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PGPR in managing root rot disease and enhancing growth in mandarin (*Citrus reticulata* Blanco.) seedlings

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ABSTRACT

Decline in general plant-health and fruit production in mandarin influenced by abiotic and biotic factors is a major threat to cultivars grown in Darjeeling and Sikkim hills. *Fusarium* root rot, caused by *F. oxysporum*, is one of the most serious diseases afflicted during early plant growth stage in *Citrus*. To address this, seven PGPR isolates - *Pseudomonas poae* (RMK03), *Bacillus stratosphericus* (RHS/CL-01), *Ochrobactrum anthropi*, *Paenibacillus lentimorbus*, *Bacillus pumilus*, *Bacillus megaterium* and *Bacillus amyloliquefaciens* were isolated from the rhizosphere of *Citrus reticulata*, *C. limonia* and *Camellia sinensis*, and used for evaluating their effect on growth of mandarin seedlings. *Pseudomonas poae* showed *in vitro* antagonism to *Fusarium oxysporum*. Better growth enhancement was noticed with *P. poae*, *B. stratosphericus*, *O. anthropi* and *B. pumilus*. Enhanced activity of chlorophyll, total protein, phenol, four major defense enzymes-chitinase, β-1,3-glucanase, peroxidase and phenylalanine ammonia lyase was observed upon application of PGPR. *P. poae* also suppressed root rot caused by *Fusarium oxysporum*. Use of PGPR, which promote growth besides reducing disease severity to some extent, may lead to use of eco-friendly approaches for controlling plant diseases.

Key words: PGPR, mandarin, root rot, *Citrus*, bacterial isolates

INTRODUCTION

Mandarin (*Citrus reticulata* Blanco.) is one of the most important fruit crops of Darjeeling and Sikkim hills. Economy of the farmers in this region is fully dependent on good, disease-free fruit production. However, several abiotic and biotic factors pose a threat to the plants, causing rapid decline in plant health resulting in decreased fruit production. Several diseases in citrus are attributed to soil-borne fungi. *Fusarium* root rot, caused by *F. oxysporum*, is one of the serious diseases in this crop. Soil application of *Bacillus subtilis* and *Streptomyces griseoviridis* has been reported to control root rot disease (Nemec *et al*., 1996; Ziedan and Eman, 2002). This has led to delivery of PGPR strains to the soil to improve population dynamics of the augmented bacterial antagonists and to suppress establishment of pathogenic microbes in horticultural crops (Esitken, 2011).

Intensive interactions take place in the rhizosphere among soil, microorganisms and microfauna which may affect plant growth and yield positively or negatively (Antoun and Prevost, 2006). It has been proved that root inoculation and/or spraying with PGPR results in increased germination, seedling emergence, and, modified growth and yield in tomato (Mayak *et al*, 2004; Woitke *et al*, 2004), lettuce (Han and Lee, 2004; Barassi *et al*, 2006), radish (Yildirim *et al*, 2008a, b) and strawberry (Karlidag *et al*, 2011). Growth stimulation in plants by PGPR application may be a direct mechanism set-in by the production of secondary metabolites such as phytohormones, riboflavin and vitamins (Dakora, 2003), or, through improved nutrient availability (Glick, 1995; Chabot *et al*, 1996a; Yanni *et al*, 1997). Three commercial bio-fertilizers: phosphorein (phosphate dissolving bacteria - *Bacillus megaterium* var. *Phosphatircum*), microbien (*Azotobacter* spp.) and Cerealien (nitrogen-fixing Cyanobacteria) controlled dry root-rot disease

of citrus caused by *Fusarium solani* (Mart.) Snyd & Hans, and improved the yield quality in mandarin (Mohamedy and Ahmed, 2009). Enhanced plant nutrition effected by PGPR is mainly through increased phosphorus uptake, by solubilization of inorganic phosphate or by mineralization of organic phosphate. Further, indirectly, PGPR act as biocontrol agents to reduce disease severity, or these stimulate other beneficial symbioses, or, protect the plant by degrading xenobiotics in contaminated soils (Jacobsen, 1997). More importantly, such PGPR may also induce systemic resistance in the host (ISR), thereby protecting plants against pathogen attack. This study aimed to understand the effect of PGPR in controlling root rot disease of mandarin caused by *F. oxysporum* using biochemical analysis during disease suppression.

MATERIAL AND METHODS

Test organisms

Plant growth promoting rhizobacteria (PGPR): *Pseudomonas poae* (RMK03), isolated from the rhizosphere of *Citrus reticulate*; *Bacillus stratosphericus* (RHS/CL-01), isolated from the rhizosphere of *Citrus limonia*; and, *Ochrobactrum anthropi*, *Paenibacillus lentimorbus*, *Bacillus pumilus*, *Bacillus megaterium* and *Bacillus amyloliquefaciens*, isolated from the rhizosphere of *Camellia sinensis*, were identified on the basis of morphological, microscopic and biochemical tests, and 16S rDNA sequencing.

Fungal pathogen: *F. oxysporum* (RHS/M534), causing root rot of mandarin, was obtained from the rhizosphere of *C. reticulata*. BLAST query of the 18S rDNA sequence of *F. oxysporum* against GenBank database confirmed its identity.

Morphological and scanning electron microscopic (SEM) studies: Pure cultures of the three isolates were streaked on Nutrient Agar (NA) plates for colony development. Individual colonies were examined for shape, size, structure and pigmentation. Gram-specific reactions of these isolates were recorded as per Buchanan and Gibbson (1974). Gram positive/negative reaction and the shape of cells was recorded. SEM view of all the seven isolates was also recorded.

