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Biological Management of Sclerotinia Rot of Bean through Enhanced Host Defense Responses Triggered by *Pseudomonas* and *Trichoderma* Species

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Plant growth promoting microorganisms have been reported to induce systemic resistance in plants. In the present study, a consortium comprising of *Trichoderma harzianum* and *Pseudomonas aeruginosa* were evaluated for their efficacy to control Sclerotinia rot of bean. Upon infection of bean plants with the *S. sclerotiorum* resulted in substantial changes in enzymatic and non-enzymatic defense related compounds. The induction of phenylalanine ammonia-lyase (PAL), peroxidase (POx), polyphenoloxidase (PPO), superoxide dismutase (SOD), and the accumulation of proline and phenols in bean leaves were studied periodically at 2 d intervals after pathogen infection. Levels of PAL and phenols were induced most rapidly and increased significantly in all the treatments after pathogen challenge and reached maximum after 2 d and 4 d of pathogen challenge, respectively. Similarly, the activities of POx, PPO, SOD, along with proline content was also found to increase consistently and reached maximum at 6 d after pathogen challenge and their content was maximum in plants treated with microbial consortium. These results demonstrated that interaction of microorganism in rhizosphere could provide enhanced tolerance against pathogen through induction of host defense-related compounds.

Key words: Biocontrol agents; Defense enzymes; Induced systemic resistance; Microbial consortium; Bean; Sclerotinia rot.

Plant's response against pathogenic microorganism's invasion is essential for success of plant resistance (Boller and Felix 2009). Induction of defense proteins makes the plant resistant to pathogen invasion (Van Loon 1997). A large number of studies have focused on the extensive description of induced systemic resistance (ISR) (Van Wees *et al*. 2008; Lungtenberg and Kamilova 2009). Studies on ISR using strains of plant growth-promoting microorganisms (PGPM) have concentrated on the use of single microbe as ISR inducers against plant pathogens. To date, few reports have examined the potential of microbial mixtures to ISR against diseases of different plants. By combining microorganisms, multiple antagonistic traits can be combined and one may assume that at least one biocontrol mechanism will be functional under the circumstances faced by the released biocontrol agents (BCAs). The use of microbial mixtures may provide improved effect on plant growth promotion and ISR (Jetiyanon 2007;

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Ryu *et al*. 2007; Akhtar and Siddiqui 2008; Pandey and Maheshwari 2007; Srivastava *et al*. 2010).

Microbes act as ISR elicitors and on perception by plants they give rise to an exaggerated immune response. ISR has been known to mobilize defense-related proteins such as those pathogenesis-related (PR) proteins, phenylalanine ammonia-lyase (PAL), peroxidase (POx), polyphenol oxidase (PPO), superoxide dismutase (SOD) and induce accumulation of proline and phenols (Chen *et al*. 2000; Jetiyanon 2007; Jain *et al*. 2012). An increased level of defense-related enzymes viz., PAL, POx and PPO, were recorded on co-inoculation of rhizobia with *Bacillus cereus* strain BS 03 and a *P. aeruginosa* strain RRLJ 04 under the stress generated by Fusarium wilt of pigeon pea (Dutta *et al*. 2008).

Sclerotinia sclerotiorum is a necrotrophic ascomycete fungus that infects virtually all dicotyledonous (>400 species) plants (Boland and Hall 1994; Bolton *et al*. 2006). Breeding programmes and management strategies like crop rotation are unsuccessful due to its broad host range. Sclerotia formation has also contributed greatly to its pathogenic success as they remain viable for many years in the soil (Li *et al*. 2003). In addition the continuous use of chemicals may develop fungicidal resistance (Hawthorne and Javis 1973; Mueller *et al*. 2002) and may cause negative effects on non target species and the environment. Use of biocontrol agents offer an alternative method to control this pathogen and can be used as a cost effective part of management programme. Several BCAs have been identified as potential agents for controlling *S. sclerotiorum* in different crops (Savchuk and Fernando 2004; Zhang and Fernando 2004; Fernando *et al*. 2007; Awais *et al*. 2008; Zhang and Xue 2010). Combining such beneficial BCAs would enhance the plant's innate resistance level against the invading pathogens. The objective of this study was to select a compatible mixture of PGPM strains with the capacity to elicit ISR in bean plants. In the present study two compatible BCAs, viz., *Trichoderma harzianum* strain THU0816 ARS culture collection number NRRL 30598 and *Pseudomonas aeruginosa* strain PJHU15 (GenBank accession no. JN099685) was evaluated for their ability to manage Sclerotinia rot of bean through ISR induction under greenhouse conditions.

