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Biological control of sclerotial blight of tea using arbuscular mycorrhizal fungus and plant growth promoting rhizobacterium

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ABSTRACT: *Glomus fasciculatum*, one of the dominant arbuscular mycorrhizal fungus (AMF) associated with tea root colonization, was selected and maintained in maize plants. Besides AMF, *Bacillus amyloliquefaciens* TRS6 isolated from tea rhizosphere, which showed *in vitro* antagonism to a number of tea root rot pathogens as well as siderophore-producing and phosphate-solubilizing activities, was selected for utilization as bioinoculants in tea plants for improvement of health status. The bacterium was applied to the soil as aqueous suspensions, and in case of *G. fasciculatum*, soil was inoculated with its spores and in joint inoculations, the AMF was inoculated prior to the bacterium. Inoculation of rhizosphere of tea plants of six different varieties (TV-18, T-17, AV-2, T-78, UP-3 and UP-26) with any of two microorganisms increased growth of plants, but the most significant increase was obtained in dual application. Plant growth was measured in terms of increase in height, increase in number of branches and leaves. Similarly, sclerotial blight of tea, caused by *Sclerotium rolfsii*, was suppressed to certain extent by *G. fasciculatum* or *B. amyloliquefaciens*, but significant suppression occurred when *G. fasciculatum* and *B. amyloliquefaciens* were applied jointly. Polyphenolics and four major defense enzymes showed enhanced activities during disease suppression. Western blot of the enzyme extracts from control and all treated plants using PAb raised against chitinase revealed strong reaction when disease suppression was evident. Population of *S. rolfsii* in soil was also determined following immunological techniques using PAb raised against the pathogen. Results of ELISA and dot-blot revealed that application of *G. fasciculatum* and *B. amyloliquefaciens* significantly reduced *S. rolfsii* population.

Keywords: Disease control; induced resistance; *Glomus fasciculatum*; *Bacillus amyloliquefaciens*; sclerotial blight; tea

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is the major plantation crop of northeast India and forms the back bone of the economy of this region. It is a perennial and survives for more than 100 years. "Biological control against root diseases" and "plant growth promotion" are two important areas which are closely linked and have great impact on present-day agriculture. In tea plantations, with the reduction in the permissible levels of chemicals which can be used, there is urgent need for identification and selection of microbes which have the potential to control diseases as well as increase productivity.

Rhizosphere soil of plants contains a wide variety of microorganisms (bacteria, fungi and actinomycetes), some of which are beneficial, while others are harmful. Among the beneficial microorganisms, the following two groups are very important agriculturally: the mycorrhizal fungi and plant growth promoting rhizobacteria (PGPR). Mycorrhizal fungi colonize plant roots and extend the root system into the surrounding soil. Estimates of amounts of mycorrhizal filaments present in healthy soil are astonishing. Several miles of filaments can be present in less than a thimbleful of soil associated with vigorously growing plants. The relationship is beneficial because the plant enjoys improved nutrient and water uptake, disease resistance and superior survival and growth. AM fungal association protects the plants from soil-borne diseases and detoxifies soil contaminants of certain metals. AM fungi increase tolerance to heavy metals, salinity and drought.[1,2] AM fungi have been shown to increase the productivity of several cereals, pulses, oilseed crops, vegetable crops, medicinal plants and also ornamental plants. The AM fungi are obligate symbionts and not host-specific.[3] The spore count, root colonization, species diversity and dominant species vary with the region and soil nutrient conditions.[4] Many bacteria are known to be able to stimulate plant growth through direct or indirect interaction with plant root and these have been classified as plant growth-promoting rhizobacteria; first defined by Kloepper and Scroth.[5] The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct, *i.e.* production of growth hormones, phosphate solubilization, nitrogen fixation or indirect, *viz*, suppression of deleterious microorganisms by siderophore production or secre-

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tion of antifungal metabolites.[6] It has been reported that many soil bacteria can solubilize inorganic phosphates which can play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. Research over the past years has demonstrated that induced systemic resistance (ISR) can be a potential mechanism by which PGPR demonstrate biological disease control.[7] ISR is dependent on colonization of the root system by sufficient numbers of PGPR. Previous studies have shown that *Serratia proteamaculans* 1-102 promotes soybean–bradyrhizobia nodulation and growth, but the mechanism is unknown.[8] Some biocontrol PGPB strains protect plants by activating gene-encoding defense enzymes (peroxidase, chitinase, phenylalanine-ammonia-lyase, β-1,3-glucanase and others) involved in synthesis of phytoalexin.[9] Chakraborty *et al.*[10] also reported that *Serratia marcescens* (TRS-1), either as aqueous suspensions or in bioformulations of saw dust, rice husk and tea waste, promoted growth in tea seedlings as evidenced by increase in height, emergence of new leaves and branches, as well as increase in leaf biomass. The bacterium also reduced brown root rot of tea caused by *Fomes lamaoensis*. It has also been reported that a highly chitinolytic bacterium, *Serratia marcescens* B2, isolated from tomato phylloplane controlled broad bean chocolate spot, caused by *Botrytis fabae* in a growth chamber.[11,12] *Serratia marcescens* NBR11213 was also reported to induce plant growth promotion and biological control of foot and root rot of betelvine caused by *Phytophthora nicotianeae* as studied in Lavania *et al.*[13]

Since microorganisms do not live in isolation in the soil, their effects on plant growth, nutrition and protection against diseases are due to their interactions. The dual activity of AMF and PGPR is generating ample evidence to act as bioprotector as well as biofertilizer which plays a significant role in sustainable agriculture.

