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Impact of arbuscular mycorrhizal fungus, *Glomus intraradices*, *Streptomyces* and *Pseudomonas* spp. strain on finger millet (*Eleusine coracana* L.*) cv Korchara* under water deficit condition

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In present study, a total of 104 bacteria and 96 actinomycetes were isolated from rhizosphere plant root of finger millet (*Eleusine coracana)* from Almora district of Uttarakhand, India. Isolates were characterized using microscopic and morphological methods followed by their biochemical test. Based on functional tests, four most promising isolates were identified using 23S rDNA primers as *Streptomyces labedae* (SB-9), *Streptomyces flavofuscus* (SA-11), and 16S rDNA primers as *Pseudomonas poae* (KA-5), and *Pseudomonas fluorescens* (KB-7). Influence of *Glomus intraradices* (G.I.) singly and along with actinomycetes and *Pseudomonas* spp. were evaluated under irrigated and water deficit conditions. Out of eight treatments studied in this study, a higher significant increase in plant height with *S. labedae* SB-9 + G.I and *P. poae* KA-5 + G.I were recorded. In well watered and water deficient conditions, maximum shoot dry matter content was recorded in P. *Poae* + P. *flurosence* + G.I and S. *labedae* + S. *flavofuscus* + G.I inoculated plants in comparison to their respective control. Under *in vitro* condition, *P. poae* KA-5 produced higher amount of indole acetic acid (IAA) among the selected isolates. Anti-oxidant enzyme, superoxide dismutase (SOD) and accumulation of free proline content in leaves were maximum in *P. Poae* + G.I inoculated plants under water deficit condition. In the presence of *Streptomyces* and *Pseudomonas* spp. a better mycorrhizal infection was observed in treated plant roots. This investigation support mutual symbiosis of *Streptomyces* and *Pseudomonas* strains with *G. intraracides* which had synergic effect on finger millet cv. Korchara plant under irrigated as well as under water stress condition.

Key words: *Glomus intraradices*, *Streptomyces*, *Pseudomonas*, anti-oxidants, plant growth promotion, IAA production

INTRODUCTION

Finger millet (*Eleusine coracana*) is a staple crop in Africa as well as in India. In India it is cultivated mainly in the tarai regions of Himalayas and the southern peninsula. Finger millet grains are rich in methionine and tryptophan, which is lacking in the diets of poor people living on starchy foods like cassava, plantain, polished rice, and

maize meal. Wheat and rice provide food security, but crops like finger millet promise nutritional security for the world (Bhatt et al., 2011). Drought, a situation of severe water deficit, recurrently occurs in various parts of the world, often with devastating effects on crop productivity. Incidentally, the arid and semiarid zones that are primarily affected by water deficit, have traditionally contributed around 40% of the total production of all categories of food grains (Thakurta, 2010). Plants protect themselves against mild drought stress by accumulating osmolytes.

Proline is one of the most common compatible osmolytes in drought stressed plants. Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans, 2008). Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). Plants protect themselves from drought induced oxidative damage, through an array of anti-oxidative enzymes like superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) etc., which act synergistically to limit the levels of reactive oxygen species. Several reports underlined direct relationship between enhanced anti-oxidant enzyme activities and increase tolerance to environmental stresses (Sayfzadeh and Rashidi, 2011; Gusain et al., 2014; Gusain et al., 2015a).

Arbuscular mycorrhizal fungi (AMF) can provide numerous benefits to their host plants, including improved nutrient uptake, drought resistance, and disease resistance (Sharma et al., 2014). Seven fungal species belonging to the genus *Glomus* were studied (Ruiz-Lozano and Azcón, 1995) for their ability to enhance the drought tolerance of lettuce plants (*Lactuca sativa* L.cv. Romana). The ranking of AMF effects on drought tolerance, based on the relative decreases in shoot dry weight, was as follows: *Glomus deserticola* > *G. fasciculatum* > *G. mosseae* > *G. etunicatum*> *G. intraradices* > *G. caledonium* > *G. occultum*. It was observed that mycorrhizal fungi had consistent effects on plant growth, mineral uptake, the CO_2 exchange rate, water use efficiency, transpiration, stomatal conductance, phosphorus use efficiency, and proline accumulation under either well-watered or drought-stressed conditions.