Genomic DNA isolation and 16S rDNA amplification by PCR

Extraction of genomic DNA was done from 24h old culture as per Stafford *et al* (2005) with modification. This was followed by spectrophotometric quantication using a Coleparmer UV–VIS Spectrophotometer and, quality was analyzed on 0.8% agarose gel. For PCR amplication, DNA was amplied by mixing the template DNA (50ng) with polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction (PCR) was performed in a total volume of 100µl, containing 78µl deionized water, 10µl 10x Taq polymerase buffer, 1µl 1U Taq polymerase enzyme, 6µl of 2 mM dNTPs, 1.5µl of 100 mM forward (50-AGAGT RTGATCMTYGCTWAC-30) and reverse (50-CGYTAMC TTWTTACG RCT-30) primers, and 3.5µl of 50ng template DNA. PCR programming was as follows: initial denaturing at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 60s, annealing at 59°C for 60s and extension at 70°C for 2 min, and final extension at 72°C for 7 min, in a Primus 96 advanced gradient Thermocycler. The PCR product (20µl) was mixed with a loading buffer (8µl) containing 0.25% bromophenol blue, 40% w/v sucrose in water, and then loaded onto 2% Agarose gel with 0.1% ethidium bromide for examination under horizontal electrophoresis. The PCR product was sent for sequencing to Chromous Biotech, Bengaluru, India.

16S rDNA sequence and phylogenetic analysis

The 16S rDNA sequences obtained from PCR products were analyzed by NCBI-BLAST and aligned with ex-type isolate sequences from NCBI GenBank for identication.

Evaluation of PGPR activity with bacterial isolates *in vitro*

Phosphate solubilization: Primary phosphate solubilizing of all eight isolates was carried out by allowing the bacteria to grow on a selective medium, i.e., Pikovskaya's agar (Pikovskaya, 1948). Appearance of a transparent halo around the bacterial colony indicated phosphate solubilizing activity of the bacterium.

Siderophore production: Production of siderophore was detected by the standard method of

Schwyn and Neiland (1987), using the blue indicator chrome azurol S (CAS).

IAA production: For detection and quantification of IAA, selected bacterial cells were grown for 24h to 48h on high C/N ratio medium. Tryptophan (0.1mM) was added to enhance indole acetic acid (IAA) production. IAA in the culture supernatant was assayed by Pillet-Chollet method (Dobbelaere *et al*, 1999).

Plant growth promotion

One-year-old mandarin seedlings were selected and maintained in 12˝ earthenware pots under open condition. Field-grown plants were watered regularly for maintenance. Growth promotion was studied in terms of increase in plant height and number of leaves. Observations were recorded at 4 and 8 months from application of the seven different bacterial strains.

In vitro antagonism

F. oxysporum was paired with *P. poae* on solid medium as per Chakraborty and Chakraborty (1989).

Inoculum preparation and application

Plant Growth Promoting Rhizobacteria: Initially, bacteria were cultured on Nutrient Broth medium (Himedia, M002-100G, ingredients: peptic digest of animal tissue 5.00g/litre, sodium chloride 5.00g/l, beef extract- 1.50g/l, yeast extract- 1.50g/l, final pH at 25°C 7.4±0.2), and allowed to grow with shaking at 37°C at 120rpm for 48h. At the end of the log phase, bacterial cultures were centrifuged at 10,000rpm for 15 min and the bacterial pellet collected. Bacterial aqueous suspension was prepared using distilled water as per requirement, to maintain a bacterial concentration of 10^8 c.f.u./ml. The aqueous suspension was then applied as foliar spray and soil-drench @ 100 ml/plant to the rhizosphere of one-year-old mandarin plants. Application of suspension was done every month, repeated in three replications.

Fungal pathogen: The pathogen (*F. oxysporum*) was grown in sand-maize meal medium (maize meal:sand:water 1:9:1 w:w:v) in autoclaved plastic bags (sterilized at 20lb pressure for 20 min) for a period of three weeks at 28ºC, until the mycelium completely covered the substrate. Mandarin seedlings were inoculated by adding 100g of previously prepared inoculum of *F. oxysporum* to the rhizosphere soil.

Disease assessment

Disease assessment was done as per Chakraborty *et al* (2006) at 15, 30 and 45d after inoculation. Disease intensity was assessed as root-rot index on a scale of 0-6, depending on underground as well as above-ground symptoms, as follows: 0=no symptom; 1=small roots turn brownish and start rotting; 2=leaves start withering and 20-40% of roots turn brown; 3=leaves withered and 50% of roots affected; 4=shoot tips also start withering and 60-70% roots affected; 5=shoots withered, with defoliation of lower withered leaves, 80% roots affected; 6=whole plants die, with upper withered leaves still attached to the shoot; roots fully rotted.

Biochemical analysis

All the biochemical analyses were performed with treated as well as Control mandarin leaves and roots.

Enzyme assay

Peroxidase (POX, EC1.11.1.7): Extraction and assay of peroxidase was done as per Chakraborty *et al* (1993). Specific activity was expressed as an increase in ΔA_{465}/g tissue/min.

Chitinase (CHT, EC 3.2.1.14): Chitinase was extracted from mandarin leaves and assayed following Boller and Mauch (1988).