MATERIALS AND METHODS

Isolation and multiplication of the pathogen

S. sclerotiorum was isolated from an infected bean plant from the Agricultural Farm of Banaras Hindu University. The sclerotium was surface sterilized in 70% ethanol for 2 min, rinsed thrice in sterile distilled water, bisected and one of the two sclerotial halves was transferred to potato dextrose agar (PDA; Himedia, MO96) in a Petri plate with the freshly cut surface towards the agar and incubated at 25±2^{0}C in darkness for 4 days. The isolate thus obtained was stored at 4^{0}C.

Selection of compatible microbes

Compatibility between the fluorescent *Pseudomonas* and *Trichoderma* strains was checked on PDA which were previously screened for their antagonistic potential against *S. sclerotiorum* (Jain *et al*., 2012). A 5 mm *Trichoderma* mycelial bit was kept at the distance of 2.5 cm away from the centre of the plate and the *Pseudomonas* strain was also streaked at a distance of 2.5 cm away in the opposite side from the centre. Any overgrowth of *Trichoderma* on bacterial streaks without a zone of inhibition was considered compatibility. Based on antagonistic activity and compatibility tests the *Trichoderma* strain THU0816 and *Pseudomonas* strain PJHU15 were selected for further experiments which were priorly identified as *Trichoderma harzianum* and *Pseudomonas aeruginosa*.

Seed treatment with BCAs and greenhouse experiment

The *Pseudomonas* strain PJHU15 was grown on NA for routine use, and maintained in Nutrient Broth (NB; Himedia MOO2) with 20% glycerol at -80^{0}C for long-term storage. Single bacterial colony was transferred to 250 ml flasks containing 100 ml of NB, and was grown on a rotating shaker (200 rev min^{-1}) for 48 h. The bacterial suspension was centrifuged at 8000 *g* for 10 min and washed twice with sterile distilled water. The final pellet was re-suspended in a small quantity of sterile distilled water and the final concentration was adjusted to 4x10^8 CFU ml^{-1} using "Thermo Scientific UV 1" spectrophotometer. Similarly, the *Trichoderma* strain THU0816was grown on PDA for 6 days at 27± 2 ^{0}C and the spores were harvested and brought to a final concentration at 2x10^7 CFU ml^{-1}.

Soil mixture containing sandy soil, vermicompost and farm yard manure (2:1:1) was sterilized in an autoclave at 15 lb pressure for 30 min for three consecutive days and were filled in plastic pots (15 x 10 cm) at the rate of 1.5 kg $pot^{-1.}$ Seeds of bean (*Phaseolus vulgaris* L.) cultivar "HUR 15" were surface-sterilized with 1% sodium hypochlorite for 30 s, then rinsed twice with sterile distilled water and dried under a sterile air stream. Following treatments were part of the experimental design: (i) *Pseudomonas aeruginosa* (PJHU15), (ii) *Trichoderma harzianum* (THU0816) and (iii) PJHU15+THU0816. The seeds were inoculated by soaking with *P. aeruginoasa* and *T. harzianum* suspensions prepared in 1% CMC, used as adhesive. Seeds were soaked in their respective suspensions for 8 h {in the case of consortia equal amount of suspension (v/v) was mixed}, after which the suspension was drained off and the seeds were dried overnight in sterile Petri plates. Two sets of untreated control plants were also maintained. For each treatment five pots were maintained in green house for 4 weeks and the growth was monitored at regular intervals. A cycle of 28/20^{o}C, day/night and the humidity 60% was maintained. Irrigation was provided as and when required or at two days interval till partial saturation. The pathogen was multiplied on bajra seed meal-sand medium (bajra seed 250 g, washed white sand 750 g, distilled water 250 ml) at 25±2^{0}C for 15 days (Sarma *et al*. 2007). Colonized culture was blended well prior to its use as inoculums. The pathogen was inoculated in the collar region of the plants in all the treatments at an amount of 50 g pot^{-1}. One set of control plants was left unchallenged.