Plant growth promoting rhizobacteria (PGPR) are usually in contact with the root surface or rhizoplane, and increase plant growth and yield by one or more mechanisms such as improved mineral nutrition, disease suppression and phytohormone production (Weller, 1988; Lugtenberg and Dekkers, 1999; Défago and Keel, 1995; Gusain et al., 2015b). An additional possibility is that the beneficial effects of some PGPR are due to their interactions with AMF (Bianciotto et al., 2001). AM fungus, *Glomus mosseae,* and fluorescent pseudomonad (Sanchez and colleagues, 2004) had similar impacts on plant gene induction, supporting the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms. Specific interactions between AM fungi and PGPR most likely occur, and certain groups of bacteria have been shown to be established to a much higher extent in the mycorrhizosphere compared with other groups.

Actinomycetes are one of the major components of the microbial populations present in soil. In addition, these bacteria are known for their economic importance as producers of biologically active substances, such as antibiotics, vitamins and enzymes (De Boer et al., 2005). Actinomycetes are also an important source of diverse antimicrobial metabolites (Lazzarini et al., 2000; Terkina et al., 2006). Actinobacteria were often found to be associated with AMF spores. Mugnier and Mosse (1987) reported that *G. mosseae* spores germinated *in vitro* only in the presence of microorganisms, including *Streptomyces orientalis* and Ames et al. (1989) found that out of 190 spores examined, 100 were colonized by one or more chitin-decomposing microorganisms; 82% were colonized by actinomycetes, 17% by bacteria, and 1% by fungi. Actinomycetes found from the rhizosphere of field grown plants *Trifolium repens* L. (Franco-Correa et al., 2010) was able to improve plant growth and nutrition, and benefit root colonization by AM fungi.

Manulis et al. (1994) described the production of the plant hormone indole-3-acetic acid (IAA) and the pathways of its synthesis by various *Streptomyces* spp. including, *Streptomyces violaceus*, *Streptomyces scabies*, *Streptomyces griseus*, *Streptomyces exfoliatus, Streptomyces coelicolor,* and *Streptomyces lividans,* while prior works had reported IAA synthesis in *Streptomyces* spp. (El- Sayed et al., 1987). This was the first confirmation of its production using modem analytical methods such as HPLC and GC-MS. Manulis et al. (1994) provided a detailed description of the IAA biosynthetic pathways in Streptomyces. This study lent further credence to the possibility that certain rhizobacteria, including the actinomycetes, may act as

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Abbreviations: SOD, Superoxide dismutase; **G.I.,** *Glomus intraradices*; **VAM,** vesicular arbuscular mycorrhiza; **AMF,** arbuscular mycorrhiza fungi; **MHB,** mycorrhiza helper bacteria; **H_2O_2,** hydrogen peroxide; **CAT,** catalase; **GPX,** guaiacol peroxidase; **APX,** ascorbate peroxidase; **PGPR,** plant growth promoting rhizobacteria; **IAA,** indole acetic acid; **CAS,** chrome azurole S; **NBT,** nitro-blue tetrazolium; **PVK,** Pikovaskaya medium.

plant growth enhancers.

There are reports available on the beneficial effect of AMF and PGPRs' on crop growth however, the combined effect of AMF, bacteria and actinomycetes has not been observed in detail especially under water deficient conditions. Therefore, the aim of this study was to evaluate the interaction effect of arbuscular mycorrhiza fungus and PGPR's (bacteria and actinomycetes) on the growth and development of finger millet under water deficit condition.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from rhizosphere of finger millet plants growing at different locations at Almora district of Uttarakhand in India. Plant roots were dug out and rhizospheric soil was taken in sterile poly begs stored at 4°C for further experimental use. Total six soil sample were collected for isolation of bacteria and actinomycetes.

Isolation and characterization of bacteria from rhizospheric soil

The isolation of bacteria was done by serially diluted soil suspension (rhizospheric soil) onto selective isolation agar medium, and incubated at 28 ± 2°C on Nutrient Agar and Actinomycetes Isolation Agar (Himedia) medium, respectively. Isolates were further purified and stored at 4°C. Characterization of bacteria and Actinomycetes was done on the basis of their colony morphology, including substrate and aerial mycelium, color, size, shape and type of colonies on isolation medium. Pseudomonas colonies giving fluorescence against reflected light were picked up and purified further by streaking on the King's B agar media. Selected isolates were identified upto the genus level by morphological and biochemical characteristics (Palleroni, 1984).

