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Effect of bioinoculant on survival and rooting characteristics of air layering in citrus species under the *Tarai* region of Uttarakhand

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Abstract
Bio-inoculants have been used for enhancing plant growth in fruits crops even in nutrient-limited soils. The present experiment was carried down to evaluate the effect of bio-inoculants such as *Pseudomonas species* + *Ochrobactrum anthropi* (DPC12+DPC9) and *Pseudomonas fluorescens* + *Pseudomonas palluoniana* (DPB15+DPB16) on Pant lemon-1, Kinnow and Grapefruit. All the treatments tested significantly increased survival percentages as well as root fresh and dry weight compared to the control. The best results were obtained from treatment of air layers with *Pseudomonas species* + *Ochrobactrum anthropi* (DPC12+DPC9) in Pant Lemon -1, followed by Kinnow and grapefruit. Hence, this treatment can be recommended for improving the propagation of Pant Lemon -1, Kinnow and grapefruit through air layering.

Keywords: Citrus, Kinnow, bio-inoculants, pseudomonas, air layering

Introduction
Citrus is an important sub-tropical fruit tree belongs to family Rutaceae and believed to have originated in China. Citrus has played a great role in the food and nutritional security and it can bring economic prosperity in the areas where it is grown. It is the one of the most important fruit crops since antique and known as a good source of vitamin-C with high antioxidant potential. It is a most valuable crop which is adaptable to wide range of soils, province, planting and cultural arrangements and over more than 100 nations recorded citrus production in 1980 (Reitz, 1984) [13]. In the world, citrus fruits are grown over an area of 8.9 million ha with 137.8 million tons production (Singh *et al.*, 2018) [14]. China, Brazil, India, USA, Mexico, Spain and Egypt are the major producers of Citrus in the world. India is the third largest citrus producing country in the world with an area of 1.02 m ha and annual production of 11.15 m tonnes (FAOSTAT 2015) [6]. Grapefruit (*Citrus paradisi*) is a famous for its sour to semi-sweet, somewhat bitter fruit and it is a hybrid between Sweet orange (*C. sinensis*) and Pomelo (*C. maxima*), both were introduced from Asia in the 17th century. It is a rich source of vitamin-C (>20% of the daily value), pink and red hues contain the beneficial antioxidant lycopene. Kinnow is a high yielding Mandarin hybrid, cultivated extensively in the Punjab region of India. It is a hybrid of two Citrus cultivar King (*Citrus nobilis*) x 'Willow Leaf' (*Citrus delicosa*) which developed by H. B. Frost. It contains 2% Vitamin A, 110% Vitamin C, 2% Iron and 4% Calcium. The cultivation of Kinnow in India for a long time and extends too many states of the country. Lemon (*Citrus limon* L.) is considered an important plant of citrus species and originated in Asia. It is used to culinary and non-culinary purposes through-out the world. Every 100 g of lemon contain 29 calories, 2.5 g sugar, 9 g carbohydrate, 1.1 g protein and 88% vitamin C. In Uttarakhand state tarai region has unique climate like humid summer and cold winters for lemon production. Citrus species like Pant lemon-1, Kinnow, and Grapefruit are generally propagated vegetatively by air layering which insure true to type of plants, uniform quality and regular bearing habits of the plants. The quality of root formation in air layers depends upon certain factors. Among these factors application of bioinoculants provide successful propagation of citrus plants. Its increase percentage of success by easy rooting formation in plants, where vegetative propagation is not easy. Bioinoculants can help in callus formation, root initiation, root development and survival percentage of air layers (Bankapur, 1957) [3]. The other practical problem in Citrus is plants with under developed root system and are generally poor in nutrient uptake from soil.

