Effect of different concentrations of Indole butyric acid (IBA), Seaweed Extract and Phloroglucinol on air layering in Kinnow

Abstract

This experiment was conducted to evaluate the best treatment combination among IBA, Seaweed Extract and Phloroglucinol concentration for assesing the success rate, survival percentage of Kinnow. The experiment was laid out in Randomized Design having 12 treatments and replicated 3 times. The T_7 IBA@2000ppm+Phloroglucinol@5000ppm recorded highest values in terms of success in rooting percentage (73.67), number of days taken for initial root formation (28.44), root length of layers (7.30cm), number of roots per layering (20.44), root thickness (3.70mm), number of new sprouts per layer (6.56-1MAP, 8.89-2MAP, 8.56-3MAP & 8.00-4MAP), number of new leaves per layer (6.67-1MAP, 10.56-2MAP, 7.09-3MAP & 12.78-4MAP), length of new shoots (4.26cm-1MAP, 5.23cm-2MAP, 7.09cm-3MAP & 9.94cm-4MAP) and survival percentage of rooted polybag transplanted air layers (68.66%) respectively in Kinnow, whereas minimum values were recorded in T_6 (IBA@3000ppm + SWE@5000ppm).

Keywords: *Air layering, Kinnow, Phloroglucinol, Citrus and IBA*

1. INTRODUCTION

Citrus fruits play a vital role in the Indian economy, with the Kinnow mandarin, a hybrid of *Citrus nobilis* Lour. and *Citrus deliciosa* Tenore, being of significant commercial importance. Developed by H.B. Frost in 1915, the Kinnow mandarin was first introduced in virus-free budwood form on Jatti Khatti (*Citrus jambhiri*) rootstock in 1959 at Punjab Agriculture University (PAU), Regional Research Station, Abohar, from the University of California (Singh, 1977). The fruit is highly valued for its freshness, high vitamin C content, aromatic flavour, low saturated fat, cholesterol, and sodium content (Mabberley, 2004). Kinnow mandarin is cultivated in various regions across India, including Punjab, Haryana, Himachal Pradesh, Uttar Pradesh, Karnataka, Kerala, and Tamil Nadu, and is ranked third in importance after bananas and mangoes (NHB 2020).

Despite the immense significance of citrus production, most citrus cultivars are the result of spontaneous hybridizations and bud sport mutations, rather than intentional and focused breeding. This is due to the unique and complex botanical features of polyembryony, in which adventive embryos are formed from nucellar cells that are identical to the mother plant, a high level of heterozygosity, and a very long juvenile phase, all of which make conventional breeding challenging for this particular group of fruits (Roose 2007). Kinnow mandarin, on the other hand, is highly productive and efficient for processing, making it a commercially valuable fruit. The fruit is medium globose to oblate in shape, with a golden orange skin and mild acidity, and ripens during January-February, producing around 50-60 kg of fruit per tree. It can be grown successfully at altitudes ranging from 500-1500 amsl in a wide range of light and degraded lands with proper drainage.

In regions where Kinnow is grown, the traditional method of propagation is through sexual means such as seeds. However, this method has several drawbacks, including a long time to reach maturity, a lack of uniformity in progeny, and a high risk of contamination by viral diseases. To overcome these issues, vegetative propagation is the preferred method, as it allows for the production of true-to-type plants with desirable traits from the mother plant. One of the most common and cost-effective methods of asexual propagation for Kinnow trees is layering.

Air layering is a well-known horticultural technique that has been used for over 2,000 years. It involves inducing roots to grow on a stem or branch that is still attached to the mother plant (Mergen 1953). In India, this method has been widely used for producing elite planting material in various fruit crops. Although there are several vegetative methods for multiplying quality stock in fruit tree species, air layering is often used when the formation of roots from cuttings is slow. With air layering, the propagation process is quicker and produces more reliable results. Air layering is a technique used to propagate plants without severing the stem from the parent plant. This method enables horticulturists to produce larger and mature plants much faster than growing them from seed or cuttings (Hartmann 1975). Moreover, the new plant that develops is identical to the parent plant in terms of fruit taste, colour, and size. Air layering is a reliable means of vegetative propagation that produces a large number of plants in a relatively short period of time. In subtropical climates, air layering can be done during the rainy season, which is favourable for rooting due to the constant moisture (Bose 1986). This method is easy, inexpensive, and advantageous because the reserve food of the parent branch induces the formation of a well-developed root system. Air layered branches have a balanced root system compared to cuttings and develop rapidly on planting out (Hartmann 1975). The season is an important factor for successful layering in woody plants, as rooting is enhanced by light and the presence of sufficient moisture and optimum temperature (Tomar 2006).