Phenylalanine Ammonia Lyase (PAL, EC 4.3.1.5): The enzyme was extracted and assayed as per Bhattacharya and Ward (1987). Enzyme activity was determined as the amount of cinnamic acid produced from L-phenyl alanine from 1g of tissue/min.

β-1,3- glucanase (β-GLU, EC 3.2.1.39): β-1,3-glucanase was extracted and assayed from leaf samples as per Pan *et al* (1991). Enzyme activity was expressed as µg glucose released /min/g tissue.

Phenolics: Phenols were extracted and estimated from leaf samples as per Mahadevan and Sridhar (1982). Quantification of total phenol and *o*-dihydroxy phenol was done using a standard of caffeic acid.

Chlorophyll

Total chlorophyll content was estimated by the method of Harborne (1973).

Protein

Soluble proteins were extracted and estimated by the method of Lowry *et al* (1951).

RESULTS AND DISCUSSION

Microscopic observations and identification of PGPR

Morphological observations of all the seven PGPR isolates showed that these were rod shaped and Gram +(ve) with the exception of RMK03 isolated from the rhizosphere of *Citrus reticulata*, which was rod shaped but Gram -(ve). All the bacilli of the group also produced endospores. Scanning electron micrographs also confirmed the structure of bacteria: *B. amyloliquefaciens*- larger, rod shaped (size 2µm), *B. pumilus* –rod shaped (size 2µm), *O. anthropi*- rod shaped (size 2µm), *P. lentimorbus*- rod shaped (size 2µm) and *B. megaterium* larger rod, shaped (size 2µm). Of the seven PGPR isolates, sequences of two isolates, RMK03 and RHS/CL-01, have been deposited with NCBI GenBank database, under accession Nos. KJ 917553.1 and KM 066950.1, for *Pseudomonas poae* and *Bacillus stratosphericus*, respectively. These two isolates, *Pseudomonas poae* and *Bacillus stratosphericus*, isolated from the rhizosphere of *Citrus reticulata* and *C. limoni*, respectively, were selected for the present study on the basis of their better response of phosphate solubilization and *in vitro* antagonism against *F. oxysporum*, causal agent of the root-rot disease in mandarin.

BLAST query of the 18S rDNA sequence of *F. oxysporum* (RHS/M534) against GenBank database confirmed its identity. The sequence has been deposited with NCBI GenBank database as accession no. KF952602.

In vitro PGPR activities

The seven bacterial strains were tested for different PGPR activity, as described already under Materials and Methods. All these seven isolates produced a clear halo zone of approx. 3cm dia in Pikovskaya's medium indicating, that, these could solubilize phosphate. Similarly, production of siderophore was confirmed by a change in colour of CAS from blue to yellow around the bacterial inoculum in the *petri* plate (Table 1).

Antagonistic effects of *P. poae* against *F. oxysporum*

Table 1. *In vitro* PGPR characteristics of bacterial isolates

Rhizosphere	PGPR isolate	Gen Bank Acc. No.	Phosphate solubilization	Characteristic siderophore production	IAA production
Citrus reticulata	*Pseudomonas poae* (RM-K-03)	KJ917553	+	+	+
Citrus limoni	*Bacillus stratosphericus* (CL-RH-01)	KM066950	+	+	+
Camellia sinensis	*Bacillus amyloliquefaciens* (TRS 6)	JN983127	+	+	+
	Ochrobactrum anthropi (TRS 4)		+	+	+
	Paenibacillus lentimorbus (TRS 5)		+	+	+
	Bacillus pumilus (BRHS/T382)	JQ765579	+	+	+
	Bacillus megaterium (TRS 7)	JX312687	+	+	+

+ = Activity present

P. poae was tested for its antagonistic effect on *F. oxysporum*. In paired culture, *P. poae* inhibited growth of *F. oxysporum* (Fig. 1).

Growth of mandarin seedlings

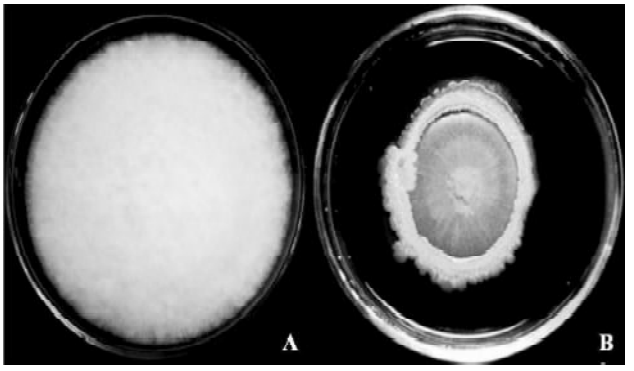


Fig 1. *In vitro* antagonistic effect of *P. poae* against *F. oxysporum* (A), *P. poae+F. oxysporum* (B)

Growth promotion was studied in terms of increase in plant height and number of leaves, in comparison to the Control. Marked by enhanced growth of one-year-old mandarin seedlings was observed with all the isolates, but, superior growth enhancement was noticed on application of *P. poae*, *B. stratosphericus*, *O. anthropi* and *B. pumilus*. Per cent increase in plant height and number of leaves in mandarin plants were recorded at 4th and 8th month after application of the seven isolates as soil drench and foliar spray (Fig. 2).