Since, availability of the nutrients to plants depends on the essential and derived structure to access these nutrients in plants. The rhizosphere of roots is rich with soil biota, amongst them microorganisms play a major role in nutrients availability, convenience and translocation (Jefwa *et al.*, 2010) [8]. PGPR are also able to produce plant growth regulators (PGRs) such as auxins, cytokinins, gibberellins and ethylene (Kloepper, 1997) [9]. Increase the plant hormone level, particularly indole-3-acetic acid (IAA) at plant rhizosphere zone helps in healthy growth and development of root. Indole-3-acetic acid is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch, 1985) [11]. Therefore, the current study aimed to define the effect of bioinoculant application on rooting and survival percentage, as well as root and growth characteristics of citrus species propagated by air layering.

Material and Methods

The present studies were carried out at HRC, Patharchatta, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand during 2017-18. The experimental site falls in humid sub-tropical with hot summers and cold winter. For air layering preparation, healthy and terminal branches which receiving good sunshine with thickness 2.5-3 cm was selected and a ring of bark measuring 3 cm in length was removed about 45 cm below the shoot apex. A layer of moist sphagnum moss treated with inoculant including *Pseudomonas species* + *Ochrobactrum anthropi* (DPC12+DPC9) *i.e.* T_2 and *Pseudomonas fluorescens* + *Pseudomonas palluonia* (DPB15+DPB16) *i.e.* T_3 and without any inoculants *i.e.* T_1 were wrapped with a piece (20x25 cm) of 300-gauge transparent white polythene sheet at the exposed woody portion on the selected branch. The entire process was done during 30th July to 3rd August 2017. All the air layers were detached from mother citrus trees according to species such as Pant lemon-1 (66-70 DAL), Kinnow (82-87 DAL) and Grapefruit (82-87 DAL) and transplanted in polybags for further study. The observations on survival percentage of air layers in root trainer were recorded after one and two months of transplanting and percent germination was calculated. After removing the plant from the poly-bag, washed them by tap water without disturbing the roots. After that fresh weight of roots was assessed with the help of electronic weighing balance and express in gram (g). Dry weight of roots was recorded with the help of electronic weighing balance and expressed in gram (g). The data pertaining to various investigations on growth and development of air layering Plants were subjected to statistical analysis RBD as per method suggested by Gomez and Gomez (1984).

Result and discussion

The application of bioinoculant had a significant effect on survivability of air layers than untreated layered plat (Table 1). The maximum survival percentage (87.03%) was recorded in Pant lemon-1 when with DPC12+DPC9 (T_2) followed by 85.18% survivability of air layers with DPB12+DPB16 (T_3) after one month of planting in poly-bag. While the minimum survival percentage was recorded in the grapefruits under T_1 (61.10%) followed by 75.92% with DPC12+DPC9. Application of plant growth promoting bioinoculants (PGPB) levels showed non-significant influence on survival percentage of air layers. Among all citrus species, Pant lemon-1 recorded the maximum survival percentage of air layers (82.71%), while the minimum survival percentage (68.47%) was noted in Grapefruit after one month of planting in poly-bag. It may be attributed to the inoculation of PGPR *viz*., *Ochrobactrum anthropi*, *Pseudomonas fluorescens*, *Pseudomonas palluonia*, and *Psudomonas* species exudates plant growth hormones which are vital for root initiation, healthy growth and higher survivability of roots. Similar results were obtained by Aseri *et al.* (2008) [2] The maximum survival percentage (81.47%) was observed in Pant lemon-1 when air layering was done with bioinoculant *i.e.* DPC12+DPC9 (T_2) followed by T_3 (77.75%). On the other hand, when air layering was done without any inoculant, it recorded minimum survival percentage (51.84%) in grapefruit followed by kinnow (62.95%) after two months of planting in poly-bag. Among all *citrus species*, Pant lemon-1 recorded the maximum survival percentage of air layers (75.29%), while the minimum survival percentage (61.10%) was recorded in Grapefruit after two months of planting in poly-bag. Application of plant growth promoting bioinoculants (PGPB) levels showed non-significant influence on survival percentage of air layers. Similar results were obtained by Aseri *et al.* (2008) [2]. Bioinoculant has a great role in promoting better uptake of water and nutrients by the plant and hence improving survival percentage (Kumar and Syamal, 2005; Cakmakci *et al.*, 2006) [10, 4]. Data in the table 3 indicated that there was significant variation in fresh weight of roots among treatments and species. The maximum fresh weight of roots (7.93 g) was recorded in Pant lemon-1 when air layering was performed with inoculation DPC12+DPC9 (T_2) closely followed by inoculation DPB15+DPB16 (T_3) 7.06 g fresh weight of root after two months of planting in poly bag. While the minimum fresh weight was recorded when layering was performed without any inoculation treatment in grapefruit (2.18 g) followed by kinnow (2.39 g). Application of plant growth promoting bioinoculants (PGPB) levels showed significant influence on fresh weight of roots after two months of planting in poly bag. The data in the table 1 revealed that the dry weight of roots of air-layered plant was significantly influenced with plant bioinoculants and citrus species. The maximum dry weight of roots (2.13g) was observed in Pant lemon-1air when layering treated with bioinoculant DPC12+DPC9 (T_2) followed by T_3 *i.e.* DPB15+DPB16 1.98 g after two months of planting in poly bag. Application of plant growth promoting bioinoculants (PGPB) levels showed significant influence on dry weight of roots. On the other hand, air layering done without any treatment recorded minimum dry weight of roots in grapefruit (0.78 g) closely followed by in Kinnow (106 g) after two months of planting in poly bag in descending order. It may be due to the production of plant growth promoting substance by beneficial microorganisms such as IAA production, inhibition of ethylene synthesis and mineralization of nutrients which leads to production of more roots (Erturk *et al.*, 2010) [5]. Similarly, Al-Karaki *et al.* (2013) [1] evaluated the response of sour orange (*Citrus aurantium L.*) seedlings to inoculation with *Pseudomonas species* and it enhanced the plant dry weight and root dry weight 34.4% and 20.2% over the non-inoculated plants, respectively.

