by exogenous application of IBA 4000 ppm. These findings also form similar results with the reports of Tyagi and Patel (2004) [28] and Baghel *et al*. (2016) [3] in guava and Das and Prasad (2014) [6] in litchi. Moss grass induced significantly maximum (0.57 and 0.60 g) dry weight of roots and minimum of it (0.41 and 0.43 g) noted under soil media. An increase in dry weight of primary roots was achieved by 39.02 and 39.53% due to moss grass over soil during respective years. This enhancement may be attributed to accumulation of food materials as well as larger root length and change in amino acid metabolism which ultimately favored dry matter accumulation. These results collaborate with the observation of Awan *et al.* (2000) [2] in litchi, Singh *et al.* (2007) in guava and Reddy *et al*. (2014) [23] in fig. Comparison of control versus treatment exhibited significant differences revealing 0.36, 0.39 g and 0.48, 0.51 g dry weight respectively. Treatments increased it to the tune of 33.33 and 30.77% over control during corresponding years of study.

The rooting at 4000 ppm IBA treatment significantly maximized (80.59 and 82.55%) during respective years. Lower concentration irrespective of growth regulators proved relatively less effective and recorded 66.24 and 72.13 per cent rooting when compared with IBA 2000 ppm during respective years (Table-1). Rooting enhanced by 21.66 and 14.45% with IBA 4000 ppm concentration. It is obviously exogenous application of auxin which might have ultimately helped to promotion of rooting per cent. These finding are accordance with the reports of Anandhanmbi *et al.* (2016) [1] and Naithani *et al*. (2018) [18] in guava. Moss grass revealing 86.01 and 88.03% rooting being significantly superior than rest of rooting media and exhibited 42.14 and 40.58% more rooting than soil during respective years. These findings are in line with the reports of Das *et al*. (2016) [7] in litchi and Urmi *et al.* (2016) [9] in guava. Influence within unit of media varied significantly and recorded maximum i.e. 50.61 and 53.07% rooting against the minimum of 43.01 and 45.16% under soil during respective years. Treatment versus control brought about significant differences. Former revealed 72.10 and 75.53% rooting and the later 46.82 and 48.96% during corresponding years of study. Interactive treatment I_2R_4 revealed the maximum (90.33 and 92.02%) rooting followed by I_1R_4 and N_2R_4 recording 87.83, 89.94% and 83.89, 85.98% rooting respectively. The minimum 53.66 and 55.61% rooting (Table-2) demonstrated under interactive treatment N_1R_1 during corresponding years of investigation.

Table 1: Effect of regulators, rooting media, controls and treatment v/s control on different root attributes in Kagzi lime air layers

Treatments	**Callus formation (cm)**		**Days required for root initiation**		**Number of primary roots**		**Length of primary roots (cm)**		**Diameter of primary roots (mm)**		**Fresh weight of primary roots (g)**		**Dry weight of primary roots (g)**		**Per cent of rooting (%)**	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
I_1	0.50	0.51	24.94	24.45	16.48	17.56	4.24	4.32	1.46	1.50	3.99	4.18	0.47	0.49	69.67	72.13
I_2	0.59	0.61	22.87	22.46	19.35	20.41	4.58	4.66	1.62	1.66	4.39	4.53	0.52	0.54	80.59	82.55
N_1	0.47	0.48	25.53	25.12	15.81	16.91	4.11	4.19	1.42	1.47	3.91	4.07	0.46	0.48	66.24	73.34
N_2	0.52	0.53	24.54	24.06	17.48	18.52	4.24	4.32	1.49	1.53	4.15	4.38	0.49	0.52	71.91	74.11
R_1	0.43	0.44	26.53	26.83	14.16	13.94	3.96	4.03	1.34	1.38	3.51	3.63	0.41	0.43	60.51	62.62
R_2	0.48	0.50	25.18	24.52	15.55	16.26	4.14	4.22	1.44	1.48	4.18	4.38	0.49	0.51	67.67	75.03
R_3	0.53	0.55	24.01	22.80	18.03	15.72	4.36	4.44	1.53	1.58	3.91	4.10	0.46	0.49	74.22	76.46
R_4	0.63	0.64	22.17	22.10	21.38	21.72	4.70	4.79	1.67	1.72	4.83	5.04	0.57	0.60	86.01	88.03
C_1	0.29	0.31	29.60	29.12	11.16	12.10	3.52	3.59	1.11	1.15	2.90	3.13	0.34	0.37	43.01	45.16
C_2	0.32	0.33	29.13	28.60	11.51	12.56	3.58	3.66	1.14	1.18	3.06	3.30	0.36	0.39	45.57	47.22
C_3	0.34	0.35	28.66	28.21	11.89	13.02	3.65	3.74	1.18	1.21	2.98	3.23	0.35	0.38	48.08	50.39
C_4	0.35	0.37	28.22	27.76	12.37	13.51	3.73	3.80	1.22	1.25	3.23	3.49	0.38	0.41	50.61	53.07
T	0.52	0.53	24.47	24.02	17.28	18.35	4.29	4.37	1.50	1.54	4.11	4.29	0.48	0.51	72.10	75.53
C	0.32	0.34	28.90	28.42	11.73	12.78	3.62	3.70	1.16	1.20	3.04	3.29	0.36	0.39	46.82	48.96
C.D. at 5%																
G	0.05	0.05	1.01	0.83	1.41	1.50	0.19	0.21	0.11	0.12	0.21	0.24	0.04	0.05	2.29	2.49
R	0.05	0.05	1.01	0.83	1.41	1.50	0.19	0.21	0.11	0.12	0.21	0.24	0.04	0.05	2.29	2.49
Within control	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	4.58	4.99
Treatment v/s	0.02	0.02	0.44	0.61	0.61	0.65	0.08	0.09	0.05	0.05	0.09	0.10	0.02	0.02	1.00	1.09