IBA is a synthetic auxin hormone that stimulates the growth and development of roots (Strader 2018). It is widely used in the horticulture industry to promote root formation in cuttings and air layers. In air layering, a paste of IBA powder is applied to the wound on the stem after the bark has been removed. This stimulates the formation of adventitious roots, which grow from the layer and penetrate into the rooting medium (Rajesh 2012). IBA is particularly effective in promoting root growth in difficult-to-root species, such as citrus.

Phloroglucinol is another plant growth regulator that has been used to enhance root formation in air layering (Ibrahim 2012). It is a natural phenolic compound that is found in various plant species, including oak trees. In air layering, a solution of phloroglucinol is applied to the wound on the stem, which promotes the formation of callus tissue. This callus tissue produces a hormone called ethylene, which stimulates root formation. Phloroglucinol is particularly effective in promoting root growth in difficult-to-root species.

Seaweed extract is a natural plant growth promoter that is derived from seaweed. It contains a range of plant hormones, micronutrients, and other growth-promoting compounds that stimulate growth and development (Rajan 2022). In air layering, a solution of seaweed extract is applied to the rooting medium before inserting the air layer. This enhances the root growth and development of the layer by providing it with essential nutrients and hormones (Ibrahim 2012). Seaweed extract has been found to be particularly effective in promoting root growth in a range of species.

When it comes to the propagation of plants, including citrus, various rooting hormones and additives are commonly used to enhance root development. Among these, Indole-3-butyric acid (IBA), seaweed extract, and phloroglucinol are frequently employed. Each of these substances has distinct benefits, and when combined, they can potentially provide synergistic effects in promoting rooting and overall plant growth. When these three substances (IBA, seaweed extract, and phloroglucinol) are combined in the rooting process, their benefits can complement each other. The synthetic auxin IBA directly stimulates root initiation, while the seaweed extract provides additional growth-promoting compounds that enhance the overall health and vigor of the cutting. Phloroglucinol, in turn, promotes lignification, reinforcing the root structure. Using only one or two of these substances may limit the potential benefits. For instance, if only IBA is used, the cutting may experience improved root initiation but may lack the additional growth-promoting effects provided by seaweed extract or the enhanced structural support provided by phloroglucinol. Similarly, using only seaweed extract might enhance overall growth but may not directly promote root initiation and lignification. By combining all three substances, the cutting benefits from multiple mechanisms that work synergistically to stimulate root development, improve overall plant vigor, and increase stress tolerance. The combination of these substances enhances both the quantity and quality of roots, resulting in a more successful and robust propagation process.

It is worth noting that the concentrations and application methods of these substances should be carefully considered and optimized for specific plant species and propagation conditions. Additionally, factors such as timing, environmental conditions, and the physiological state of the layered trees should also be taken into account to ensure optimal rooting success.

2. MATERIALS AND METHODS

The experiment was conducted from August 2022 to February 2023, at the Horticulture Research Farm, Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj (U.P.) which is located at 25^0 39‟ 42” N latitude, $81^0$67‟‟56” E longitude and 98 m altitude above the mean sea level.