Effect of *P. poae* on root-rot of mandarin

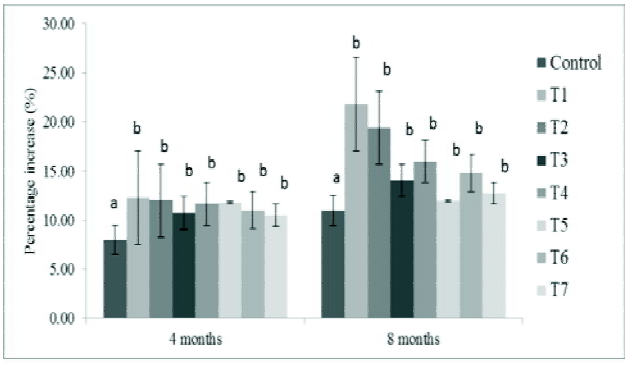


Fig 2. Effect of PGPR application on growth of mandarin seedlings at 4 and 8 months. T1- *P. poae*, T2- *B. statosphericus*, T3- *B. amyloliquefaciens*, T4- *O. anthropi*, T5- *P. lentimorbus*, T6- *B. pumilus* and T7- *B. megaterium* treated. Differences in height determined through t-test between Control and treatments significant at p=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

P. poae reduced significant root-rot (Table 2). Disease index value of root-rot in one-year-old mandarin saplings following the treatment with *F.oxysporum* at 15 days after inoculation was 2.3, whereas, this value fell to 0.6 in plants treated with *P. poae* and challenge-inoculated with *F.oxysporum*. Even at 45 days after inoculation, root-rot index value was low in *P. poae+F. oxysporum* treatment, in comparison to the plants inoculated with *F. oxysporum* alone.

Chlorophyll and phenol content

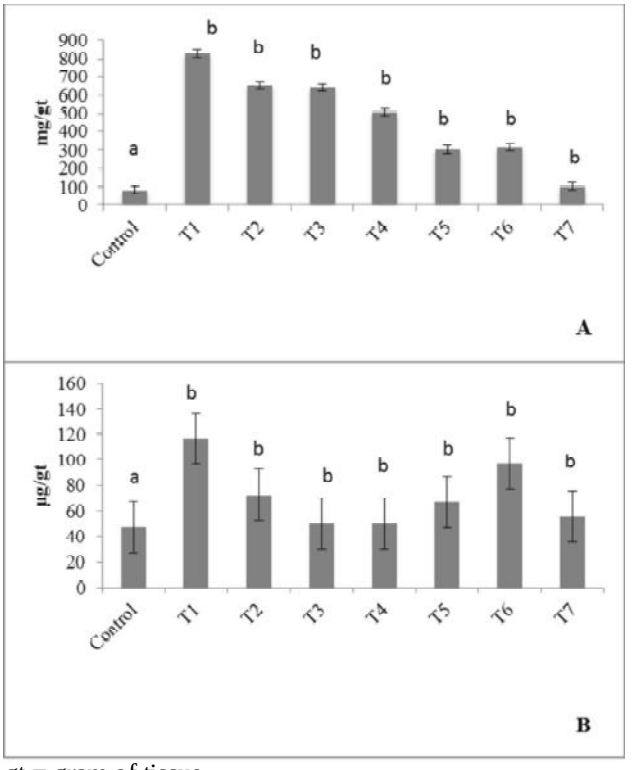
Table 2. Disease index for root rot incidence in one-year old mandarin saplings following treatment with *Pseudomonas poae* and pathogen challenge

Days of inoculation	Disease index	
	Root inoculated with *F. oxysporum* alone	Plant treated with *P. poae* and pathogen- *F. oxysporum*
15	2.3±0.145[a]	0.6±0.145[b]
30	3.4±0.115[a]	1.2±0.115[b]
45	5.9±0.057[a]	2.8±0.115[b]

Rot index: 0- No symptoms; 1- Small roots turn brownish and start rotting; 2- Leaves start withering and 20-40% of roots turn brown; 3- Leaves withered and 50% of roots affected; 4- Shoot tips also start withering; 60-70% roots affected; 5- Shoots withered with defoliation of lower (withered) leaves, 80% roots affected; 6- Whole plant dies, with upper withered leaves still attached to shoot, roots fully rotted

Average of three replicates; Differences between *F. oxysporum* treatment and *P. poae* + *F. oxysporum* treatment significant at p = 0.05 (Student's t-test)Difference between F. oxysporum and *P. poae* + *F. oxysporum* teatments significant at p = 0.05 (students t-test) where superscript (a, b) are different ; where seperate same, difference insignificant

Biochemical tests were performed to evaluate changes brought about by application of the PGPR isolates. Enhanced accumulation of chlorophyll and total phenol was observed in the treated seedling, as compared to the Control (Fig. 3).



gt = gram of tissue

Fig 3. Effect of application of rhizobacterial strains on total chlorophyll content (A) and total phenol content (B) in mandarin leaves. T1- *P. poae*, T2- *B. statosphericus*, T3- *B. amyloliquefaciens*, T4- *O. anthropi*, T5- *P. lentimorbus*, T6- *B. pumilus* and T7- *B. megaterium* treated; mg /gt =milligram/gm tissue; Differences in chlorophyll and phenol content determined through t-test between Control and treatment significant at p=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

Defense enzymes and protein

The activity of defense enzymes and protein on seedling are presented in Table 3. There was considerable increase in the activity of the defense enzymes, viz, PAL, POX (Fig. 4 A-B), CHT and β-1,3-GLU (Fig. 5 A-B), with all seven PGPR isolates. Significant increase in the activity of defense enzymes was observed in *P. poae* + *F. oxysporum* treatments in comparison to *P. poae* or *F. oxysporum* alone, or in Control plants (Fig. 6 A-B; Fig. 7 A-B). Chitinase content increased with increase in period of inoculation in both leaves and roots, whereas, peroxidase activity decreased with increase in the number of hours. Leaves showed a significant drop in peroxidase activity within 96 hours of inoculation. Protein content also increased significantly in plants following bacterial application, compared to that in untreated Control plants.