Sample collection for biochemical analysis

Leaves were carefully collected from each treatment without causing any damage at 2 day intervals after the pathogen inoculation up to 8th day. The samples were washed in running tap water and stored in a deep freezer (-80 ^{0}C) until used for biochemical analysis.

Phenylalanine ammonia lyase (PAL) assay

Leaf tissue (0.1 g) from each of the treatments was homogenized in 2 ml of ice cold 0.1 M sodium borate buffer (pH 7.0) containing 1.4 mM β-mercaptoethanol and centrifuged at 16000 x g at 4^{0}C for 30 min. The supernatant was used as enzyme source. To 0.2 ml of enzyme extract, 0.5 ml of 0.2 M borate buffer (pH 8.7), and 1.3 ml of water were added. Reaction was initiated by the addition of 1 ml of 0.1 M L-phenylalanine (pH-8.7) and incubated for 30 min at 32 ^{0}C. The reaction was terminated by addition of 0.5 ml of trichloroacetic acid (TCA, 1 M). PAL (*EC 4.1.3.5)* activity was measured following the formation of trans-cinnamic acid at 290 nm as described by Brueske (1980), and was expressed in terms of µM TCA g^{-1} fresh weight (FW).

Total phenolic content (TPC)

The total phenolic content was determined following the method of Zheng and Shetty (2000). Leaf sample (0.1 g) was placed in 5 ml of 95% ethanol and kept at 0 ^{0}C for 48 h. The samples were homogenized individually and centrifuged at 13000 x g for 10 min. The reaction mixture consisted of 1 ml of the supernatant, 1 ml of 95% ethanol, 5 ml of SDW and 0.5 ml of 50% Folin-Ciocalteau regent were added and the content was mixed thoroughly. After 5 min., 1 ml of 5% sodium carbonate was added, the reaction mixture was allowed to stand for 1h and the absorbance of the colour developed was recorded at 725 nm. Standard curves were prepared for each assay using various concentrations of gallic acid (GA; Sigma, USA) in 95% ethanol. Absorbance values were converted to mg GA equivalents (GAE) g^{-1} FW.

Polyphenol Oxidase (PPO) assay

Leaf samples (0.1 g) were homogenized in 2 ml ice cold phosphate buffer (0.1 M, at pH 6.5). The homogenate was centrifuged at 16000 x g for 30 min at 4^{0}C. The reaction mixture contained 0.4 ml catechol (1mM) in 3 ml of 0.05 M sodium phosphate buffer (pH 6.5) and 0.4 ml of the supernatant. Reaction mixture containing only substrate served as control. Catechol was used as substrate for PPO (EC 1.14.18.1) and increase in absorbance was recorded at 405 nm (Gauillard *et al*. 1993). The linear portion of the activity curve was used to express PPO enzyme activity as change in O.D. min^{-1} g^{-1} FW.