Keeping these in view, the present study was undertaken to determine how does a combination of two microorganisms originally isolated from the tea rhizosphere itself – *Glomus fasciculatum* and *Bacillus amyloliquefaciens*, singly, or in combination, influence the growth of tea plants, development of sclerotial blight and induction of resistance in the host.

Materials and Methods

In the plains, tea plants grow best at a temperature of 28–30°C, with a slightly acidic soil (~pH 5) with an annual rainfall of 2500 mm. Six varieties of tea (TV-18, T-17, AV-2, T-78, UP-3 and UP-26) were selected for experimental purposes. The selected tea seedlings were maintained in 12″ earthenware pots. Potted tea plants were watered regularly for proper maintenance.

Spores of arbuscular mycorrhizal fungi were isolated from rhizosphere soil of six different varieties of tea by wet sieving and decanting method.[14] Approximately 250 ml of soil was suspended in 1 L water. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through sieves of decreasing size (BS 60, BS 80, BS 100, BS 150 and BS 200). Pores were fine enough to remove the larger particles of organic matter, but coarse enough to allow the desired spores to pass through. The suspension that passed through these sieve was saved and stirred to resuspend all particles. The heavier particles were allowed to settle for a few seconds, and the liquid decanted again through the sieve and spores, and then particles were collected by fine brushes and kept in different petriplates according to their size and colours. Moreover for further observations or purification of AMF spores, the sucrose gradient centrifugation method was used. In sucrose gradient centrifugation,[15] spores and minimal amount of organic particles could be further purified by suspending sieving in the 40% sucrose solution and centrifuging at 2,000 rpm (approximate 370 × *g*) for 1 min. The supernatant (with spores) was poured through the sieve of 400 mesh and rinsed with distilled water to remove sucrose residue. With the help of a simple microscope (20×) parasitized spores, plant debris, *etc.* were separated, and clean spores were stained with Melzar's reagent and studied microscopically. Spores of Glomus fasciculatum were separated from the mass of other AM spores by fine tweezers under dissecting microscope and were washed by distilled water several times to remove the adhered debris, followed by inoculation in the roots of 7–10-day-old seedlings of maize and sorghum plants grown in black plastic pots (12″) having autoclaved soil to discard the presence of other fungal propagules. After 45 days the presence of spores of *G. fasciculatum* were verified and inocula were prepared by mixing the chopped roots of maize/sorghum plants with the potted soil where extra radical spores of *G. fasciculatum* were present. Approximately >175 spores per 100 g could be considered as potent inocula for application.

Bacillus amyloliquefaciens TRS6 was isolated from the rhizosphere soil of 90-year-old tea bushes of Hansqua Tea Estate (Latitude: 26°41′51.59″N, Longitude: 88°17′26.81″E), Terai, West Bengal, India, and preliminarily identified on the basis of morphological, microscopic and biochemical tests. *In vitro* PGPR tests *viz.* IAA production, siderophore production, phos-

phate solubilization, chitinase, protease production[16–20] and *in vitro* antagonism of *B. amyloliquefaciens* to fungal pathogens – *Sclerotium rolfsii*, *Poria hypobrunnea*, *Fomes lamaoensis*, *Sphaerostilbe repens*, obtained from the culture collection of Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, were determined by paired culture as described by Chakraborty *et al.*[21] The identity of the bacterium was confirmed from the Plant Diagnostic and Identification Services, UK. The 16s rRNA gene sequence of *B. amyloliquefaciens* was also deposited in NCBI with Accession No. JN 983127. To prepare the inoculum, initially *B. amyloliquefaciens* was cultured in Nutrient Broth medium (Himedia, M002-100G, ingredients – peptic digest of animal tissue: 5.00 g/L; sodium chloride: 5.00g /L, beef extract: 1.50 g L^{-1}; yeast extract: 1.50 g L^{-1}; final pH 25°C –7.4±0.2) and was allowed to grow properly, with shaking at 37°C at 120 rpm for 48 hr. At the end of the log phase, bacterial culture was centrifuged at 10,000 rpm for 15 min, and the supernatant was discarded, selecting the bacterial pellet. Pellet was scraped into sterile distilled water. The aqueous suspensions were diluted as necessary to maintain the bacterial concentration at 108 *c.f.u*/ml. The aqueous suspension was then applied as a soil drench, at the rate of 100 ml per plant to the rhizosphere of tea plants 1 month after transplantation. Application was done at an interval of 1 month and three applications were done.