Genomic DNA extraction from isolates

Seven days old pure culture grown in ken knight broth was pelleted in 50 ml centrifuge tube by centrifugation at 10,000 rpm X *g* for 15 min at room temperature. Supernatant was discarded and the cells pellets were resuspended in lysis buffer (TE buffer, 10 % SDS and Proteinase K) followed by C-TAB method (Rogers and Bendich, 1994). DNA was collected and loaded onto 1% agarose gels containing 0.5 µg of ethidium bromide ml^{-1} and run for 1 h at 60 V in 1X TAE. DNA fragments of approximately 15 kb, which represented the vast majority of the extracted DNA, was observed under UV trans-illuminaton (Sambrook et al., 1989).

PCR amplification

The amplification was done from total genomic DNA using universal primers of actinomycetes (23S rRNA) Actino 23 F- CCGANAGGCGTAGBCGATGG, Actino 23 R - CCWGWGTYGGTTTVSGGTA, and universal primers for bacteria (16S rRNA) GM 3 F- AGAGTTTGATCMTGG, GM 4 R – TACCTTGTTACGACTT. These primers amplify approximately 361 (bp) and 1500 base pairs (bp), respectively. The (50 µl) PCR mixture contained 25 pmol of each primer, 2.5 mM of each dNTP (dATP, dGTP, dTTP, dCTP (GeNei), 10 XReaction buffer (100 mM Tris/HCl, 500 mM KCl, 15 mM $MgCl_2$) (GeNei), 0.5 unit of Taq DNApolymerase (Larova) and 10 to 50 ng of genomic bacterial DNA. The PCR program was performed on a thermal cycler (Biometra/Biored) system. The DNA and ddH_2O were subjected to a denaturation step of 98°C for 2 min followed by addition of the rest of the PCR mix and 30 cycles of 97°C for 30 s, 55°C for 30 s, 72°C for 1 min; a final re-annealing at 55°C and extension at 72°C for 15 min (Gao and Gupta, 2005). Following thermal cycling, the PCR products were visualized by agarose gel electrophoresis and captured (Bio-red) Gel Doc. Sizes of the amplicons were assessed by comparison with a 1 kb ladder (GeNei) run in the agarose electrophoresis.

Phosphate solubilization by isolates

All the isolates were tested to solubilize the insoluble phosphate on Pikovaskaya agar medium (Himedia). Bacterial cultures were incubated up to 48 h and actinomycetes cultures were incubated up to 4 days at 28 ± 2°C. Appearance of clear zones around the colonies indicated positive test.

Starch hydrolysis by isolates

The ability of isolates to excrete hydrolytic enzymes capable of degrading starch was tested on starch agar medium (Tryptone 10 g/l, starch 3 g/l, yeast extract 10 g/l, K_2HPO_4 5 g/l, agar 20 g/l). The isolates were inoculated onto starch agar plate and incubated for 48 h at 28 ± 2°C. Starch in the presence of iodine produce blue colorization on the plate and yellow zone around the colonies showed amylolytic activity considered as positive result.

Siderophore production by isolates

Siderophore production test was conducted with selected isolates; Chrome Azurole S (CAS) agar test method was adopted as reported (Schwyn and Neilands, 1987). Bacterial isolates were inoculated on CAS agar plate by spot inoculation using autoclaved tooth pick, incubated at 28 ± 2°C for 48 h (bacteria) and 4 days for actinomycetes. Yellow-orange color hollow zone around the colony was considered positive for siderophore production. Based on diameter the ability of isolates was measured.

IAA estimation

IAA production by actinomycetes and bacteria were estimated according to Gordon and Weber (1951) method by inoculating the isolates in 5 ml Luria Bertanni broth supplemented with 0.01% tryptophan separately and incubated for 3 days at 28 ± 2°C. The cells were centrifuged at 3000 rpm for 5 to 7 min, supernatant were collected in separate tubes. Appearance of pink colour after the addition of 4 ml of Salkowski's reagent to 2 ml of culture supernatant confirmed the production of IAA. Quantitative measurement of IAA was determined by recording absorbance at 535 nm by using UV/VIS spectrophotometer (Ray-Leigh UV 2601).

Growth conditions

Total 14 isolates were selected to investigate plant growth promotion activity on finger millet without Mycorrhiza (*Glomus intraradices*). To examine plant growth promotion effect of these isolates, a pot experiment was conducted under maintained glasshouse conditions, supplementary light at 40 µE $m^{-2}s^{-1}$, with a 16/8 h day/night cycle at 28°C and 50% humidity. Based on the

results (data not mentioned) from pot experiment, four most potent strains, that is, KA-5, KB-7, SB-9 and SA-11, were selected to study interaction and symbiosis with mycorrhiza (*G. intraradices*) on finger millet plants under well watered and water deficit condition.