Superoxide Dismutase (SOD) assay

SOD (EC 1.15.1.1) activity was assayed following the method of Fridovich (1974) by measuring the ability of enzyme extract from samples to inhibit photochemical reduction of nitroblue tetrazolium (NBT) chloride. Leaf tissues (0.1 g) from each of the treatments were homogenized in 2.0 ml extraction buffer (0.1 M

phosphate buffer containing 0.5 mM EDTA at pH 7.5) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. The reaction mixture contained 200 mM methionine, 2.25 mM NBT, 3 mM EDTA, 100 mM phosphate buffer (pH 7.8), 1.5 M sodium carbonate and enzyme extract. The final volume was maintained to 3 ml. Reaction was started by adding 2 µM riboflavin (0.4 ml) and the tubes were illuminated with two 15-W fluorescent lamps for 15 min. Reaction mixture without enzyme served as control. The reaction was terminated by putting the light off and keeping the tubes in dark until the absorbance was recorded at 560 nm. One unit of the SOD activity was defined as the amount of enzyme reducing the absorbance to 50% in comparison to control lacking enzyme.

Peroxidase (POx) assay

POx (EC 1.11.1.7) activity was assayed by the method of Hammerschmidt *et al.* (1982), with slight modification. Leaf samples (0.1 g) were homogenized separately in 2 ml of 0.1 M phosphate buffer (pH 7.0), at 4 ^{0}C, centrifuged at 16000 x *g* at 4 ^{0}C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml pyrogallol (0.05 M), 0.05 ml enzyme extract and 0.5 ml H_2O_2 (1% v/v). Reaction mixture without enzyme served as control. The changes in the absorbance at 420 nm were recorded after 30s intervals for 3 min. The enzyme activity was expressed as change in the U min^{-1} g^{-1} FW.

Proline content

Proline content was measured as described by Bates *et al.* (1973). 0.1 g leaf samples were homogenised with 5 ml of sulphosalicylic acid (3%), in a prechilled mortar and pestle. The homogenate was centrifuged at 12000 x *g* for 15 min. 2 ml of the extract was reacted with 2 ml glacial acetic acid and 2 ml ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until resolved) in a water bath (100°C) for an hour. The reaction was terminated in an ice bath to stabilize the purple colour of the extract and was brought to room temperature. 4 ml toluene was added to each tube, and vortexed for 15-20s. The absorbance of top purple aqueous layer was recorded at 520 nm. The concentration of proline samples was determined according to the standard curve plotted with known concentrations of L-proline.

Statistical Analysis

Values from different experiments shown in figures are mean ± standard deviation (SD) of at least three replications of each treatment. Data collected in this study were subjected to analysis of variance (ANOVA). The treatment mean values were compared by Duncan's multiple range test (DMRT) at *p* d" 0.05 significance level. The software used for analysis was SPSS version 16.

RESULTS

Selection of microbes

Among 40 fluorescent *Pseudomonas* and 20 *Trichoderma* isolates tested, *P. aeruginosa* PJHU15 and *T. harzianum* THU0816 were found compatible with each other and were used for further experimentation.

Effect of microbial consortium on TPC in leaves

Maximum phenolic content was found in plants treated with microbial agents and were significantly higher when compared with untreated plants challenged or unchallenged with the pathogen (Fig. 1). When BCAs were used singly or in consortium, this increase was more pronounced at 4th day after pathogen inoculation, which declined thereafter. Seeds treated with a consortium of *P. aeruginosa* and *T. harzianum* showed highest phenolic content, which was 1.72 and 5.33 times higher than its corresponding challenged control and untreated unchallenged control, respectively at 4th day after pathogen challenge.

Effect of microbial consortium on PAL and PPO activity

PAL activity increased significantly in all BCA treatments up to 2nd day, followed by a gradual decline (Fig. 2). Two species microbial consortium i.e. *P. aeruginosa* and *T. harzianum* followed by singly treated microbes induced higher PAL activity at all the sampling stages compared to the untreated challenged as well as unchallenged controls. The two microbe consortium supported 1.62 and 2.66 fold higher PAL activity at 2nd day after pathogen challenge compared to their corresponding challenged and unchallenged controls, respectively and was significantly higher when compared to all other treatments. However, maximum activity of PPO was observed at 6th day in all the treatments which declined thereafter (Fig.