Seven PGPR isolates, *Pseudomonas poae* (RMK03), *Bacillus stratosphericus* (RHS/CL-01), *Ochrobactrum anthropi*, *Paenibacillus lentimorbus*, *Bacillus pumilus*, *Bacillus megaterium* and *Bacillus amyloliquefaciens*, were tested on mandarin seedlings in pot culture experiment, for plant growth promoting activity when applied as an aqueous suspension to non-sterile soil with the natural rhizospheric microflora. Of the seven PGPR isolates, sequence of two isolates (RMK03 and RHS/CL-01) has been deposited with NCBI GenBank database under the accession Nos. KJ 917553.1 and KM 066950.1 for *Pseudomonas poae* and *Bacillus stratosphericus*, respectively. The sequence of *F. oxysporum* (RHS/M534) has been

Table 3. Protein content in leaf and root upon application of various PGPR isolates

Treatment	Protein content	
	Leaf	Root
Untreated (Control)	63.0 ± 0.57[a]	2.68 ± 0.397[a]
Plant treated with:		
Pseudomonas poae	136.8 ± 1.18[b]	8.58 ± 0.881[b]
Bacillus stratosphericus	106.4 ± 1.16[b]	6.37 ± 0.867[b]
Bacillus amyloliquefaciens	107.7 ± 1.74[b]	4.04 ± 1.15[b]
Ochrobactrum anthropi	104.2 ± 1.15[b]	6.29 ± 1.15[b]
Paenibacillus lentimorbus	104.2 ± 0.58[b]	5.77 ± 1.73[b]
Bacillus pumilus	76.4 ± 1.16[b]	5.81 ± 0.57[b]
Bacillus megaterium	86.8 ± 1.19[b]	2.88 ± 0.57[b]

Average of three replicates; Differences between Control and treated significant at p=0.05 (Student's t-test)Difference between control and treated significant at p = 0.05 (student's t-test) where superscript (a, b) are different ; where superscipt same, difference insignificant

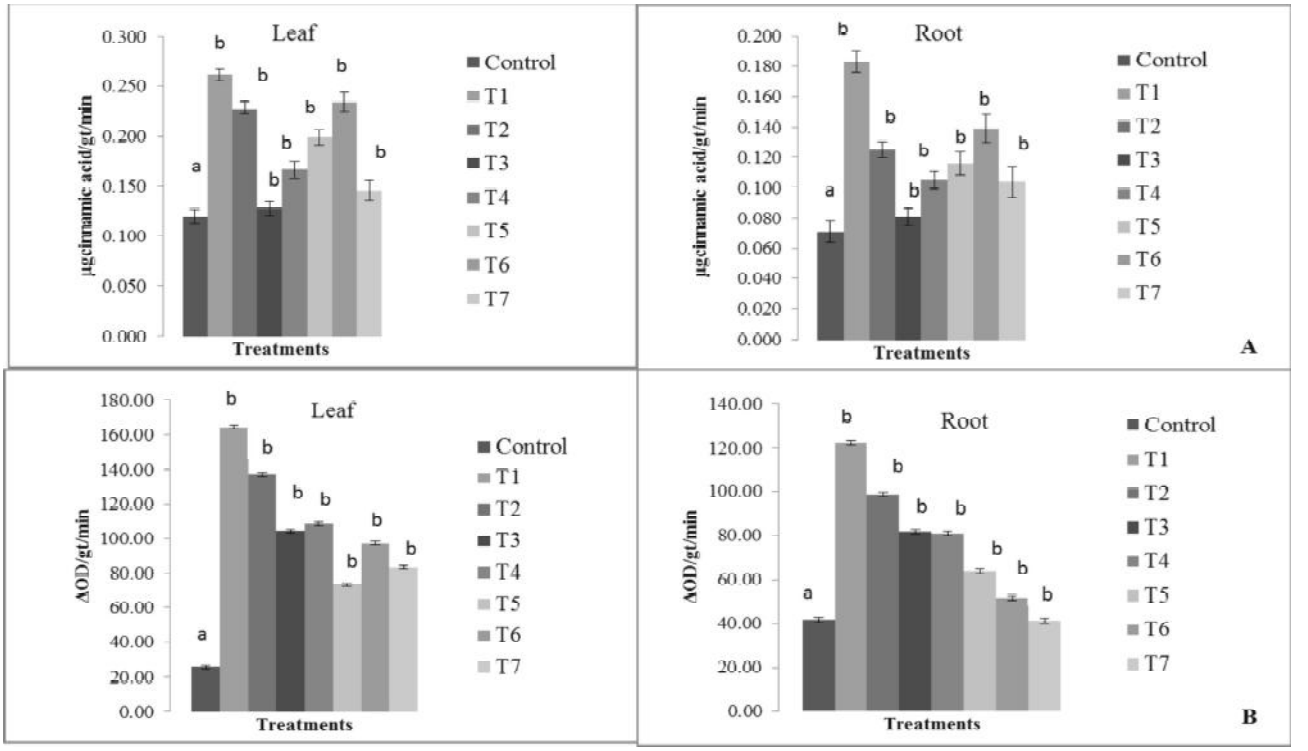


Fig 4. Activity of phenylalanine ammonia lyase (A) and peroxidase (B) in leaf and root of mandarin following application of PGPR. T1- *P. poae*, T2- *B. statosphericus*, T3- *B. amyloliquefaciens*, T4- *O. anthropi*, T5- *P. lentimorbus*, T6- *B. pumilus* and T7- *B. megaterium* treated;µgcinnamic acid/gt/min=µgcinnamic acid/gm tissue/min; "O.D/gt/min="O.D/gm tisuue/ min;Differences in PAL and POX activities determined through t-test between Control and treated leaf / root, significant at *p*=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