Test organisms

Mycorrhizal spore inoculum of *G. intraradices* (G.I.) were collected from Rhizosphere Biology Lab, (by Dr. A. K. Sharma) Department of Biological Sciences, CBSH, GBPUAT, Pantnagar, India, two *Pseudomonas* spp. strains and two actinomycetes from rhizosphere of finger millet from Almora district of Uttarakhand, India were used for greenhouse experiment.

Mycorrhizal (*G. intraradices*) inoculum development

Mycorrhizal spore of *G. intraradices* were isolated following wet sieving and decantation method proposed by Gerdemann and Nicolson (1963). Spores were mass produced in sand and soil (2:1) mixture which was autoclaved three times at regular interval of 24 h at 121°C and 15 lbs. Maize plants were grown as host crop. Pots were irrigated regularly to maintain moisture and water level. Inoculum was harvested at 90 days of inoculation after achieving adequate number of spores and percentage root colonization (Krishna et al., 1981). Roots were chopped to make fine pieces of 1 to 2 cm length and mixed with substrate along with spores properly to make homogenized mixture of AM inoculum.

Screening of mycorrhiza in roots

Root samples from each replication were carefully separated and washed with tap water followed by de-ionized water for microscopic examination. Root segments were cut into 0.5 to 1 cm in length, kept in 10% KOH for at 95°C for 1 h and then immersed in 5% HCl for 10 min. Finally, roots were stained with trypan blue (Phillips and Hayman, 1970) for overnight. AM infection was quantified under microscope (Biermann and Lindermann, 1981).

Streptomyces and *Pseudomonas* spp. interactions between *Glomus intraradices*

A pot experiment was conducted to investigate the effect of Streptomyces and *Pseudomonas* spp. interaction with AM fungi on plant growth and enzyme activity under two water levels (well water and water deficit) conditions. Finger millet seeds were surface sterilized with 2% sodium hypochlorite for 2 min (Guo et al., 2004), and sown in sterilized soil and sand (1:2) mixture. AMF inoculum was supplied in holes having spore quantity of 40 to 50 g^{-1} soil with infected roots and mycelium. Bacterial and actinomycetes strains were inoculated on rootlets system during transplanting at a concentration of 10^8 and 10^5 cells/pot, respectively. There were eight treatments Control (T1), G. *intraradices* (G.I) (T2), *Pseudomonas Poae* + G.I. (T3), *Pseudomonas flurosence* + G.I. (T4), P. *Poae* + P. *flurosence* + G.I. (T5), S. *labedae* + G.I (T6), S. *flavofuscus* + G.I. (T7) and S. *labedae* + S. *flavofuscus* + G.I. (T8). Each treatment had three replicate. Plants were irrigated with water on every 2 to 3 days as needed and with Hoagland's (Hoagland and Arnon, 1950) solution weekly. Pots were placed in green house chamber with complete randomized order. Plants were grown for 45 days and water deficit stress was inflict at 45 day old plant, by withholding irrigation, until the soil water content declined progressively to 45% of the soil water holding capacity, which occurred after eight days of withholding water. All the plants were irrigated on alternate days and maintained at 75 to 80% of soil water capacity (SWC). First harvesting was done after 45 days of sowing at 0 day of drought stress (75 to 80% swc), second harvesting /sampling was done after 10 days of imposing stress (45% swc). After harvesting plant fresh weight were taken immediately and sample were placed in -80°C for determination of biochemical assays. After taking the plant sample for proline content and antioxidant activity, the remained sample were dried at 60°C for 78 h to measure the dry biomass of the sample.

Plant growth parameters

Plant height

The plant height was measured from base of the plant up to the tip of the fully opened top leaf at 45 and 55 days of plant growth and expressed in centimetres.

Plant biomass

The root and shoot portions of the uprooted plants were separated and oven dried at 60°C for 24 h. The dry weight is expressed in grams per plant.

Influence of *Pseudomonas* and *Streptomyces* strains on enzyme activity

Estimation of free proline content

Free proline was estimated by the method of Bates et al. (1973). Leaf material (0.1 g) was homogenized in 4 ml sulfosalicyclic acid (3% w/v in distilled water), centrifuged at 10,000 rpm for 30 min at room temperature. Chromophore was extracted in toluene by vigorous stirring. The absorbance of chromophore was measured at 520 nm. Concentration of proline samples was computed from standard curve of L–proline.