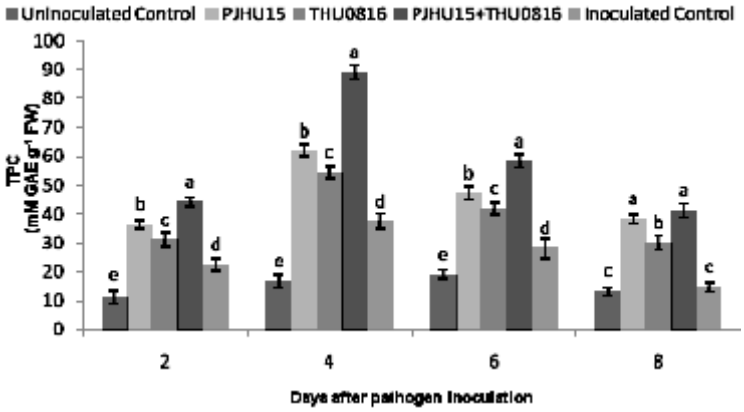

Fig. 1. Effect of singly or in consortium treated plants on total phenolic content in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatment results taken at the same time interval according to DMRT at $p \leq 0.05$

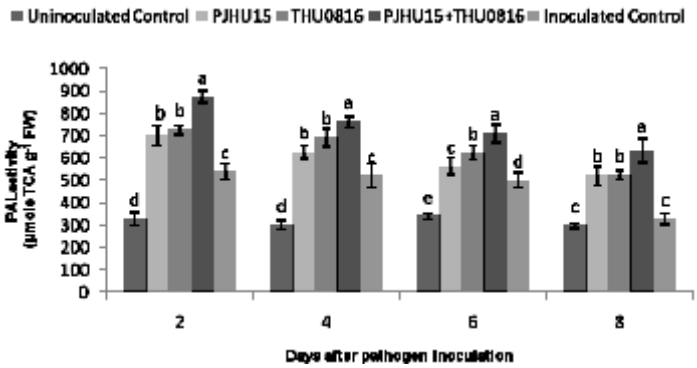

Fig. 2. Effect of BCAs singly or in consortium treated plants on PAL activity in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatments within the results taken at the same time interval according DMRT at $p \leq 0.05$

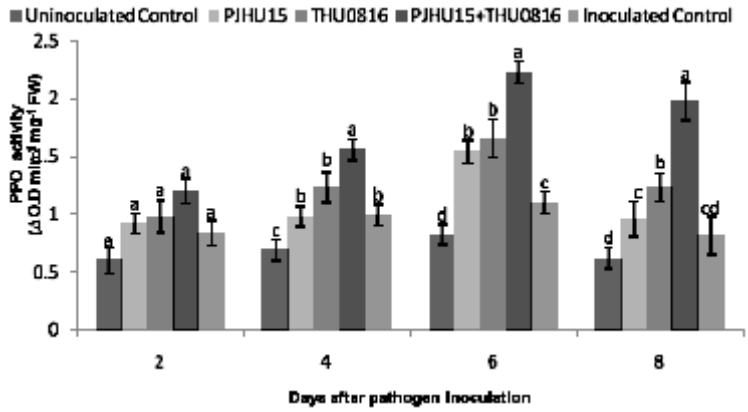

Fig.3. Effect of BCAs singly or in consortium treated plants on PPO activity in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatments within the results taken at the same time interval according DMRT at $p \leq 0.05$

