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Influence of plant growth promoting bioinoculants on root development of different *Citrus species*

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Abstract
Plant growth promoting bioinoculants have beneficial properties to increase plant growth. The present experiment was carried out at HRC, Patharchatta, Department of Horticulture, G.B. Pant University of Agriculture and Technology, Pantnagar to study the effect of bioinoculants on rooting of different citrus species. *Citrus species viz*., Pant lemon-1, kinnow and Grapefruit were selected for the experiment. PGPR species *viz*., *Pseudomonas species, Ochrobactrum anthropi* (DPC12+DPC9), *Pseudomonas fluorescens* and *Pseudomonas palluoniana* (DPB15+DPB16) were used in the study. They were applied in each air layer in all three citrus species under proposed treatments used for preparation of air layers. Among all citrus species, Pant lemon-1 recorded the minimum days taken to root initiation (25.66 days), maximum average number of roots (33.90), maximum average length of roots (7.66 cm), maximum fresh weight of root (3.26 g) and the maximum dry weight of root (0.97 g) as compared to Kinnow and Grapefruit.

Keywords: Citrus, bioinoculants, air layering, pant lemon

Introduction
Citrus is an important sub-tropical fruit tree belongs to family Rutaceae with chromosome number (2n=18) and believed to have originated in China. Citrus is the most valuable fruit crops since antique and known as a good source of vitamin-C with high antioxidant potential. India ranks sixth in citrus producing counties in the world. In India mostly citrus growing states are Andhra Pradesh, Maharashtra, Haryana, Karnataka, Uttar Pradesh, Punjab, Meghalaya, Uttarakhand, Himanchal Pradesh, Arunachal Pradesh, Rajasthan, Orissa, Assam and Tripura. 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. It is an asexual propagation method that is best suited for plants that do not perform well with conventional layering. Generally layering is a comparatively easy and convincing method of propagation in citrus species. Air layering is advantageous for producing large sized plants in compressed time. Quality of root formation in air layers depends upon certain factors. Among these factors application of bioinoculants provide successful propagation of citrus plants. The use of various bioinoculants increase percentage of success by easy rooting formation in plants, where vegetative propagation is not easy. Plant growth promoting bioinoculants like *Pseudomonas species, Ochrobactrum anthropi, Pseudomonas fluorescens* and *Pseudomonas palluonia* have beneficial properties to increase plant growth because they are ACC deaminase producer, Siderophore producer, Phosphorus solubilizer, Ammonia producer and Nitrogen fixers which change the morphology of plant. The response of *Citrus species* varies to different treatments of plant growth promoting bioinoculants and change in physiological and environmental conditions of plant. Bioinoculants help in callus formation, root initiation, root development and survival percentage of air layers. Microorganisms are bio resources that may become potential mechanism for providing massive benefits in agriculture and they are the key elements for plant formation under nutrient-inequality conditions. Constructive soil microbes can help to enhance the growth of plant, nutrition and competitiveness and plant acknowledgment to external stress factors by an array of mechanisms (Vessey, 2003; Lucy *et al*., 2004) [7, 3]. They can also constrain soil-borne plant pathogens and activate plant resistance. Requena *et al*. (1997) [6] have verified that the ultimate benefit to the plant host arises from native plant beneficial microbes like Plant growth-promoting rhizobacteria (PGPR) compared with commercial or introduced forms.

The beneficial effect of Plant growth promoting bioinoculants is to enhance nutrients uptake in host plant, particularly phosphorus (P) and other micronutrients (Al-Karaki, 2002) [1]. Bioinoculants absorbs inorganic P either from the soluble P pools in the soil or from insoluble forms such as rock phosphate as well as from insoluble organic sources. Therefore, the present research was carried out to study the effect of bioinoculants on rooting of different citrus species.