The present investigation was carried out on semi-hardwood stems of Kinnow. Plant growth regulator Auxins (IBA), Phloroglucinol and Seaweed Extract (*Kappaphycus alvarezii*) was used as a root induction chemical at twelve different concentrations and combinations replicated thrice in randomized block design. Cocopeat was used as root growth media in layering and also soil, sand and vermicompost are used as growth media in polybags at the time of transplantation of rooted air layered plants. Treatments used are T_1 (IBA@2000ppm+SWE@3000ppm), T_2 (IBA@2000ppm+SWE@4000ppm), T_3 (IBA@2000ppm+SWE@5000ppm), T_4 (IBA@3000ppm+SWE@3000ppm), T_5 (IBA@3000ppm+SWE@4000ppm), T_6 (IBA@3000ppm+SWE@5000ppm), T_7 (IBA@2000ppm + PG@5000ppm), T_8 (IBA@2000ppm+PG@6000ppm), T_9

(IBA@2000ppm + PG@7000ppm), T_{10} (IBA@3000ppm + PG@5000ppm), T_{11} (IBA@3000ppm + PG@6000ppm) and T_{12} (IBA@3000ppm + PG@7000ppm)

To conduct air layering on Kinnow mandarin trees, healthy branches of uniform vigour that were one year old and of pencil thickness were selected. Only disease-free branches were chosen, and a careful girdling of the bark, about 2-2.5 cm, was done just below the bud. Two circular cuts were made around 45-60 cm below the top end of the selected shoot, being careful not to harm the underlying wood. A pre-prepared growth regulator formulation with IBA, Phloroglucinol and bio stimulant seaweed extract was applied evenly on all sides of the upper cut of the ringed portion. The cut portion was then covered with various types of rooting media and white polythene wrappers as per the treatment combination. Observations were made after 1, 2, and 3 month(s) from the day of operation, noting the success percentage of layering and various root parameters such as the number of days for root formation (initial rooting), number of roots, root length and root thickness. After detaching the air layers by making a cut just below the lowest end of the ringed surface with sharp secateurs, they were transplanted into the fruit nursery of the Department of Horticulture at SHUATS. Shoot parameters were calculated after 1, 2, 3, and 4 months after planting into polybags and the survival percentage of the layered plants was calculated after 4 months

3. RESULTS AND DISCUSSION

3.1 Root parameters

The maximum success rate in Kinnow (73.67) was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm) and significantly similar with treatment T_9 (IBA@2000ppm + Phloroglucinol@7000ppm) (67.00). The successful application of IBA and Phloroglucinol in air layering of Kinnow may be attributed to their correct concentration. IBA is known to enhance the synthesis of food material in plants, which leads to quicker healing and better callusing (Rymbai et al. 2010) while Phloroglucinol promotes the accumulation of food material. The results showed that an optimal concentration of IBA and PG led to a decrease in amyloplast and cambium activities and stimulated the mobilization of food material to the root-promoting sites (Singh *et al.* 2014). Additionally, cocopeat proved to be an effective medium in promoting the success percentage in air layering (Abad 2002). This may be due to its ability to provide good aeration and water retention, resulting in higher success rates for air layers. The minimum number of days taken to initial root formation in Kinnow (28.44) was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm) and significantly similar with T_9 (IBA@2000ppm + Phloroglucinol@7000ppm) (30.56). Days taken to rooting was found gradually increased with decrease in the IBA concentration. These results are also in agreement with previous studies of Jan *et al*. 2003**.** This might be due to the wound response signaling pathways are initiated at the location of the wound (ring site) as a part of plant protection mechanism to induce the formation of callus at the wound site. These signaling pathways control the cell division and differentiation that leads to a new root primordium (Tyagi *et al*. 2004). Application of auxin (IBA) and PG at wound site leads to adventitious root formation. These adventitious roots may then develop into new root primordia and eventually form a complete root system. The longest root length is (7.30cm) was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm). In comparison to layers treated with Seaweed Extract and IBA, layers treated with Phloroglucinol and IBA at different concentrations showed longer root lengths. These results were also in agreement with previous studies of (Baghel *et al.* 2016)**.** This could be attributed to the fact that the layering was carried out on younger mother plants that naturally produce longer roots due to the presence of actively dividing cells and higher levels of growth hormones. The exogenous application of IBA may have also played a vital role in inducing cell elongation and division, leading to an increase in root length (Nathani *et al*. 2018). Additionally, the proteins from IBA and Phloroglucinol can break hydrogen bonds between cellular microfibrils, promoting cell wall loosening and elongation (Kumar *et al*.2015). The optimum external application of IBA can also enhance the rate of cambium dedifferentiation, leading to increased hydrolytic activity and callus formation, ultimately resulting in better root length. Maximum root thickness in Kinnow (3.70mm) was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm). IBA and Phloroglucinol combination at different concentrations might have affected the root thickness & timing with frequency of application must have promoted root growth along with wound size and location on the stem also might have affected root growth, with larger wounds producing more callus tissue and more roots. The concentration of carbohydrates in the roots affects the thickness and differentiation of the root system Adequate nutrient availability in media and temperature might have promoted the thickness of the roots.