Tea roots were inoculated with *G. fasciculatum* alone and in combination with *B. amyloliquefaciens* which was applied as soil drench. Growth promotion was studied in terms of increase in height, number of leaves, number of shoots in six varieties of tea plants. Plants were grown under natural conditions of light and temperature (30±2°C). Observations were recorded after 1 and 2 months of application. For each treatment, 10 replicates were taken, and average of the 10 replicate plants was analysed. Leaf number and number of branches were also determined visually after 2 months of 1st dual application of *G. fasciculatum* and *B. amyloliquefaciens*. For improvement of plant health, the rhizosphere soil was further analysed to determine the soil texture, pH, moisture content, phosphorus, potassium, organic carbon and nitrogen to co-relate the changes followed by combined application of AMF and PGPR.

Leaves of tea plants of six varieties grown in treated or control soil were used for all biochemical analyses. Leaves were collected for assay 72 hr after inoculation. Peroxidase (POX, EC1.11.1.7), Chitinase (CHT, EC 3.2.1.14), Phenylalanine ammonia lyase (PAL, EC 4.3.1.5), β-1,3-glucanase (β-GLU, EC 3.2.1.39) and phenolics 22–26 were assayed.

For disease assessment, culture of *S. rolfsii* was grown in sand–maize meal medium (maize meal: sand: water – 1:9:1.5 w:w:v) in autoclavable plastic bags (sterilized at 20 lbs. pressure for 20 min) for a period of 3 weeks at 28°C until the mycelia completely covered the substrate. Rhizosphere of tea plants were inoculated by adding 100 g of previously prepared inoculum of S. *rolfsii* to the rhizosphere soil. Inoculation was done 3 days after final application of *G. fasciculatum* and *B. amyloliquefaciens*, singly or jointly. Disease assessment was performed following the method of Chakraborty *et al.*[21] after 15, 30 and 45 days of inoculation.

Polyclonal antibodies were raised against chitinase, bacteria as well as fungal pathogen in white, male rabbit following the procedure described by Alba and Devay.[27] Before immunization, normal sera were collected from rabbit. Following injection schedule with antigens, blood samples were collected and kept at 37°C for 1 h for clotting, followed by centrifugation at 5,000 rpm for 10 min at room temp. IgG was purified from the serum as described by Clausen.[28]

Plate-trapped antigen ELISA was performed following the method as described by Chakraborty *et al.*[29] The antigen (chitinase extracted from treated and control tea plants and soil antigen after bacterial as well as fungal inoculation to the rhizosphere) was diluted with coating buffer and IgG was diluted to 1:125 with phosphate buffer saline – Tween (PBST) containing 0.5% BSA. goat antirabbit IgG antiserum labelled with alkaline phosphatase and 4-nitrophenyl phosphate (pNPP), as enzyme substrate were used. Absorbance values in wells not coated with antigens were considered as blanks. The absorbance was determined in an Multiscan Ex (Labsystems) ELISA Reader at 405 nm. Dot immunobinding assay was carried out using the rhizosphere soil antigen and PAb raised against *S. rolfsii* following the procedure suggested by Lange *et al.*[30]

All results were analysed statistically by determining standard error, "*t*" test and ANOVA wherever appropriate.

Results

Population of different species of AM fungi isolated from the rhizosphere of six tea varieties were determined. Among the AM fungi, Glomus comprises 60–65% followed by Acaulospora 15–20%, Gigaspora 10–15%, Scutellospora 6–8% and Entrophospora 2%.Glomus

Table 1: Population of AMF Spores found in Different Varieties of Tea

AM fungi	AM species	AM spores in soil
Glomus	*G. aggregatum*	60–65%
	G. constrictum	
	G. fasciculatum	
	G. intraradices	
	G. microaggregatum	
	G. mosseae	
	G. pansihalos	
	G. albidum	
Acaulospora	*A. bireticulata*	15–20%
	A. delicata	
	A. laevis	
	A. scrobiculata	
	A. spinosa	
	A. undulata	
Gigaspora	*G. albida*	10–15%
	G. rosea	
	G. gigantea	
	G. margarita	
Scutellospora	*S. rubra*	6–8%
	S. pellucida	
	S. calospora	
Entrophospora	*E. colombiana*	2%

fasciculatum could be determined as the predominant one (as shown in Table 1 and Fig. 1). On the basis of consistent association of *Glomus fasciculatum* and easy recognition, it was selected. *B. amyloliquefaciens* was tested for different PGPR activities as described in section Materials and Methods. Results revealed that the bacterium could solubilize phosphate, produce siderophore, protease, chitinase, HCN and also IAA (29 µg ml^{-1}). *B. amyloliquefaciens* was tested for its antagonistic activities against several plant pathogenic fungi. In paired culture, it inhibited the growth of fungi such as *Poria hypobrunnea*, *Sclerotium rolfsii*, *Fomes lamaoensis* and *Sphaerostilbe repens* significantly (Table 2). In solid medium, inhibition ranged from 39% to 74%, while in liquid medium it ranged from 24% to 59%.