Material and Methods

The present was carried out at Horticulture research centre, Patharchatta, Department of Horticulture, G.B. Pant University of Agriculture and Technology, Pantnagar, district U.S. Nagar (Uttarakhand). *Citrus species viz*., Pant lemon-1, kinnow and Grapefruit were selected for the experiment. Sphagnum moss grass was taken from HRC, Pattarchatta. Moss grass is generally preferred over other material used in air layering which gives a satisfactory result. Before use of sphagnum moss grass, all the unwanted materials were removed from the sphagnum moss grass and then washed with tap water followed by sun drying. After sun drying, moss grass was filled in autoclavable polythene bag for autoclaving. Autoclaving was carried out in an autoclave (Model No.YSU-405, Horizontal Cylindrical Autoclave) at 121^0C with 15 pound-force per square inch (PSI) for 15 min. Bioinoculants (DPC12+DPC9 and DPB15+DPB16) were collected from Rhizosphere Laboratory, Department of Biological Sciences, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar. PGPR species *viz*., *Pseudomonas species* and *Ochrobactrum anthropi* (DPC12+DPC9) and *Pseudomonas fluorescens* and *Pseudomonas palluoniana* (DPB15+DPB16) were used in the study. They were given for each air layer under proposed treatments used for preparation of air layers. For air layering preparation, healthy and terminal branches, which received good sunshine with thickness 2.5-3 cm was selected. During preparation of air layers, a ring of bark measuring 3 cm in length was removed about 45 cm below the shoot apex. The cambium layer was rubbed off and woody portion was exposed. A layer of moist sphagnum moss treated with DPC12+DPC9 and DPB15+DPB16 inoculants were wrapped with a piece (20x25 cm) of 300 gauge transparent white polythene sheet at the exposed woody portion on the selected branch. After wrapping, the polythene was tied properly at both ends with sutli to ensure supply of proper moisture from moist moss grass to facilitate the development of roots. Root initiation was observed from air layers of *Citrus species*. The air layers were detached from mother tree carefully by the help of secateurs, without damaging to the root system. The number of roots were counted and reported on mean basis. The length of root was measured from base to top, and average length per layer was worked out by dividing the total length of roots by the total number of roots in each treatment. Whole roots were detached from the air layers of *Citrus species*, washed in tap water and then fresh weight of root were recorded with the help of electric balance after cutting of roots and average fresh-weight/air-layer was calculated. After taking fresh weight of roots, they were kept in brown paper bags separately and put in oven for drying at 60 ± 2^0C temperature for 48 hrs. The dry weight of root was recorded with the help of electric balance after dryness of roots. And average dry-weight/air-layer was calculated. The number of rooted air-layers was counted after detachment of air-layered twigs from the mother plants after operation. The data were compiled and success in rooting percentage was calculated.

Result and Discussion

Root Initiation

The effect of DPC12+DPC9 and DPB15+DPB16 inoculation on days taken to root initiation is presented in Table 1. The minimum days for root initiation (21.66 days) by Pant lemon-1air layers was observed in those air layers, which were treated with T_2 (DPC12+DPC9) followed by T_3 (25.61 days). In Kinnow, application of T_2 (DPC12+DPC9) recorded the minimum days (40.88) for root initiation followed by T_3 (42.22 days) and T_1 (45.66). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the minimum days (61) for root initiation followed by T_3 (42.22 days) and T_1 (45.66). Among all citrus species, Pant lemon-1 recorded the minimum days for root initiation (25.66 days), while the maximum days taken to root initiation (63.49 days) was recorded in Grapefruit. Root exudates are natural sources of tryptophan for the rhizosphere microflora, which enhance auxin biosynthesis in the rhizosphere (Martens and Frankenberger, 1994). Lucy *et al*. (2004) [3] found that IAA-producing PGPR increase root growth and root length resulting in greater root surface area, which enables the plant to access more nutrients from soil. In the present investigation, inoculation of PGPR *viz*., *Pseudomonas species* and *Ochrobactrum anthropi* (DPC12+DPC9) gave best result and *Pseudomonas fluorescens* + *Pseudomonas palluonia* (DPB15+DPB16) found better as compare to control. This effect might be due to production of IAA by these PGPRs, which increase root growth and early emergence of root.

Number of Root per Air Layer

Application of plant growth promoting bioinoculants (PGPB) levels showed significant influence on average number of root. The maximum average number of roots (38.66) by Pant lemon-1air layers was observed in those air layers, which were treated with T_2 (DPC12+DPC9) followed by T_3 (32.72 roots). These treatments were better over control which recorded 30.33 roots. In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum roots (31.33), followed by T_3 (28.22 roots) and T_1 (24.83) roots. In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum roots (28), followed by T_3 (24.88 roots) and T_1 (20.61) roots. Among all citrus species, Pant lemon-1 recorded maximum average number of roots (33.90), while the minimum average number of roots (24.49) was recorded in Grapefruit.

Table 1: Effect of bioinoculants on root initiation and number of roots per air layer in citrus species

Bioinoculants treatments	Days taken to root initiation			Average number of roots per air layer		
	Pant lemon-1	Kinnow	Grapefruit	Pant lemon-1	Kinnow	Grapefruit
T_1	29.72	30.33	30.33	30.33	24.83	20.61
T_2	21.66	38.66	38.66	38.66	31.33	28.00
T_3	25.61	32.72	32.72	32.72	28.22	24.88
SEm±	0.70	0.34	0.34	0.34	0.43	0.36
CD at 5%	2.03	0.98	0.98	0.98	1.25	1.05