3.2 Shoot parameters

Maximum number of new sprouts in Kinnow at 1, 2, 3 and 4 month(s) after transplanting (6.56), (8.89), (8.56) and (8.00) was observed in treatment T_7 (IBA@2000ppm + Phloroglucinol@5000ppm) Number of new sprouts per layer followed an increasing pattern with IBA and PG when compared to Seaweed Extract and IBA. These results are in close conformity with the reports of (Gilani *et al*. 2018)**.** IBA and phloroglucinol might have played crucial roles in promoting both root and shoot growth during air layering. IBA stimulates the growth of new roots from the cut stem, providing the plant with necessary nutrients and water for developing new sprouts. On the other hand, phloroglucinol triggers the production of cytokinins, plant hormones that promote cell division and growth. When phloroglucinol is applied to the air layering medium, it stimulates the formation of new sprouts from the stem tissue by signalling the plant to start dividing cells and producing new tissue. Thus, the combination of IBA and phloroglucinol might have created an environment that supports both root and shoot growth, with IBA encouraging the growth of new roots and phloroglucinol stimulating the formation of new sprouts. Maximum length of new shoots in Kinnow at one 1, 2, 3 and 4 month(s) after transplanting (4.27cm), (5.23cm), (7.09cm) and (9.94cm)was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm). The application of IBA in air layering might have led to the stimulation of shoot growth, resulting in a longer and more robust stem or shoot. Similarly, the use of phloroglucinol might have promoted the growth of new cells, increasing the length of the shoot and leading to the development of larger and more vigorous plants. When used in combination, these plant hormones might have created a synergistic effect that could have further promoted shoot growth in citrus plants. It is important to note that other factors, such as light, temperature, and water under nursery conditions, might have also contributed to plant growth. Maximum number of new leaves in Kinnow at 1, 2, 3 and 4 month(s) after transplanting (6.67), (10.56), (10.00) and(12.78) was observed in T_7 (IBA@2000ppm + Phloroglucinol@5000ppm). Similar results were reported by (Singh *et al.* 2017)**.** The application of IBA, phloroglucinol, and seaweed extract might have potentially stimulated the growth and development of new roots and shoots, which in turn may have led to the production of new leaves. The presence of auxins, cytokinins, gibberellins, and essential nutrients in seaweed extract might have contributed to the growth and development of new leaves in plants. Overall, these supplements might have potentially improved the overall health and vigour of the plants, leading to the growth of new leaves and potentially contributing to the overall growth and development of the plant.

3.3 Survival Percentage

The maximum survival percentage in Kinnow (68.33) was observed in $\mathbf{T_7}$ (IBA@2000ppm + Phloroglucinol@5000ppm). These results are in concurrence with reports of (Lalramhluna *et al*. 2016) on their studies on air layering in Citrus. The high survival rate of layers treated with combined IBA and Phloroglucinol might have been due to the increased number of roots and root length. This could have led to better absorption of nutrients and water from the soil, ultimately resulting in a higher survival rate. The balance of rooting co-factors and their interaction with auxin and nutritive substances might have also played a role in the response of the growing media (Gonalez *et al.* 2009). The maximum number of roots and increased root length at this concentration could have contributed to the better absorption of nutrients, food material, and moisture from the soil, leading to a higher survival percentage of layers.

4. Conclusion

From the present investigation it is concluded that effect of Treatment T_7 i.e., IBA@2000ppm + Phloroglucinol@5000ppm was found to be best in terms of Success rate, Number of days to root formation (initial rooting), Root length(cm), Root thickness(mm), Number of roots per layer, Survival percentage, Number of new sprouts per layered plant, Number of new leaves per layered plant and length of new shoot(cm) in Kinnow. Moreover, the above mentioned treatment combination also provides true to type and elite planting material of Kinnow mandarin to the fruit growers.