Application of *G. fasciculatum* and *B. amyloliquefaciens* in the rhizosphere of tea plants maintained in glasshouse and field conditions led to an increase in the growth in terms of increase in height and number

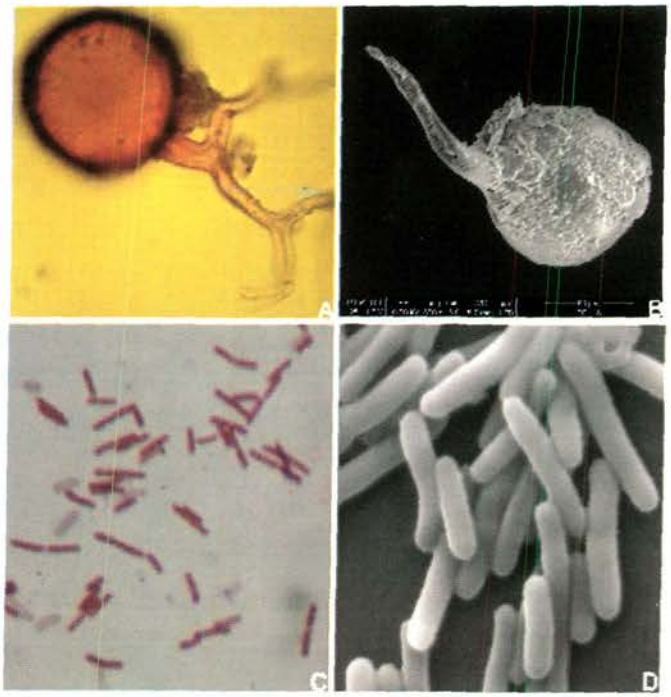

Figure 1. (A) *Glomus fasciculatum*, **(B)** SEM image of *G. fasciculatum*, **(C)** Light microscopic view and **(D)** SEM image of *Bascillus amyloliquefaciens*.

of leaves. Determination of height in seedlings, after 2 months of application, showed an increase in all varieties. On the other hand, increase in number of leaves was highly significant after 2 months of application in all varieties. But, joint inoculation with both the microorganisms gave most significant results in % increase in height as well as number of leaves after 2 months of application (Fig. 2). Highest % increase in height in T-78, UP-3 and UP-26 varieties were observed when tea plants were treated with joint inoculation of *G. fasciculatum* and *B. amyloliquefaciens*. Statistical analysis (ANOVA) also revealed that there was no significant difference among the varieties. Total phenol contents were estimated in tea leaves of different tea varieties following application of *G. fasciculatum*, *B. amyloliquefaciens* and challenge inoculation with *S. rolfsii*. In all cases, young leaves were selected from tea plants of all six varieties and analyses were done immediately after 72 hr of bacterial inoculation to the rhizosphere. There was a significant increase in phenol contents of leaves in all treatments (S.r, B.a+S.r, G.f+S.r and G.f +B.a +S.r), but most significant increase was when there was challenge inoculation (Fig. 3). Statistical analyses (ANOVA) revealed that there was significant differences in all treatments in case of total and ortho-phenol contents during disease development except between control and *S. rolfsii*-treated plant in determination of total phenol contents. In case of defense enzymes-POX, CHT (Fig. 4), PAL and

Table 2: *In Vitro* Antagonistic Tests of *B. amyloliquefaciens* against Tea Rootpathogens in Liquid Culture

Treatment	Dry Weight* (g)
Fomes lamaoensis	0.259±0.058
F. lamaoensis + *B.amyloliquefaciens*	0.105±0.028
Poria hypobrunnea	0.279±0.017
P. hypobrunnea + *B.amyloliquefaciens*	0.173±0.023
Spherostilbe repens	0.529±0.057
S. repens + *B.amyloliquefaciens*	0.321±0.069
Sclerotium rolfsii	0.465±0.015
S. rolfsii + *B.amyloliquefaciens*	0.254±0.046

*8 days of growth in PDB; Average of 3 replicates, ±S.E.

GLU (Fig. 5) also, similar results were obtained. But, no significant differences was observed between control and *S. rolfsii*-treated tea plants in case PAL and GLU activities.

Rhizosphere of tea was inoculated by *G. fasciculatum* and *B. amyloliquefaciens* prior to challenge inoculation with *Sclerotium rolfsii*. Development of blight was determined after 15, 30 and 45 days of inoculation on the basis of disease index. Results revealed that both microorganisms could reduce sclerotial blight, but maximum suppression of disease was due to joint inoculation (Table 3).

In order to confirm the induction of enhanced activities of defense enzymes due to treatment of *G. fasciculatum* and *B. amyloliquefaciens* with or both, immu-

Table 3: Effect of Application of Bioinoculants on Sclerotial Blight Disease of Tea

Tea Varieties	Disease index (45 days after inoculation) Pre treated with*			
	None	*G.f.*	*B.a.*	*G.f.* + *B.a.*
TV-18	4.1	1.3	3.2	1.9
TV-17	5.9	1.9	4.4	2.7
AV-2	3.5	1.1	3.0	1.6
T-78	4.0	2.0	2.9	2.1
UP-3	4.0	1.9	2.8	2.2
UP-26	5.4	2.4	3.4	2.5
CD ($P = 0.05$)	(treatment)		0.486	
	(varieties)		0.595	