Average length of roots (cm) per air layer

The data regarding average length of roots in air-layering indicates that the maximum average length of roots (8.47 cm) by Pant lemon-1air layers was observed in those air layers which were treated with T_2 followed by T_3 (7.75 cm). These treatments were better over control which recorded 6.78 cm length of roots. In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum length of root (7.83 cm) followed by T_3 (7.03 cm) and T_1 (5.42 cm). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum length of root (4.09 cm) followed by T_3 (3.25 cm) and T_1 (3.07 cm). Among all citrus species, Pant lemon-1 recorded the maximum average length of roots (7.66 cm), while the minimum average length of roots (3.47 cm) was recorded in Grapefruit. The effect of bioinoculants on average length of roots, studied by Mia *et al*.(2010) [5] found increase in root growth like root length (33-34%), due to PGPR inoculation in banana plantlets planted in nitrogen free hydroponics condition.

Fresh weight of roots

The data indicated that there was significant variation in fresh weight of roots among treatments and species (Table 2). The maximum fresh weight of roots (4.05g) by Pant lemon-1air layers was observed in those air layers which were treated with T_2 (DPC12+DPC9), followed by T_3 (3.71 g). These treatments were better over control, which recorded 2.05g fresh weight of roots. In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum fresh weight of roots (2.20 g) followed by T_3 (1.74 g). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum fresh weight (1.98 g) followed by T_3 (1.33 g) and T_1 (1.05 g). Among all *citrus species*, Pant lemon-1 recorded the maximum fresh weight of root (3.26 g), while the minimum fresh weight of root (1.45 g) was recorded in Grapefruit. In the present investigation, inoculation of PGPR *viz*., *Ochrobactrum anthropi*, *Pseudomonas fluorescens* and *Pseudomonas palluonia* gave best result as compare to control. This effect might be due to production of phosphorus and IAA by these PGPRs which increase on fresh weight of roots.

Table 2: Effect of bioinoculants on root length and fresh weight of roots in citrus species

Bioinoculants treatments	Average length of roots (cm)			Fresh weight of roots (g)		
	Pant lemon-1	Kinnow	Grapefruit	Pant lemon-1	Kinnow	Grapefruit
T_1	6.78	5.42	3.07	2.05	1.13	1.05
T_2	8.47	7.83	4.09	4.05	2.20	1.98
T_3	7.75	7.03	3.25	3.71	1.74	1.33
SEm±	0.15	0.14	0.11	0.10	0.06	0.03
CD at 5%	0.45	0.42	0.31	0.30	0.18	0.08

Dry weight of roots (g)

The maximum dry weight of roots (1.19g) by Pant lemon-1air layers was observed in those air layers which were treated with T_2 (DPC12+DPC9) followed by T_3 (1.04 g). These treatments were better over control, which recorded 0.71g dry weight of roots. In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum dry weight of roots (1.01 g) followed by T_3 (0.97 g). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum (0.96 g) dry weight followed by T_3 (0.57 g) and T_1 (0.29g). Among all *citrus species*, Pant lemon-1 recorded the maximum dry weight of root (0.97 g), while the minimum dry weight of root (0.60 g) was recorded in Grapefruit. Khalid *et al*. (2004) demonstrated increase in the elongation of root up to17.3%, root dry weight (up to 13.5%) due to inoculation with PGPR.

Survival percentage of air layers on mother tree

Survival percentage of *Citrus species* air layers on mother tree varied significantly over control. The maximum survival percentage (94.44%) by Pant lemon-1air layers was observed in those air layers which were treated with T_2 (DPC12+DPC9) followed by T_3 (90.73%) survivability of air layers. In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum (88.88%) survivability of air layers followed by T_3 (83.32%) and T_1 (77.77%). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum survivability (81.47%) followed by T_3 (75.92%) and T_1 (68.51%). Among all citrus species, Pant lemon-1 recorded the maximum survival percentage of air layers (88.88%), while the minimum survival percentage (75.30%) was recorded in Grapefruit.

Table 3: Effect of bioinoculants on dry weight of roots and survival percentage in citrus species

Bioinoculants treatments	Dry weight of roots (g)			Survival percentage (%)		
	Pant lemon-1	Kinnow	Grapefruit	Pant lemon-1	Kinnow	Grapefruit
T_1	0.71	0.44	0.29	81.47	77.77	68.51
T_2	1.19	1.01	0.96	94.44	88.88	81.47
T_3	1.04	0.97	0.57	90.73	83.32	75.92
SEm±	0.05	0.04	0.03	5.72	6.90	8.44
CD at 5%	0.13	0.11	0.10	NS	NS	NS

Conclusion

The above study reveals that bioinoculant *Ochrobactrum anthropi* (DPC12+DPC9) can be successfully used in citrus species particularly in Pant lemon for maximum rooting and its survival.

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