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Table 1. Success rate and Root parameters as effected by different combinations and concentrations of IBA, Seaweed Extract & Phloroglucinol

Treatment	Treatment Combination	Success rate in rooting	Days to root formation	Root length (cm)	Root Thickness (mm)	No. of Roots
T1	IBA@2000ppm + SWE@3000ppm	62.67	30.78	5.71	1.55	16.56
T2	IBA@2000ppm + SWE@4000ppm	61.67	34.78	5.40	1.83	17.22
T3	IBA@2000ppm + SWE@5000ppm	57.33	37.33	5.83	1.73	15.22
T4	IBA@3000ppm + SWE@3000ppm	56.33	39.44	6.11	2.28	17.11
T5	IBA@3000ppm + SWE@4000ppm	64.33	40.11	5.41	1.84	12.89
T6	IBA@3000ppm + SWE@5000ppm	41.33	42.33	4.67	1.43	10.33
T7	IBA@2000ppm + PG@5000ppm	73.67	28.44	7.30	3.70	20.44
T8	IBA@2000ppm + PG@6000ppm	65.33	31.00	6.56	2.58	18.11
T9	IBA@2000ppm + PG@7000ppm	67.00	30.56	7.20	3.55	19.78
T10	IBA@3000ppm + PG@5000ppm	54.67	31.56	6.87	3.16	18.22
T11	IBA@3000ppm + PG@6000ppm	47.33	32.00	6.72	2.11	15.89
T12	IBA@3000ppm + PG@7000ppm	48.67	33.33	6.28	2.22	15.56
S.E. (m) (±)		2.72	0.65	0.29	0.27	0.69
CD (5%)		7.99	1.92	0.85	0.79	2.03

Table 2. Survival percentage and Shoot parameters as effected by different combinations and concentrations of IBA, Seaweed Extract & Phloroglucinol

Treatment	Number of new sprouts				Length of new shoots				No. of new leaves				Survival %
	1MAP	2MAP	3MAP	4MAP	1MAP	2MAP	3MAP	4MAP	1MAP	2MAP	3MAP	4MAP	
T1	5.56	7.33	7.22	6.44	3.98	4.99	6.19	7.76	6.11	8.67	8.11	10.40	60.56
T2	6.00	7.22	6.56	6.33	4.06	4.94	6.59	7.93	6.00	8.56	8.11	9.11	61.44
T3	5.44	7.78	7.56	7.22	3.97	5.04	6.64	8.16	6.00	7.78	8.67	9.22	54.56
T4	5.44	7.22	7.56	7.22	3.94	4.54	6.37	7.98	5.67	7.22	7.78	10.30	58.11
T5	5.89	7.22	7.33	7.78	3.96	5.06	6.62	8.31	6.30	7.56	6.78	10.42	61.67
T6	4.22	5.00	5.44	5.89	3.64	4.74	6.13	6.90	4.89	6.78	5.33	8.67	50.67
T7	6.56	8.89	8.56	8.00	4.27	5.23	7.09	9.94	6.67	10.56	10.00	12.78	68.33
T8	5.44	7.00	7.44	7.44	3.84	4.89	6.71	8.28	6.00	8.44	7.22	10.11	58.22
T9	6.33	7.78	7.67	7.78	4.07	5.12	6.80	8.67	6.44	8.78	9.11	10.67	64.11
T10	4.56	6.44	6.78	6.00	3.77	4.87	6.61	8.66	6.40	7.33	7.44	10.44	60.78
T11	4.56	5.56	6.22	6.22	3.69	4.76	6.50	8.17	6.33	7.89	7.56	9.33	60.67
T12	4.78	5.22	5.67	5.89	3.67	4.80	6.36	8.21	5.60	7.56	8.00	9.56	60.89
S.E. (m) (±)	0.29	0.42	0.30	0.33	0.09	0.09	-	0.30	-	0.38	0.41	0.51	1.25
CD (5%)	0.85	1.23	0.89	0.98	0.27	0.28	-	0.87	-	1.11	1.21	1.49	3.66