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Effect of plant growth promoting bioinoculants on morphological parameters of air layers in different citrus species

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Abstract
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 plant growth promoting bioinoculants on morphological parameters 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. Among all citrus species, Pant lemon-1 recorded the minimum days for leaf initiation (23.69 days), maximum average number of leaves (21), maximum number of branches (7.25) and maximum plant height (37.79 cm) as compared to Kinnow and Grapefruit after the application of plant growth promoting bioinoculants.

Keywords: Citrus, PGPR, bioinoculant, air layering

Introduction
Citrus is one of the important sub-tropical fruit crop which is grown globally. India ranks sixth in citrus producing counties in the world. Citrus species like Pant lemon-1, Kinnow, and Grapefruit are mainly propagated by air layering. 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 increase percentage of success by easy rooting formation in plants, where vegetative propagation is not easy. 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. Plant growth promoting rhizobacteria are the soil bacteria inhabiting around the root surface and are directly or indirectly involved in promoting plant growth and development like production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally plant growth promoting rhizobacteria (PGPR) facilitate the plant growth directly by either assisting in resource acquisition (phosphorus, nitrogen and essential minerals) or modulating plant hormone levels, directly or indirectly by decreasing the inhibitory effects of various pathogens on to the plant growth and development in the forms of bio-control agents. Bacteria that colonize the rhizosphere enhance plant roots and plant growth by any mechanism is revered to as plant growth-promoting rhizobacteria (PGPR) (Dey *et al*., 2004, Herman *et al*., 2008) [3, 8]. 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 morphological parameters of different citrus species.

Material and Methods
The present was carried out at HRC, Patharchatta, 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 and sun dried. After sun drying, moss grass was filled in autoclavable polythene bag for autoclaving. 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. Sprouting of new leaves was observed in air layers after planting in poly-bags. After that, days to initiation of new leaves were counted from the date of planting in poly-bag and then reported on mean basis. Numbers of leaves of *Citrus species* were counted at 30 days after planting of air-layers from field by proper supervision. Numbers of branches were counted at 30 days after planting of air-layers in field of different species of citrus. Plant height was measured with the help of meter scale and measured in centimeter (cm).

Result and Discussion

Days taken to new leaf initiation

The data pertaining to the effect of DPC12+DPC9 and DPB15+DPB16 inoculation on days taken to new leaf initiation is presented in Table 1. The minimum days taken to leaf initiation (20.38 days) by Pant lemon-1 was observed in air layers, which were treated with T_2 (DPC12+DPC9) followed by T_3 (23.27 days). In Kinnow, application of T_2 (DPC12+DPC9) recorded minimum days (22.88) for leaf initiation followed by T_3 (23.66 days) and T_1 (29.50 days). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the minimum days (24.77) for leaf initiation followed by T_3 (25.66 days) and T_1 (30.94 days). Among all *citrus species*, Pant lemon-1 recorded the minimum days for leaf initiation (23.69 days), while the maximum days for leaf initiation (27.12 days) was recorded in Grapefruit. Janos *et al*. (2001) [9] found that PGPR treated plants shows improved leaflet expansion as early as 120 days after inoculation and subsequently enhanced height, growth and leaf production. In present investigation inoculation of PGPR *viz*., *Ochrobactrum anthropi*, *Pseudomonas fluorescens*, *Pseudomonas palluonia* and *Psudomonas species* reduced the days to new leaf initiation. This effect might be due to ammonia producing ability of PGPR *viz*., *Pseudomonas palluonia* and *Ochrobactrum anthropic*; whereas, *Pseudomonas palluonia*, *Ochrobactrum anthropi* and *Pseudomonas fluorescens* are Nitrogen fixers.

Average number of leaf appear per plant

The maximum number of leaves (25.72) in Pant lemon-1 was observed under T_2 (DPC12+DPC9) followed by T_3 (22.88 leaves). In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum leaves (17.05) followed by T_3 (14.66 leaves) and T_1 (11.50 leaves). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum leaves (18.50) followed by T_3 (15.61 leaves) and T_1 (9.61 leaves). Among all citrus species, Pant lemon-1 recorded the maximum average number of leaves (21), while the minimum average number of leaves (14.42) was recorded in Grapefruit. Similar finding were observed by Chawla and Mehta (2015) [2].

Table 1: Effect of bioinoculants on leaf initiation and leaf appear per air layer in citrus species

Bioinoculants treatments	Days taken to new leaf initiation			Average number of leaf appear per plant		
	Pant lemon-1	Kinnow	Grapefruit	Pant lemon-1	Kinnow	Grapefruit
T_1	27.44	29.50	30.94	14.38	11.50	9.16
T_2	20.38	22.88	24.77	25.72	17.05	18.50
T_3	23.27	23.66	25.66	22.88	14.66	15.61
SEm±	0.30	0.26	0.28	0.40	0.30	0.37
CD at 5%	0.89	0.74	0.81	1.15	0.85	1.08

Average number of branches appears per plant

Application of plant growth promoting bioinoculants (PGPB) levels showed significant influence on average number of branches. The maximum number of branches (8.72) in Pant lemon-1 was observed under T_2 (DPC12+DPC9) followed by T_3 (8 branches). In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum branches (6.88) followed by T_3 (6.11 branches) and T_1 (4.21 branches). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum branches (5.33) followed by T_3 (6.97 branches) and T_1 (3.38 branches). Among all citrus species, Pant lemon-1 recorded the maximum number of branches (7.25), while the minimum number of branches (4.53) was recorded in Grapefruit. Karakurt and Aslantas (2010) [10] revealed that plant-growth promoting bacteria are important in managing plant growth because of their effects on soil conditions and observed bacteria applications significantly affected number of annual shoots.

Plant height (cm)

The average plant height per air-layer was found to deviate significantly due to treatments of various plant growth promoting bioinoculants. Plant growth promoting bioinoculants significantly increased the plant height as compared with control. The maximum average height (42.16 cm) in Pant lemon-1air was observed in T_2 (DPC12+DPC9) followed by T_3 (40.66 cm). In Kinnow, application of T_2 (DPC12+DPC9) recorded the maximum plant height (32.83 cm) followed by T_3 (30.16 cm) and T_1 (25.88 cm). In Grapefruit, application of T_2 (DPC12+DPC9) recorded the maximum plant height (26.88 cm) followed by T_3 (24.22 cm) and T_1 (21.50 cm). Among all *citrus species*, Pant lemon-1 recorded the maximum plant height (37.79 cm), while the minimum plant height (24.20 cm) was recorded in Grapefruit.

Table 2: Effect of bioinoculants on number of branches and plant height per air layer in citrus species

Bioinoculants treatments	Average number of branches appear per plant			Plant height (cm) of air layers		
	Pant lemon-1	Kinnow	Grapefruit	Pant lemon-1	Kinnow	Grapefruit
T_1	5.05	4.21	3.38	30.55	25.88	21.50
T_2	8.72	6.88	5.33	42.16	32.83	26.88
T_3	8.00	6.11	4.88	40.66	30.16	24.22
SEm±	0.24	0.24	0.23	0.47	0.37	0.27
CD at 5%	0.69	0.70	0.67	1.34	1.07	0.77

Conclusion
From the above study, it can be concluded that bioinoculant *Ochrobactrum anthropi* (DPC12+DPC9) can be successfully used in citrus species for increasing number of leaves, number of branches and plant height.

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