Measurement of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed spectrophotometrically at the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) by proposed method of Beyer and Fridowich (1987). The reaction mixture consisted of 50 mM Na-phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µM NBT, 10 µM EDTA.Na_2, 2.0 µM riboflavin and 0.3 ml enzyme extract. Reaction mixture containing tubes were incubated for 10 min in 4,000 lux at 35°C. One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560 nm.

Total protein estimation

Protein concentration in all samples was determined using Bradford dye binding method (Bradford, 1976).

Statistical analysis

The data were analyzed using SPSS software. Measurements were performed in three replicates for each treatment (n = 3). The data were subjected to one way analysis of variance (ANOVA), with treatment (non-stressed and stressed) used for evaluating isolates and the differences between the means were evaluated using least significant difference (LSD) at $p < 0.05$. Different letters denote significant differences among treatments in non-stressed and stressed condition.

Table 1. Sample collection site and number of bacterial and actinomycetes colonies isolated on agar medium.

Sample number	Location of collected soil	Decoding	Number of isolated bacteria	Number of isolated actinomycetes
1	Chosli (site 1)	CA	24	22
2	Chosli (site 2)	CB	14	10
3	Kosi (site 1)	KA	11	19
4	Kosi (site 1)	KB	29	8
5	Sunoli (site 1)	SA	7	21
6	Sunoli (site 2)	SB	19	16

Table 2. Biochemical activities of selected isolates.

Strain	Starch hydrolysis	Phosphate solubilization	Siderophore activity	Catalase activity	Organism
KA-5	+	+++	++	++	*Pseudomonas poae*
KB-7	-	+++	++	++	*Pseudomonas flurosence*
SB-9	+	+	-	+	*Streptomyces labedae*
SA-11	+	-	+	+	*Streptomyces flavofuscus*

-, Negligible; +, moderate; ++, good; +++, intensive.

RESULTS AND DISCUSSION

Isolation and characterization of P-solubilising bacteria

A number of actinomycetes (96) and bacteria (104) were obtained by serial dilution method from six rhizospheric soil of healthy plant roots of finger millet. All isolates were designated as shown in Table 1. Isolates were characterized by their different morphology, size, shape, color and pigments. Actinomycetes and bacterial colonies distinctly different were picked and transferred to test the ability to phosphorus and starch solubilization, siderophore production, and catalase activity. Four promising isolates were selected for interaction and symbiosis study (Table 2).

Identification of actinomycetes and bacteria

Genomic DNA from actinomycetes and bacteria were isolated with some modifications in CTAB method, amplified using universal primers 23S rDNA (Gao and Gupta, 2005) and 16S rDNA (Muyzer et al., 1995), respectively. All bacterial isolates were sequenced and further BLAST analysed (http://blast.ncbi.nih.gov/Blast.cgi). Four most potential bacteria were closely similar to *P. paoe* KA-5 (KF719959), *P. flurosence* KB-7 (KF719960), *S. labedae* (SB-9) and *S. flavofuscus* (SA-11). Artursson et al. (2005) used molecular tools to bypass the problems commonly encountered with culture-based approaches to visualize changes in actively growing bacterial community compositions as a result of *G. mosseae* inoculation or plant species. They discovered that mostly 'uncultured bacteria' and *Paenibacillus* sp. were active in the *G. mosseae* inoculated soil, suggesting that many species of interest may be missed if relying on culturing alone.

Phosphate solubilization activity by actinomycetes and bacteria

Pseudomonas strains KA-5 and KB-7 exhibited very intensive activity of phosphate solubilization while lesser in actinomycetes strains (Table 2). Balakrishna et al. (2012) and Srivastav et al. (2004) found good phosphate solubilization activity of actinomycetes, *Bacillus* sp. and *Pseudomonas* sp. at different pH level in phosphate rich media for more than 10 days of incubation. Many soil microorganisms especially *Pseudomonas* spp. Are effective in releasing P from inorganic and organic pools of total soil P through solubilization or mineralization and are known as phosphate-solubilizing microorganisms (PSM). Some actinomycete strains have been tested (Marcela et al., 2010) to have the ability of solubilizing sparingly available inorganic P sources or mineralizing some P from the organic P sources in soil. In fact, 70% of then solubilized inorganic P in the PVK medium and all of them acidified the SRSM-1 media.

Quantitative estimation of IAA

Quantitative estimation of IAA by actinomycetes and bacterial strains grown in LB broth with and without

Table 3. Quantitative estimation of IAA (µg/ml) of selected isolates.