*10pots/treatment;*B.a. = *Bacillus amyloliquefaciens*; G.f. = *Glomus fasciculatum*. Disease (Sclerotial Blight) Index computed on a scale of 0–6 on the basis of underground and above ground symptoms. Disease intensity was assessed as rot index on a scale of 0–6, depending on both underground and above ground symptoms as follows: 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 plants die, with upper withered leaves still remaining attached; roots fully rotted.

nological tests were done using PAbs raised against chitinase. Enzyme extracts were used as antigens and PTA–ELISA and Dot Blot were carried out. Results revealed that ELISA values of reaction of PAbs of chitinase with enzyme extracts from leaves grown in treated

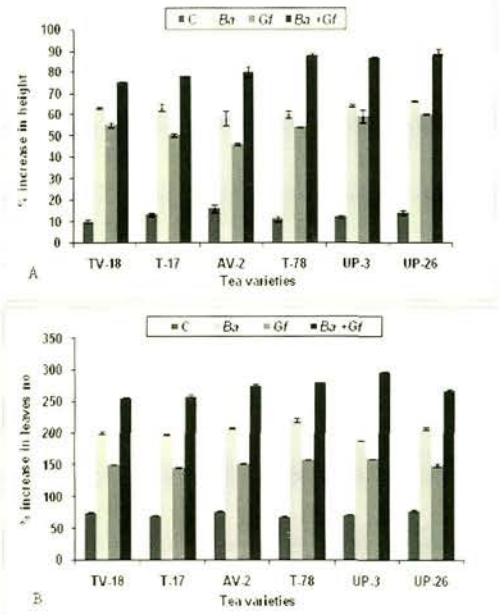

Figure 2. Effect of application of *Glomus fasciculatum* and *Bacillus amyloliquefaciens* on growth (%increase in height **A** and no. of leaves **B**) of plotted tea plants.

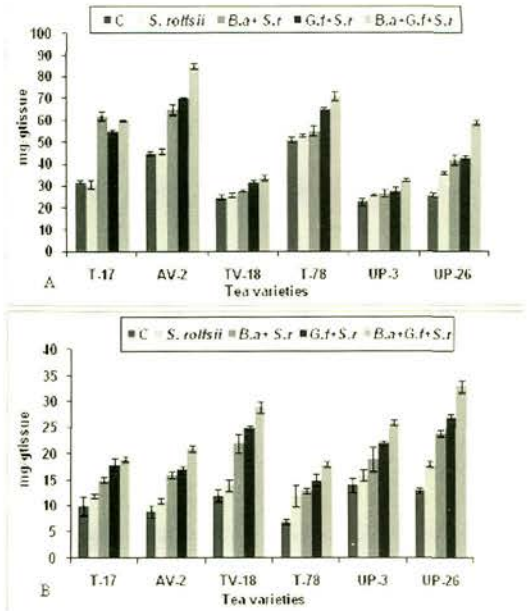

Figure 3. Total **(A)** and O-dihydroxy **(B)** phenols in leaves of tea varieties after different treatments.

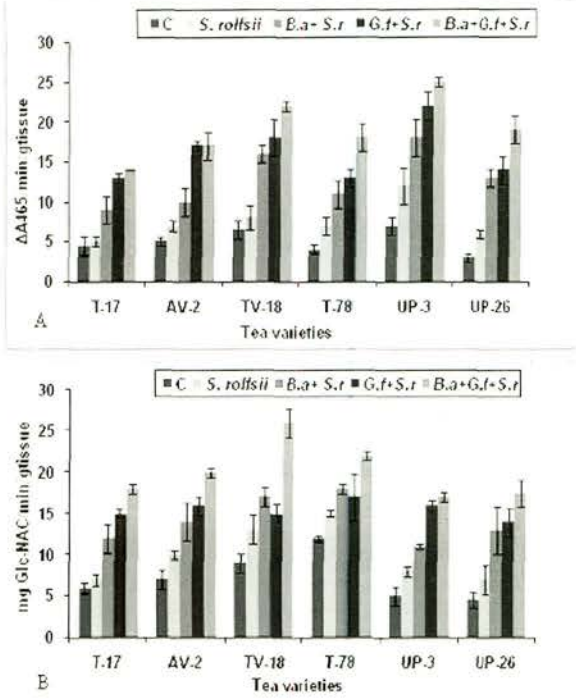

Figure 4. Peroxidase **(A)** and chitinase **(B)** activities in leaves of tea varieties after different treatments.