Table 1: Effect of bioinoculants on survival percentage of air layer after 1 month of planting in poly bag

Bioinoculants treatments	Survival percentage after one month of planting in poly-bag		
	Pant lemon-1	Kinnow	Grapefruit
T_1	75.92	72.21	61.10
T_2	87.03	83.33	75.92
T_3	85.18	81.47	68.51
SEm±	4.10	5.42	4.74
CD at 5%	NS	NS	NS

Table 2: Effect of bioinoculants on survival percentage of air layer after 2 months of planting in poly bag

Bioinoculants treatments	Survival percentage after two months of planting in poly-bag		
	Pant lemon-1	Kinnow	Grapefruit
T_1	66.66	62.95	51.84
T_2	81.47	74.07	66.66
T_3	77.75	72.21	64.81
SEm±	4.74	4.81	3.98
CD at 5%	NS	NS	NS

Table 3: Effect of bioinoculants on fresh weight of roots after 2 months of planting in poly bag

Bioinoculants treatments	Fresh weight of roots (g) after two months of planting in poly-bag		
	Pant lemon-1	Kinnow	Grapefruit
T_1	4.73	2.39	2.18
T_2	7.93	4.33	3.99
T_3	7.06	3.41	2.92
SEm±	0.13	0.10	0.08
CD at 5%	0.39	0.30	0.22

Table 4: Effect of Bioinoculants on dry weight of roots after 2 months of planting in poly bag

Bioinoculants treatments	Dry weight of roots (g) after two months of planting in poly-bag		
	Pant lemon-1	Kinnow	Grapefruit
T_1	1.26	1.06	0.78
T_2	2.13	1.93	1.74
T_3	1.98	1.55	1.24
SEm±	0.01	0.02	0.01
CD at 5%	0.02	0.05	0.03

Conclusion

From the above results, it can be concluded that best results were obtained with *Pseudomonas species* + *Ochrobactrum anthropi* (DPC12+DPC9) in Pant Lemon -1, followed by Kinnow and grapefruit. Hence, this treatment can be recommended for improving the propagation of Pant Lemon -1, Kinnow and grapefruit through air layering.

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