Strain	IAA Production (µg/ml)
Pseudomonas poae KA-5	59.55
Pseudomonas flurosence KB-7	38.83
Streptomyces labedae SB-9	18.32
Streptomyces flavofuscus SA-11	12.29

Table 4. Effect of inoculation *Pseudomonas* and *Streptomyces* strains with AMF (*Glomus intraradices*) on growth parameters of finger millet.

Treatment	Well water condition				Water deficit condition			
	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length(cm)	Shoot dry weight(g)	Root dry weight (g)
T1 (Control)	53.67^{a}	37.83^{a}	0.75^{a}	0.34^{ab}	68.50^{a}	38.17^{b}	1.71^{ab}	0.40^{a}
T2 (*G. intraradices* (G.I.)	64.08^{bc}	31.33^{a}	1.11^{bc}	0.41^{b}	74.67^{b}	28.67^{a}	1.72^{abc}	0.43^{a}
T3 (*P. Poae* + G.I.)	68.50^{c}	38.33^{a}	1.01^{abc}	0.25^{a}	79.33^{b}	46.83^{c}	2.13^{bc}	0.48^{a}
T4 (*P. flurosence* + G.I.)	58.50^{ab}	30.67^{a}	0.94^{ab}	0.29^{ab}	76.50^{b}	31.67^{a}	1.90^{abc}	0.44^{a}
T5 (*P. Poae* + *P.Flurosence* + G.I.)	65.25^{bc}	42.67^{a}	1.28^{c}	0.40^{b}	73.83^{ab}	39.67^{b}	1.91^{abc}	0.46^{a}
T6 (*S. labedae* + G.I.)	66.08^{bc}	26.67^{a}	1.00^{abc}	0.26^{a}	79.33^{b}	39.50^{b}	1.94^{abc}	0.39^{a}
T7 (*S. flavofuscus* + G.I.)	65.00^{bc}	31.83^{a}	1.14^{b}c	0.39^{ab}	76.00^{b}	40.67^{b}	1.52^{a}	0.37a
T8 (*S. labedae* + *S. flavofuscus* + G.I.)	66.33^{bc}	30.83^{a}	1.09^{bc}	0.37^{ab}	77.67^{b}	47.33^{c}	2.21^{c}	0.52^{a}

Mean followed by same letter are not significantly different (P < 0.05) for a particular trait in two cultivars at all the level of stress.

tryptophan were estimated. All four strains differed in their capacity to produced IAA. The IAA production was found maximum by *Pseudomonas* strain KA-5 (59.55 µg/ml) and KB-7 (38.83 µg/ml), while strain *S. labedae* SB-9 and *S. flavofuscus* SA-11 produced a significant amount (18.32 and 12.29 µg/ml), respectively, when culture medium was supplemented with 1 mg/ml L-Tryptophan at 28°C incubation (Table 3). Maximum amount of IAA produced by *Pseudomonas* sp. were reported recently (Kaur and Sharma, 2013) that PGPR-3 (70.05 µg/ml), PGPR-2 (66.79 µg/ml) in the presence of L-Tryptophan as precursor of IAA. Studies on endophytic actinomycetes (Gangwar et al., 2012), reported the amount of IAA by actinomycetes in the range of 18 to 42 µg/ml. Several reports on IAA and GA3 by actinomycetes, isolated from different source have also been indirected by (Khamna et al., 2009; Strzelczyk and Pokojska, 1984; Mohandas et al., 2013; Shrivastava et al., 2008; Kamal and Sharma, 2014).

Effect of *G. intraradices*, *Streptomyces* and *Pseudomonas* spp. on plant growth

Majority of the plant inoculated AM fungus with actinomycetes and *Pseudomonas* spp. or all the three in combination showed higher effect on growth parameter as compare to their respective mycorrhizal and non-mycorrhizal control under both well watered (0 days) and water deficit condition (10 days) (Table 4). So, the present study indicated that the *G. intraradices* along with selected actinomycetes and *Pseudomonas* spp. strain can enhanced plant growth under normal and water stress condition. However, antagonistic interaction between AM fungus and actinomycetes has been reported (Krishna et al., 1982) when added simultaneously. The growth and phosphorus nutrition of finger millet on a sterile, phosphorus-deficient soil was improved by inoculation with either the *Glomus fasciculatus* or with *Streptomyces cinnamomeous.* Streptomyces reduced spore production and development of infection by *Glomus*, while Glomus reduced the multiplication of streptomyces. This antagonism stimulated plant growth less than individual inoculations. When plants were examined to mild and severe water stresses, the drought stressed mycorrhizal plants inoculated with actinomycetes sp. strain SB-9 (*S. labedae*) and *Pseudomonas* sp. strain KA-5 (*P. poae*) enhanced plant height (15.40 and 15.05%, respectively). *S. labedae* SB-9 increased total dry matter (48.19%) in well water condition while in water deficit condition combined effect of both strains *S. labedae* and *S. flavofuscus* (T8) was achieved 35.89% followed by T6, 32.95% over control plants (Table 4). The synergic effect of three actinomycetes strains (Marcela et al., 2010) improved AM (*G. mosseae*) mycelial growth, stimulated spore germination in clover (*T. repens* L.) plants. Actinomycetes and *G. mosseae* co- inoculation promoted