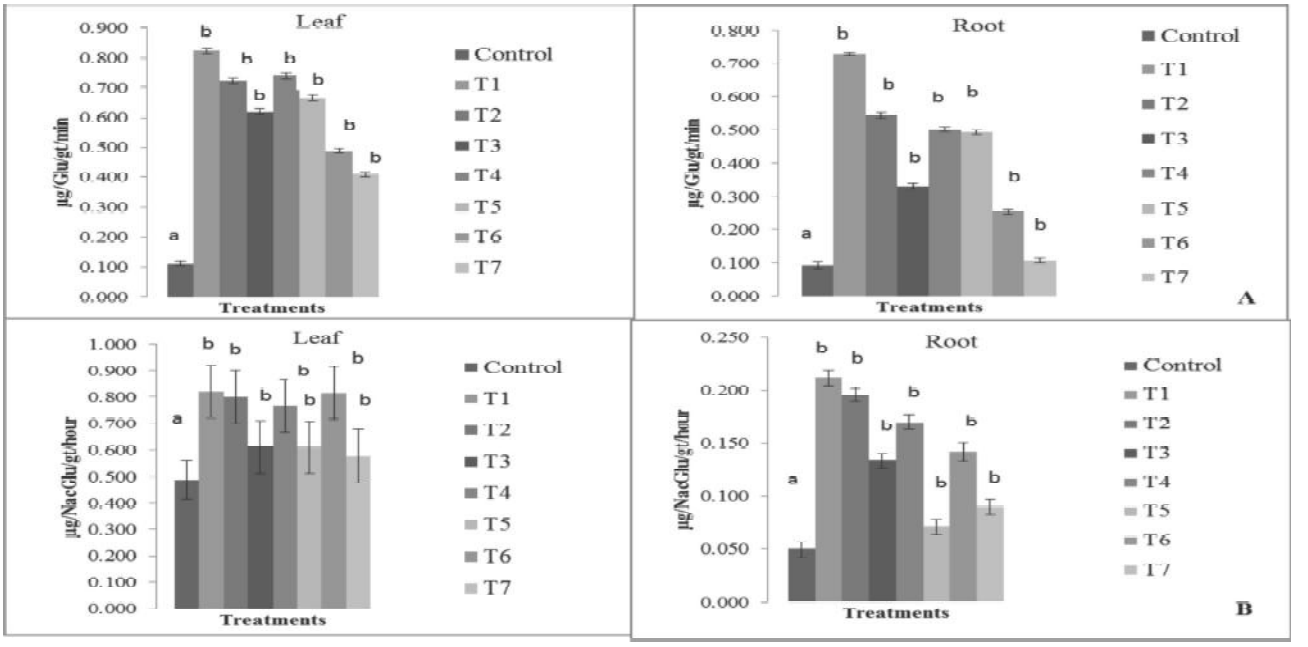


Fig 5. Activity of β-1,3 glucanase (A) and chitinase (B) in leaf and root of mandarin following application of PGPR. T1- *P. poae*, T2- *B. statosphericus*, T3- *B. amyloliquefaciens*, T4- *O. anthropi*, T5- *P. lentimorbus*, T6- *B. pumilus* and T7- *B. megaterium* treated; µgglu/gt/min=µgglu/gm tissue/min; µgNacGlu/gt/hour=µgNacglu/gmtissue/hour; Differences in GLU and CHT activity determined through t-test between Control and treated leaf / root, significant at *p*=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