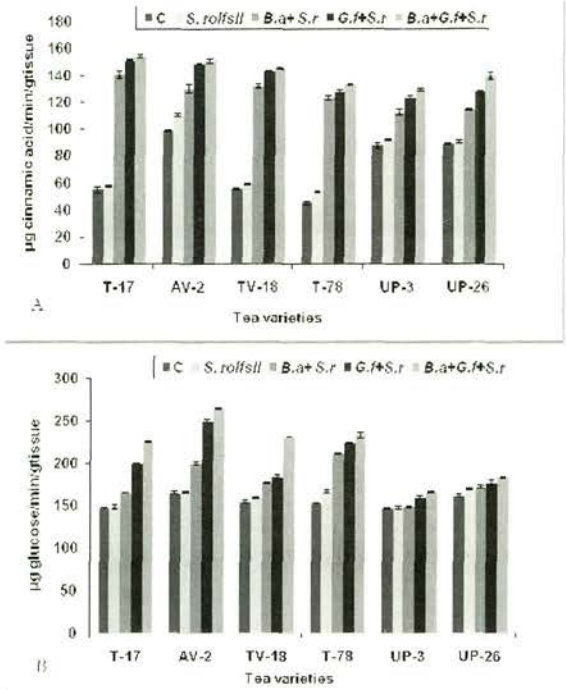

Figure 5. Phenyl alanine ammonia lyase **(A)** and β-1,3 glucanase **(B)** activities in leaves of tea varieties after different treatments.

soil were higher than the control values. Similarly, in dot-blot, more intense dots were observed in treated plants (Table 4). Besides, immunodetection of *S. rolfsii* in soil by ELISA and dot blot showed a significant reduction of population due to joint application of *G. fasciculatum* and *B. amyloliquefaciens* (Table 5).

Discussion

High population of AM fungi such as species of Glomus, Gigaspora, Scutellospora and Acaulospora were obtained. Of all of these, *Glomus fasciculatum* showed highest percentage of occurrence and it was selected for further tests. *Bacillus amyloliquefaciens*, isolated from the rhizosphere of tea, showed *in vitro* characteristics of plant growth-promoting bacteria such as phosphate solubilization, siderophore production. It was also antagonistic to a large number of fungi *in vitro*. *Glomus fasciculatum* and *Bacillus amyloliquefaciens* showed good growth

Table 4: Serological Assays of Chitinase Activity in Tea Leaves Following Application of AMF, and PGPR after Inoculation with *S. rolfsii* using PAb of Chitinase

Antigen source*	ELISA A 405	Dot-Blot Colour intensity#
Control	0.032±0.04	+
S. rolfsii inoculated	0.035±0.02	+
S. rolfsii + *G. fasciculatum*	0.470±0.03	++
S. rolfsii + *B. amyloliquefaciens*	0.423±0.18	++
S. rolfsii + *G. fasciculatum* + *B. amyloliquefaciens*	0.980±0.07	+++

* Enzyme extracts from leaves of plants treated as mentioned;
# Colour Intensity: += Light pink; ++ = Dark pink ; +++ = Deep pinkish.
±= S.E.

Table 5: Immunodetection of *S. rolfsii* in Soil after Treatments with Bioinoculants using PAb of *S. rolfsii*

Soil Antigen*	ELISA A 405	Dot-Blot Colour intensity**
Uninfested soil	0.306±0.09	–
Treatments		
S. rolfsii	1.058±0.05	++
G. fasciculatum	0.008±0.00	–
B. amyloliquefaciens	0.009±0.00	–
S. rolfsii + *G. fasciculatum*	0.438±0.003	–
S. rolfsii + *B. amyloliquefaciens*	0.457±0.002	–

Average of 3 replicates, ± = S.E. PAb (*S. rolfsii*) dilution: 1:500, * Sample collected 30 days after inoculation with pathogen, ** Colour intensity- Pinkish red- ++++; Bright pink- +++; Pink- ++; Light pink- +; No colour.

promotion in tea in glasshouse and field conditions and also suppression of blight by *Sclerotium rolfsii*. Both the microorganisms alone, or jointly, could promote growth of the seedlings in terms of increase in height and leaf number. However, joint application gave better results. AM fungi shows synergistic interaction with beneficial soil microbes such as nitrogen fixers and phosphate solubilizers. They also improve the activity of nitrogen-fixing organisms in the root zone.[31] AM fungi have been reported to occur naturally in alkaline or saline environments[32] and have been linked with increased plant biomass and development in saline alkaline soils.[33] Numerous studies have shown a substantial increase in plant growth and seed yield, following inoculation with PGPR strains.[34] Mathivanan *et al.*[35] also obtained synergistic effect of *Pseudomonas fluorescens* and *Trichoderma viride* in plant growth-promotion, yield enhancement and disease suppression in rice. Synergistic effect of *Rhizobium* sp. with either *P. putida*, *P. fluorescens* or *B. cereus* was obtained in pigeon pea, resulting in a significant increase in plant growth, nodulation and enzyme activity.[36]