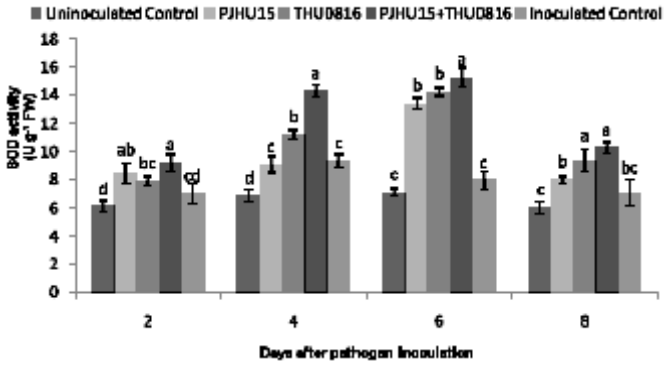

Fig.4. Effect of BCAs singly or in consortium treated plants on SOD activity in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatments within the results taken at the same time interval according DMRT at $p \leq 0.05$.

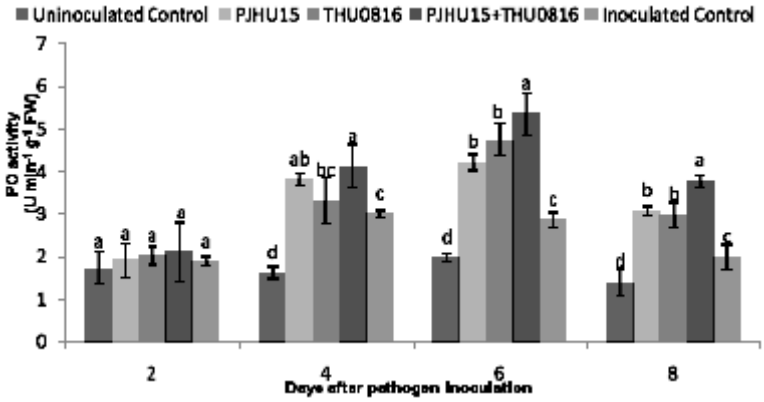

Fig. 5. Effect of BCAs singly or in consortium treated plants on PO activity in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatments within the results taken at the same time interval according DMRT at $p \leq 0.05$

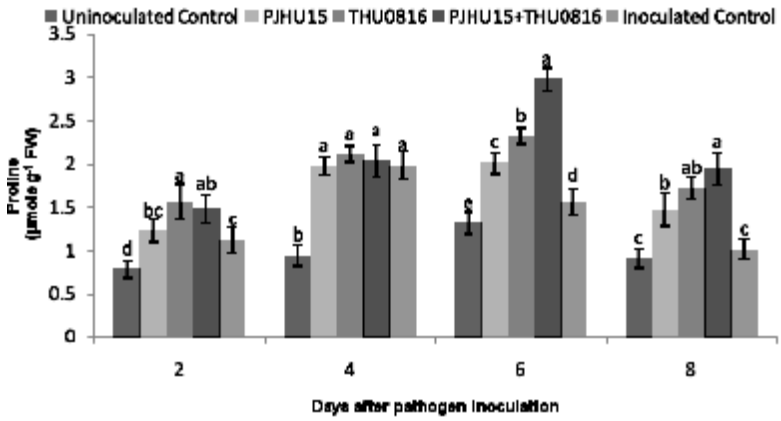

Fig. 6. Effect of BCAs singly or in consortium treated plants on free proline content in bean leaves after challenge inoculation with soft rot pathogen. Results are expressed as means of three replicates and vertical bars indicate SD of the means. Different letters indicate significant differences among treatments within the results taken at the same time interval according DMRT at $p \leq 0.05$

4). The two microbe mixture recorded 2.08 and 2.72 fold higher PPO activity compared to pathogen challenged and unchallenged controls, respectively. A small activity of PAL and PPO was consistently recorded in unchallenged healthy control as well.