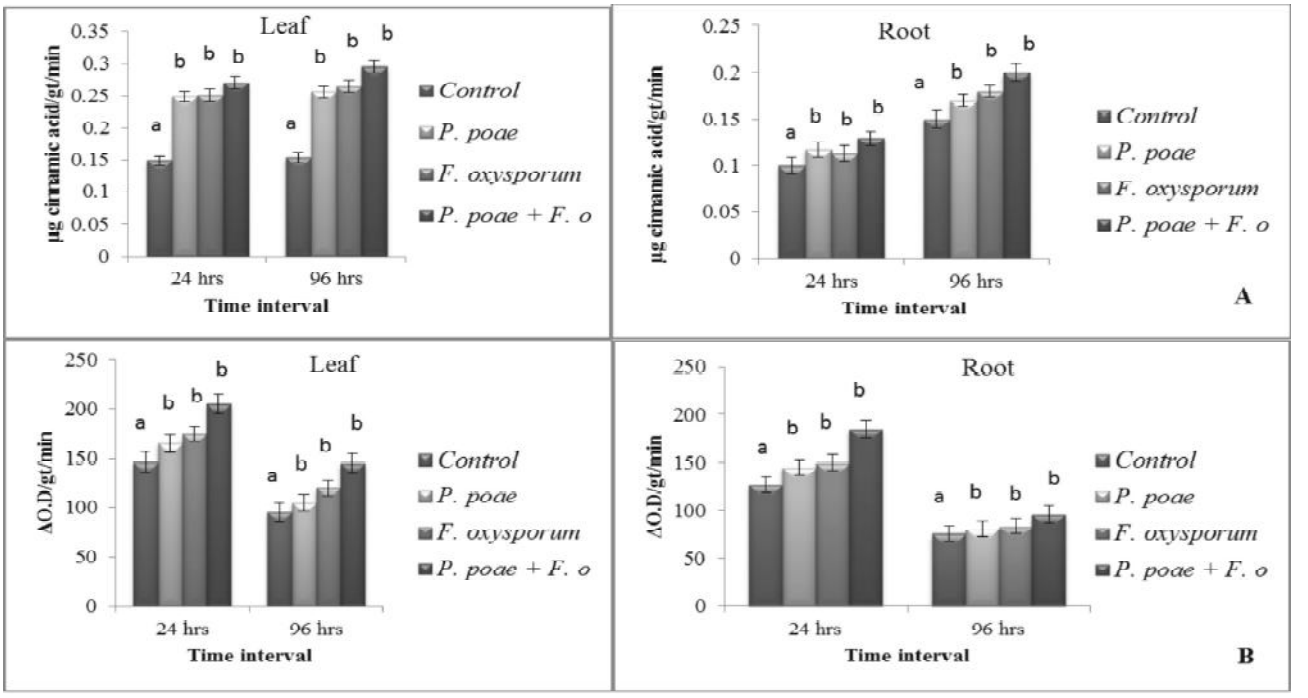


Fig 6. Activities of phenylalanine ammonia lyase (A) and peroxidase (B) in leaf and root of mandarin seedlings following application of *P. poae* and *F. oxysporum* at 24 and 96 hours after inoculation;µgcinnamic acid/gt/min=µgcinnamic acid/gm tissue/min; "O.D/gt/min="O.D/gm tisuue/min; Differences in PAL and POX activity determined through t-test between Control and treated leaf / root, significant at p=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

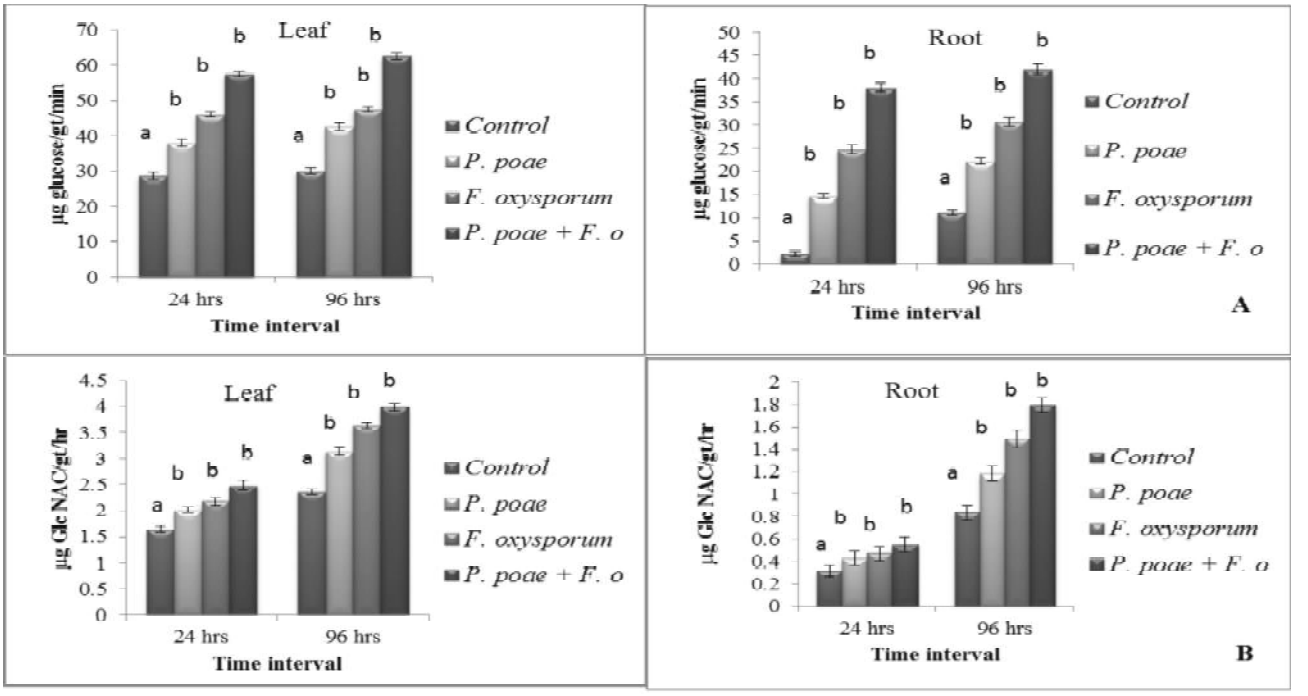


Fig 7. Activity of β-1,3 glucanase (A) and chitinase (B) in leaf and root of mandarin seedlings following application of *P. poae* and *F. oxysporum* at 24 and 96 hours after inoculation;µgglu/gt/min=µgglu/gm tissue/min; µgNacGlu/gt/hour=µgNacglu/gm tissue/hour;Differences in GLU and CHT activity determined through t-test between Control and treated leaf / root, significant at p=0.05.Different letters (a,b) above bars indicate significant difference in t-test at p=0.05 between control and treated.