Since *B. amyloliquefaciens* showed antagonism to *S. rolfsii in vitro*, it could have reduced the population of the pathogen in the soil, and hence, reduce disease severity. However, PGPR are known to act both by direct and indirect mechanisms, and biopriming with such PGPR can cause induction of resistance in the host. In the present study, activities of the different enzymes were analysed in tea following treatments with pathogen and microorganisms as follows: *S. rolfsii*, *B. amyloliquefaciens* + *S. rolfsii*, *G. fasciculatum* + *S. rolfsii*, *G. fasciculatum* + *B. amyloliquefaciens* + *S. rolfsii* as well as in control. Activities in phenolics and defense-related enzymes increased significantly during disease suppression. It is quite evident that, in the present study in addition to other mechanisms of action reported for *B. amyloliquefaciens* involving siderophore production, IAA production, antifungal metabolites and phosphate solubilization, induction of defense mechanisms play an important role in disease control and plant growth-promotion. Chakraborty *et al.*[37] reported that *Ochrobactrum anthropi* TRS-2, isolated from tea rhizosphere, could solubilize phosphate, produce siderophore and IAA *in vitro* and also exhibited antifungal activity against six test pathogens. Application of an aqueous suspension of *O. anthropi* to the rhizosphere of nursery-grown tea seedlings of five varieties of tea (TV-18, T-17, HV-39, S-449 and UP-3) led to enhanced growth of the treated plants, as evidenced by increase in height, in the number of shoots and number of leaves per shoot. Sundaresan *et al.*[38] also reported that in cowpea plants which had mycorrhizal association, accumulation of phytoalexins was much higher. Increased activity of chitinase, β-1,3-glucanase and peroxidase were obtained in sugar beet which was induced by treatment with *B. mycoides*.[40] Lavania *et al.*[13] also reported enhanced accumulation of phenolics and defense enzymes in betelvine treated with *S. marcescens* and challenge inoculated with *Phytophthora nicotineae*. In the present study, induction of activities of defense enzymes following application of *G. fasciculatum* or *B. amyloliquefaciens* was further confirmed by immunological tests using PAbs raised against chitinase. Results of ELISA and dot-blot revealed that joint application of *G. fasciculatum* and *B. amyloliquefaciens* significantly reduced *S. rolfsii* population.

Conclusion

In conclusion, it may be stated that *G. fasciculatum* was responsible mainly for induction of resistance within the host, where as *B. amyloliquefaciens* TRS-6 has been found to be a plant growth promoter with the ability to reduce sclerotial blight disease of tea which acts both by direct and indirect mechanisms in the host. Combined application of both microorganisms gave better results.

Acknowledgements

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References

1. Henning K. 1993. Response of roots to heavy metal toxicity. *Env Exploratory Bot* 33: 99–119.
2. Wan MT, Rahe TE, & Watts RG. 1998. A new technique for determining the toxicity of pesticides to the vesicular arbuscular mycorrhizal fungus, *Glomus intraradices*. *Env Toxi Chem* 17: 1421–1428.
3. Bonfonte-Fasolo P. 1987. Vesicular arbuscular mycorrhizae fungus plant interactions at the cellular level. *Symbiosis* 3(2): 249–254.
4. Singh R & Adholeya A. 2002. AMF biodiversity in wheat agrosystem of India. *Mycorrhiza News* 14(3): 21–23.
5. Kloepper JW & Schroth MN. 1978. Plant growth promoting rhizobacteria on radishes. IV. International Conference on Plant Pathogenic Bacteria, Angers, France, Vol. 2, pp. 879–882.
6. Kloepper JW. 1993. Plant growth promoting rhizobacteria as biological control agents. In: F Metting (Ed.), *Soil Microbial Ecology – Applications in Agricultural and Environmental Management*. Marcel Dekker: New York, pp. 255–274.