Effect of microbial consortium on SOD and POx activity

SOD and POx activities attained their maximum levels at 6th day in all the primed plants (Figs. 4, 5). Maximum SOD activity was found in two species microbial consortium and it was 1.91 and 2.14 fold higher than challenged and unchallenged controls, respectively. A gradual decline in SOD activity was however observed at 8th day in all treatments except untreated pathogen challenged plants which showed a sharp decline in POx activity at 6th day. A similar increase in POx activity was observed in *P. aeruginosa* and *T. harzianum* treated plants and an increase of 1.87 and 2.72 fold was recorded compared to challenged and unchallenged controls, respectively, at 6th day. The POx activity was found to be significantly higher compared to other treatments. In healthy untreated control plants a significantly lower level of POx and SOD activity was detected which remained nearly unchanged during the entire experimental period.

Effect of microbial consortium on proline content

Proline accumulation increased consitently in all the treatments upto 6th day (Fig. 6). Maximum proline content was found in the two-microbe consortium of *T. harzianum* and *P. areuginosa*, followed by single microbe treated plants. The two-microbe consortium recorded 1.91 and 2.26 fold increase in proline content at 6th day compared to unchallenged and challenged controls. A little amount of proline was detected in unchallenged healthy control plants but it remained nearly unchanged during the experimentation period.

DISCUSSION

Understanding host-microbe interactions requires elucidation of the biochemical pathways for both pathogenesis and generation of defence responses. Priming plants with consortium of beneficial microbes would accelerate faster defence responses compared to plants treated singly. Results obtained from the present study indicated that the bean plants pre-treated with microbial consortium exhibited higher defense enzymes activity and phenol content in leaves upon challenge against *S. sclerotiorum*. Two compatible microbe *P. aeruginosa* and *T. harzianum* in combination interacted with the host positively providing benefits for management of soft rot. Expression of defense gene products upon pathogen challenge in the presence/absence of BCAs is crucial to understand the mechanism of bioprotection conferred by microbial consortium and to develop innovative strategies to control the soft rot pathogen.

PAL is the entry point enzyme in the phenylpropanoid biosynthesis pathway. Branch pathways lead to the synthesis of compounds such as phytoalexin or phenols (Karthikeyan *et al*. 2003), that have diverse functions in plants. PAL has an important role in the biosynthesis of various defense-related phenolic phytoalexins and is an extremely sensitive indicator of stress conditions and fungal challenged (Sarma *et al*. 2002). The results indicated that *P. aeruginosa* and *T. harzianum* combined treated plants elicited the phenylpropanoid pathway maximum under pathogen stress among all the treatments. Our results are in agreement with the studies conducted on banana, coconut and pigeonpea, where increased PAL activity was observed on colonization by beneficial microorganisms and challenge by pathogens (Sangeetha *et al*. 2010; Karthikeyan *et al*. 2006; Dutta *et al*. 2008).

Plant phenolics are natural products formed biogenetically from the activation of the phenylpropanoid pathway that share a major role amongst the families of various biomolecules responsible for imparting PGPR-mediated ISR responses (Sarma *et al*. 2002). A significant increase in phenolic content was positively related to the degree of plant resistance against the pathogens and phenols play a key role in antimicrobial defense arsenal of plants (Shoresh and Harman 2008; Abo-Elyousr *et al*. 2009). Maximum TPC was observed in the plants treated with the consortium of *P. aeruginosa* and *T. harzianum* at 4th day after pathogen challenge, which can be further correlated with the results of PAL activity and highest level of disease suppression in this treatment. Similar, accumulation of phenolics by

prior application of *P. fluorescens* in pea has been reported against *Pythium ultimum* and *Fusarium oxysporum* f. sp. *pisi* (Benhamou *et al*. 1996).

PPO is a copper containing ubiquitous enzyme reported to catalyze oxidation of phenolics to more toxic quinones. Increased activity of PPO is directly proportional to the increased antimicrobial activity and decrease in accumulation of toxic products of oxidation and therefore correlated to greater degree of resistance against pathogen. Maximum PPO activity was observed at 6th day in bean plants treated with both *P. aeruginosa* and *T. harzianum* and was significantly higher amongst all the treatments. The increased level of phenolics recorded at 4th day in the present study could also be correlated with increased POx and PPO levels. Phenols would also provide an adequate substrate to oxidative reactions catalysed by PPO and/or PO that consume oxygen and produce fungitoxic quinones, making the environment unfavourable for further development of pathogens (Lattanzio *et al*. 2006).