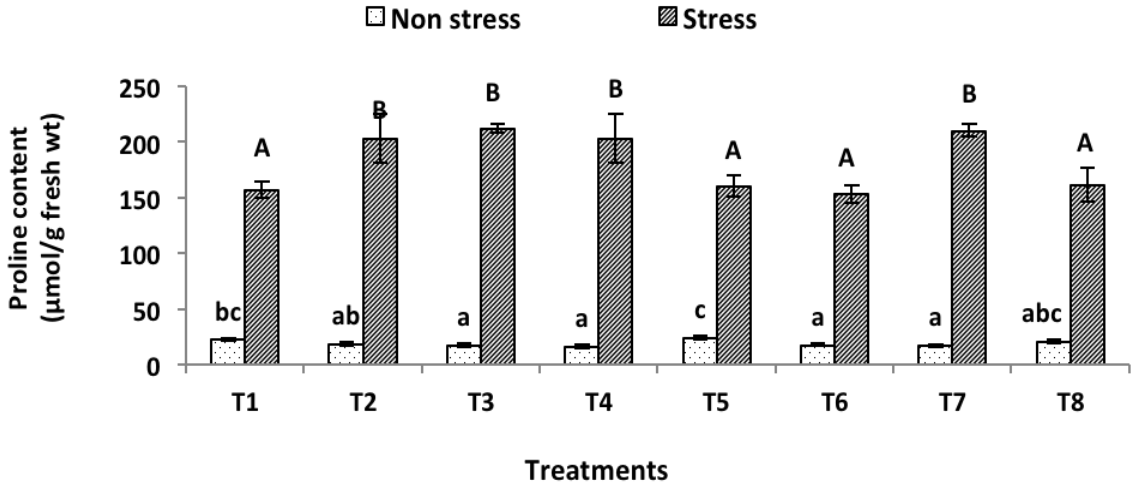

Figure 1. Free Proline content in different treatments T1, Control (without mycorrhiza); T2, *Glomus intraradices* (G.I.); T3, (G.I.) + KA-5 (*Pseudomonas poae*); T4, (G.I.) + KB-7 (*Pseudomonas fluoresence*); T5, (G.I.) + SB-9 (S. labedae); T6, (G.I.) + SA-11(*S. flavofuscus*), T7- (G.I.) + KA-5 + KB-7 (Consortia) and T8- (G.I.) + SB-9 + SA-11 (Consortia) exposed to water stress (10 days), different letters denote significant differences ($P < 0.05$) exposed to water stress (10 days), different letters denote significant differences ($P < 0.05$).

plant growth and N acquisition. Mohandas et al., 2013 examined association of *G. mosseae* with actinomycetes (*Streptomycetes canus*) from ten months old guava (*Psidium guajava* L.cv Mridula), significant increase in plant height and dry matter over control recorded.

Effect on proline content and SOD activity under stress condition

Non–mycorrhiza and mycorrhiza alone treated with bacteria evaluated for determination of free proline content were significantly variable. A significant increase in proline content was recorded in the leaves of stressed plant in all the treatments (Figure 1). In this experiment, three replicates of each treatment were studied, a maximum increase of 1.34 and 1.33 (T3) fold in proline content was recorded followed by 1.01 and 1.0 (T7), over non-mycorrhiza (T1) and mycorrhiza control (T2), respectively. Increased accumulation of proline under drought confers ability of lower osmotic potential as well as to protect DNA, enzyme and cellular membranes from oxidative damage (Liu et al., 2009; Gomes et al., 2010). Treatment T4 recorded the lowest increase of 1.29 fold in proline level under water deficit condition over non-mycorrhiza (T1) plants there were no such increase when compared to mycorrhiza (T2) control plants. A significant increase in superoxide dismutase activity in treatment (T3) 2.69 and 1.32 fold were recorded (Figure 2) in oxidative stress condition over non-mycorrhiza (T1) and mycorrhiza control (T2), respectively. In stress condition, induced SOD help in reducing reactive oxygen species such as superoxide radicals and hydrogen peroxide (H_2O_2) oxidative stress and thus lowering risk of cellular damage and increased stress tolerance. Kohler et al. (2009) demonstrated the greater activity of antioxidants in lettuce plants under drought conditions when inoculated with *Pseudomonas mendocina* and AMF (*G. intraradices* or *G. mosseae*) and suggested that the use of inoculants to alleviate the oxidative damage elicited by drought. Saravanakumar et al. (2011) reported *Pseudomonas* strains when inoculated to green gram and observed a significant increase in antioxidant enzymes when plants were exposed to water stress condition.