control																

I_1- IBA 2000 ppm, I_2-IBA 4000 ppm, N_1- NAA 2000 ppm, N_2- NAA 4000 ppm, R_1- Soil, R_2- F.Y.M., R_3- Vermicompost, R_4- Moss grass, C_1- Unit of control (soil), C_2- Unit of control (F.Y.M.), C_3- Unit of control (Vermicompost), C_4- Unit of control (Moss grass), G- Growth regulator, R- Rooting media

Table 2: Interactive effect of growth regulators and rooting media on different root attributes in kagzi lime air layers

Treatments	Callus formation (cm)		Days required for root initiation		Number of primary roots		Length of primary roots (cm)		Diameter of primary roots (mm)		Fresh weight of primary roots (g)		Dry weight of primary roots (g)		Per cent of rooting (%)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
I_1R_1	0.42	0.42	26.88	26.39	13.77	14.73	3.91	3.98	1.32	1.36	3.40	3.56	0.40	0.42	58.81	61.02
I_1R_2	0.43	0.44	26.45	25.93	14.21	15.39	3.98	4.05	1.35	1.39	3.90	4.08	0.46	0.48	61.28	63.88
I_1R_3	0.50	0.52	24.62	24.16	16.14	17.21	4.23	4.32	1.49	1.52	3.74	3.99	0.44	0.47	70.77	73.70
I_1R_4	0.65	0.66	21.81	21.33	21.82	22.90	4.84	4.93	1.70	1.75	4.91	5.09	0.58	0.60	87.83	89.94
I_2R_1	0.53	0.54	24.18.	23.73	16.69	17.88	4.29	4.37	1.53	1.57	3.56	3.66	0.42	0.43	73.31	75.38
I_2R_2	0.54	0.56	23.75	23.28	17.22	18.20	4.33	4.42	1.56	1.60	4.49	4.74	0.53	0.56	75.88	77.97
I_2R_3	0.63	0.65	22.13	21.91	21.26	23.38	4.77	4.85	1.67	1.71	4.33	4.39	0.51	0.53	82.85	84.85
I_2R_4	0.67	0.69	21.44	20.94	22.25	23.19	4.92	5.02	1.74	1.78	5.17	5.35	0.61	0.63	90.33	92.02
N_1R_1	0.38	0.39	27.75	27.51	12.87	13.94	3.79	3.87	1.25	1.28	3.58	3.62	0.42	0.43	53.66	55.61
N_1R_2	0.47	0.48	25.49	25.06	15.16	16.26	4.11	4.19	1.42	1.46	3.82	4.01	0.45	0.47	65.52	87.87
N_1R_3	0.45	0.46	25.98	25.44	14.68	15.72	4.05	4.13	1.39	1.47	3.66	3.81	0.43	0.45	63.79	65.70
N_1R_4	0.59	0.60	22.90	22.46	20.55	21.72	4.49	4.57	1.62	1.66	4.58	4.83	0.54	0.57	81.98	84.17
N_2R_1	0.40	0.41	27.31	26.33	13.32	14.43	3.84	3.92	1.28	1.33	3.49	3.68	0.41	0.45	56.26	58.46
N_2R_2	0.50	0.51	25.04	24.52	15.63	16.58	4.16	4.24	1.45	1.49	4.51	4.70	0.53	0.55	68.01	70.41
N_2R_3	0.56	0.58	23.31	22.80	20.06	21.03	4.39	4.48	1.59	1.62	3.92	4.22	0.46	0.52	79.48	81.59
N_2R_4	0.61	0.63	22.52	22.10	20.91	22.06	4.57	4.66	1.64	1.68	4.68	4.91	0.55	0.58	83.89	85.98
C.D. at 5% G×R	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	4.58	4.99

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