The enzymes SOD and POx, work together with other enzymes of the ascorbate–glutathione cycle to promote scavenging of free radicals (Hernandez *et al*. 2001). Generation of antioxidants including SOD and POx in the host plants upon pathogen attack as an ISR response against diverse pathogens has been reported previously (Jetiyanon 2007, Silva *et al*. 2004). SOD represent a group of multimeric metalloenzymes catalyzing the disproportionation of superoxide free radicals generated by univalent reduction of molecular oxygen to less toxic H_2O_2 and O_2 in different cellular compartments (Fridovich 1974). POx represents another component of an early response in plants to pathogen-mediated oxidative stress and play a key role in the biosynthesis of lignin and other oxidative phenols, although other proteins may also be involved (Quiroga *et al*. 2000). In the present study, analysis of plants after pathogen infection showed that plants treated with the combination of *P. aeruginosa* and *T. harzianum*, followed by *T. harzianum* treated plants exhibited maximum POx and SOD activities at 6th day as compared to other treatments. A proteomic approach to identify *Trichoderma* induced enzymes also showed increased levels of SOD and POxs, and other detoxifying enzymes in maize leaves (Shoresh and Harman 2008). In a similar study, maximum induction of PAL, POx, PPO and accumulation of phenolics was observed in banana fruits treated with antagonist mixture comprising of non-fluorescent *Pseudomonas* NFP6, *P. fluorescens* Pf3a, and *B. subtilis* BS1 compared to individual and two-way combination of antagonist mixtures (Sangeetha *et al*. 2010).

Proline acts as an osmoregulant and helps to maintain the water potential of plant to extract water from soil (Hanson *et al*. 1979). Significant increase in proline accumulation was observed in plants treated with *P. aeruginosa* and *T. harzianum* at 6th day after pathogen challenge. A similar increase in proline content was also demonstrated by Sarvanakumar *et al*. (2011) in green gram plants bacterized with *P. fluorescens* Pf1 followed by *B. subtilis* EPB22. Increased proline content in the BCAs treated plants in the present study may be correlated to an early response strategy of bean plants towards the invading pathogen. A similar increase in proline, phenolic content, PAL, POx, PPO was also demonstrated by Jain *et al*. (2012) in pea plants treated with microbial consortium comprising of *P. aeruginosa* PJHU15 *P. aeruginosa* PJHU15, *T. harzianum* TNHU27 and *B. subtilis* BHHU100 and challenged with *S. sclerotiorum*.

In recent years considerable attention has been given to PGPM that can mediate ISR responses. Beneficial microbes may alter host physiology and reprogram metabolism, in response to specific stimuli, which would be visible upon subsequent challenge with pathogen. Plants primed with BCAs respond faster and show stronger activation of cellular defense responses after pathogen challenge compared to unprimed plants (Conrath *et al*. 2006). From the results obtained in the present study it can be concluded that microbial consortium comprising of *P. aeruginosa* and *T. harzianum* increased plant's resistance against infection by *S. sclerotiorum*. Increased activities of defense related enzymes and increased accumulation of phenols and proline by the two species microbial consortium than single microbe treated plants showed that in consortium mode these microbes act in an additive or synergistic way of imparting increased resistance against the pathogen. The question that still awaits an answer is to how speciûcally these microbes interact in the surrounding rhizosphere and what host physiological functions are affected upon

microbial association? The present study though gives evidence that combined interactions of microorganisms are responsible for activation of host defense signalling pathway. The study further gives evidence of the ecological importance of interspecies signalling taking place in the rhizosphere.

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