Conclusion

From the results of this experiment we can assume that the *G. intraradices* provide some degree of defence alone and when inoculated with *P. poae* strain KA-5 and *S. labedae* strain SB-9 under water deficit condition. *Pseudomonas* and *Streptomyces* Strains showed synergic effect at plant growth as well as enhancement in anti-oxidative enzymes such as proline and SOD under water deficit condition. It was observed that *Streptomyces* and *Pseudomonas* strains had mutual symbiosis with *G. intraradices* on finger millet. This co-inoculum can be used in rain-fed agriculture where rainfall occurs irregularly, could provide some benefits to plants under such conditions. These bacteria can be considered as Mycorrhiza helper bacteria (MHB), therefore this study support mutual symbiosis of *S. labedae* strain SB-9 and *P. poae* strain KA-5 between *G. intraradices* having beneficial effects on finger millet plant under normal and water deficit condition.

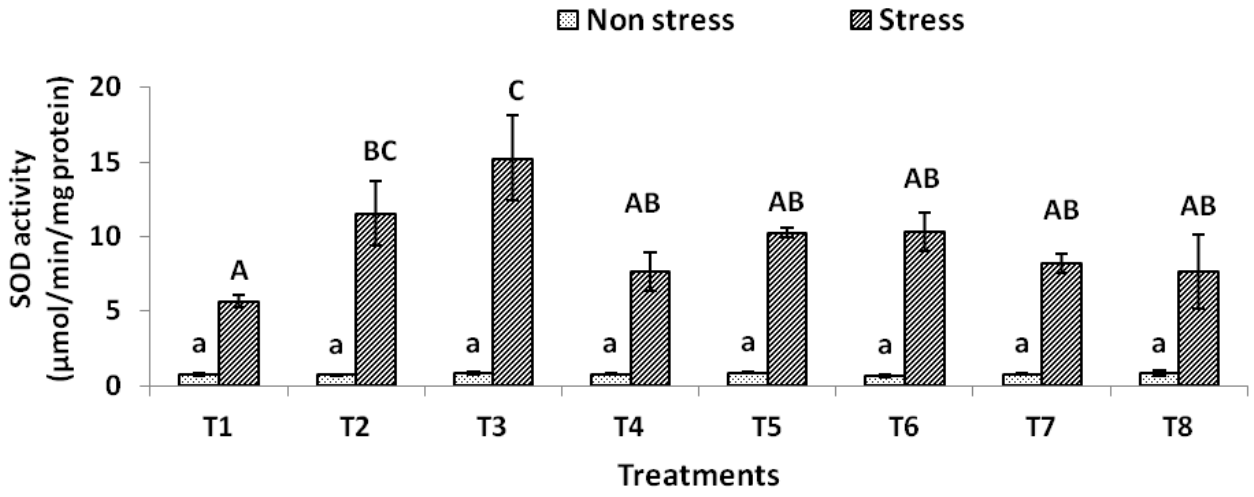

Figure 2. Superoxide dismutase (SOD) activity in different treatments. T1, Control (without mycorrhiza); T2, *Glomus intraradices* (G.I.); T3, (G.I.) + KA-5 (*Pseudomonas poae*); T4, (G.I.) + KB-7 (*Pseudomonas fluoresence*); T5, (G.I.) + SB-9 (S. labedae); T6, (G.I.) + SA-11(*S. flavofuscus*), T7- (G.I.) + KA-5 + KB-7 (Consortia) and T8- (G.I.) + SB-9 + SA-11 (Consortia) exposed to water stress (10 days), different letters denote significant differences ($P < 0.05$) exposed to water stress (10 days), different letters denote significant differences ($P < 0.05$).

Conflict of interests

The authors did not declare any conflict of interest.

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