7. Kloepper JW, Zehnder GW, Tuzun S, Murphy JF, Wei G, Yao C, & Raupach GS. 1996. Toward agricultural implementation of PGPR-mediated induced systemic resistance against crop pests. In: T Wenhua, RJ Cook, & A Rovira (Eds), *Advances in Biological Control of Plant Diseases*, China Agricultural University Press: Beijing, pp. 165–174.
8. Bai Y, Souleimanov A, & Smith DL. 2002. An inducible activator produced by a *Serratia proteamaculans* strain and its soybean growth promoting activity under green house conditions. *J Exp Bot* 53: 1495–1502.
9. M'Piga P, Belanger RR, Paulitz TC, & Benhamou N. 1997. Increased resistance to *Fusarium oxysporium* f.sp. radicis – lycopersici in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 6328. *Physiol Mol Plant Pathol* 50: 301–320.
10. Chakraborty U, Chakraborty BN, & Chakraborty AP. 2010. Influence of *Serratia marcescens* TRS-1 on growth promotion and induction of resistance in *Camellia sinensis* against *Fomes lamaoensis*. *J Plant Interact* 5(4): 261–272.
11. Iyozumi H, Akutsu K, Hirayae K, Tsuchiya K, Hibi T, & Okuyama S. 1993. Biological control of broad bean chocolate spot disease by *Serratia marcescens* B2. *Ann Phytopathol Soc Jpn* 59: 723.
12. Yamamoto M, Iyozumi H, Akutsu K, Hirayae K, Hibi T, & Okuyama S. 1992. Suppressive effects by *Serratia marcescens* B2 against *Botrytis fabae* induced chocolate spot disease of broad bean. *Ann Phytopathol Soc Jpn* 58: 582–583.
13. Lavania M, Chauhan PS, Chauhan SV, Singh HB & Nautiyal CS. 2006. Induction of plant defense enzymes and phenolics by treatment with plant growth promoting rhizobacterial *Serratia marcescens* NBRII213. *Curr Microbiol* 52: 363–368.
14. Gerdemann JW & Nicolson TH. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc* 46: 235–244.
15. Daniels BA & Skipper HD. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: NC Schenck (Ed.), *Methods and Principles of Mycorrhizal Research*. The American Phytopathology Society, pp. 29–36.
16. Pikovskaya RI. 1948. Mobilization of phosphorous in soil connection with the vital activity of some microbial species. *Microbiologiya* 17: 362–370.
17. Schwyn B & Neiland JB. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160: 47–56.
18. Dobbelaere S, Croonenberghs A, Thys A, Vande Broek A, & Vanderleyden J. 1999. Photostimulatory effects of *Azospirillum brasilense* wild type and mutant strain altered in IAA production on wheat. *Pl Soil* 212: 155–164.
19. Kamil Z, Rizk M, Saleh M, & Moustafa S. 2007. Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol. *Glob J Mol Sci* 2: 57–66.
20. Walsh GA, Murphy RA, Killen GF, & Headon Power RF. 1995. Technical note: Detection and quantification of supplemental fungal β-glucanase activity in animal feed. *J Animal Sci* 73: 1074–1076.
21. Chakraborty U, Chakraborty BN, & Basnet M. 2006. Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. *J Basic Microbiol* 46: 186–195.
22. Chakraborty U, Chakraborty BN, & Kapoor M. 1993. Changes in the levels of peroxidase and phenyl alanine ammonia lyase in *Brassica napus* cultivars showing variable resistance to *Leptosphaeria maculans*. *Folia Microbiol* 38: 491–496.
23. Boller T & Mauch F. 1988. Colorimetric assay for chitinase. *Meth Enzymol* 161: 430–435.
24. Bhattacharya MK & Ward EWB. 1987. Biosynthesis and metabolism of glyceollin I in soybean hypocotyls following wounding or inoculation with *Phytophthora megasperma* f. sp. glycinea. *Physiol Mol Plant Pathol* 31: 401–409.
25. Pan SQ, Ye XS, & Kue J. 1991. A technique for detection of Chitinase, β-1,3-glucanase and protein patterns after a single separation using polyacrylamide gel electrophoresis and isoelectric focusing. *Phytopathology* 81: 970–974.
26. Mahadevan A & Sridhar R. 1982. *Methods in Physiological Plant Pathology* (2nd edition). Sivakami Publication India.
27. Alba APC & Devay JE. 1985. Detection of cross-reactive antigens between *Phytophthora infestans* (Mont.) de Bary and Solanum species by indirect enzyme- linked immunosorbent assay. *Phytopathology* 112: 97–104.
28. Clausen J. 1988. Laboratory Techniques in Biochemistry and Molecular Biology, Vol 1, Part 3, RH Burdon &PH Van Kinppenberg (Eds), pp. 64–65.
29. Chakraborty U, Basu P, Das R, Saha A, & Chakraborty BN. 1995. Evaluation of antiserum raised against *Pestalotiopsis theae* for the detection of grey blight of tea by ELISA. *Folia Microbiologica* 41: 413–418.
30. Lange L, Heide M, & Olson LW. 1989. Serological detection of *Plasmodiophora brassiceae* by dot immunobinding and visualization of the serological

by scanning electron microscopy. *Phytopathology* 79: 1066–1071.

31. Mosse B, Powell CL, & Hayman DS. 1975. Plant growth response to VAM IX interaction between VAM, rock phosphate and symbiotic nitrogen fixation. *New Phytol* 76: 316–342.
32. Pond EC, Menge JA, & Jarrell WM. 1984. Improved growth of tomato in salinized soil by vesicular arbuscular mycorrhizal fungi collected from saline soils. *Mycologia* 76: 74–84.
33. Poss JA, Pond E, Menge JA, & Jarrell WM. 1985. Effect of salinity of Mycorrhizal onion and tomato in soil with and without additional phosphate. *Pl Soil* 88: 307–319.
34. Wani PA, Khan MS, & Zaidi A. 2007. Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea. *J Plant Nutr Soil Sci* 170: 283–287.
35. Mathivanan N, Prabhavathy VR, & Vijayanandraj VR. 2005. Application of talc formulations of *Pseudomonas fluorescens* Migula and *Trichoderma viride* Pers.ex S.F. Gray decrease the sheath blight disease and enhance the plant growth and yield in rice. *J Phytopathol* 153: 697–701.
36. Tilak KVBR & Reddy BS. 2006. *Bacillus cereus* and *B. circulans* – Novel inoculants for crops. *Curr Sci* 90: 642–644.
37. Chakraborty U, Chakraborty BN, Basnet M, & Chakraborty AP. 2009. Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc-based formulation for enhancement of growth of tea plants and management of brown root rot disease. *J Appl Microbiol* 107(2): 625–634.
38. Sundaresan P, Raja Ubalthoose N, & Gunasekaran P. 1993. Induction and accumulation of phytoalexins in cowpea roots infected with a mycorrhizal fungus *Glomus fasciculatum* and their resistance to *Fusarium wilt* disease. *J Biosci* 18: 291–301.
39. Bargabus RL, Zidack NK, Sherwood JE, & Jacobsen BJ. 2004. Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. *Biol Control* 30: 342–350.