deposited in NCBI GenBank database under the accession no. KF952602. Markedly enhanced growth of mandarin seedlings was observed with application of PGPR; however, superior growth enhancement was noticed with *P. poae, B. stratosphericus, O. anthropi* and *B. pumilus* application. All these isolates solubilized phosphate and produced siderophores *in vitro*. Application of *B. pumilus* along with *G. mosseae* in the rhizosphere of *Citrus* plants led to an increase in growth of seedlings in terms of increase in plant height and number of leaves (Chakraborty *et al,* 2011). Acharya *et al* (2013) reported soil application and foliar spray of PGPR to be effective in promoting overall growth of *Sualu* plants, as well as a increase in the level of defense-related enzymes, phenols and protein in the leaves of treated plants. Similar results were recorded in tea seedlings upon application of *Ochrobactrum anthropi* (Chakraborty *et al,* 2009). Nelson (2004) reported significant control of plant pathogens, or direct enhancement of plant development, by PGPR. The author also pointed out recent progress in understanding diversity, colonizing ability, mechanism of action, formulation and application of PGPR in management of sustainable agricultural systems. Lavania *et al* (2006) reported the ability of *Serratia marcescens* NBR11213 to promote growth and control foot and root rot disease of betel vine caused by *Phytophthora nicotianeae*, while, Chakraborty *et al* (2010) reported positive influence of *S. marcescens* (TRS-1) on growth of tea seedlings. The ability of *B. megaterium* to produce IAA (used for lateral-root induction) and to promote growth in *Lactuca sativa*, alone, or in combination with arbuscular mycorrhiza, was reported by Marulanda-Aguirre *et al* (2008). Orhan *et al* (2006) reported plant growth promoting effects of two *Bacillus* strains, OSU-142 (N2-fixing) and M3 (N2-fixing and phosphate solubilizing). *Bacillus* M3 strain stimulated plant growth and resulted in significant yield increase in raspberry (cv. Heritage) plants in terms of yield, growth and nutrient composition of leaf. Chakraborty *et al* (2013) reported that *Bacillus amyloliquefaciens*, *Serratia marcescens* and *B. pumilus* enhanced seedling growth in tea varieties in nursery as well as in the field. Effect of bacteria on metabolism in mandarin seedlings was also determined. To determine if the bacteria could induce systemic resistance in mandarin plants, accumulation of defense-related enzymes and phenolics was studied. Results revealed that the seven PGPR isolates also enhanced the activity of defense-related enzymes peroxidase, chitinase, β-1,3-glucanase, phenylalanine ammonia lyase, protein content as well as total phenol. Greater increase in the activity of defense enzymes was observed in *P. poae* + *F. oxysporum* treatments, in comparison to *P. poae* or *F. oxysporum*-treated or Control plants. Enhanced activity of chlorophyll was also observed. Antibiotic-producing *Pseudomonas chlororaphis* strains DF190 and PA23, *Bacillus cereus* strain DFE4, and *Bacillus amyloliquefaciens* strain DFE16 were tested for elicitating induced systemic resistance (ISR) and direct antibiosis for control of black-leg in canola, caused by the fungal pathogen *Leptosphaeria maculans*. Bacteria were shown to control the black-leg disease in canola (Ramarathnam *et al,* 2011). Twenty-one isolates of *Pseudomonas fluorescens* were isolated and their identity confirmed through various biochemical tests, of which five tested positive for 2,4-DAPG production, with specific primers. The biocontrol potential of these isolates on groundnut stem-rot pathogen (*Sclerotium rolfsii*) was determined through *in vitro* dual culture assays. Eight isolates were found effective against *S. rolfsii* (causing up to 75% inhibition) in the dual culture method. All the five 2,4-DAPG-producing plant growth-promoting rhizobacteria isolates were highly antagonistic to *S. rolfsii* (Asadhi *et al,* 2013).

Biological control represents a promising approach for protection of plants against soil-borne pathogens. *Fusarium* wilt of cucumber, caused by *F. oxysporum* f. sp. *Cucumerinum*, has been successfully controlled by *B. subtilis* SQR 9, both *in vitro* and *in vivo*. Wilt incidence reduced significantly by 49%–61% (Cao *et al,* 2011). Yuan *et al* (2012) established that volatile compounds produced by *B. amyloliquefaciens* NJN-6 reduced mycelial growth and germination of spores in *F. oxysporum* f. sp. *cubense in vivo* by about 30-40%, and strongly antagonized *F. oxysporum* in the soil as well. Akhtar *et al* (2010) studied the effect of *Bacillus pumilus*, *Pseudomonas alcaligenes* and *Rhizobium* sp. on wilt of lentil caused by *F. oxysporum* f. sp. *lentis*. Combined application resulted in the greatest increase in plant growth, number of pods, nodulation, root colonization by rhizobacteria, and, reduced wilting.

CONCLUSION

Application of different PGPR to *Citrus reticulata* resulted in improved growth of the crop, with simultaneous enhancement in activity of defense

enzymes, and higher proteins, phenolics and chlorophyll. Root rot disease was successfully managed using one of the PGPR strains, *Pseudomonas poae*. These PGPR can be potentially developed as plant growth promoters having disease suppressing ability.

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