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Impact of *Piriformospora indica*, *Pseudomonas* species and *Mesorhizobium cicer* on growth of chickpea (*Cicer arietinum* L.)

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Abstract: The present study was conducted to examine synergistic interactions among *Piriformospora indica* (PI) with potential plant growth promoting rhizobacteria (PGPR) and *Mesorhizobium cicer* (LGR33, MR) in two chickpea (*Cicer arietinum* L.) varieties *viz. desi* PBG1 and *kabuli* BG1053. Different PGPR species were used *viz.* native isolates of *Pseudomonas argentinensis* (LPGPR1), *Pseudomonas sp.* (LPGPR2) along with national check *Pseudomonas sp.* (LK884). Compatibility of MR, PI and different *Pseudomonas* spp. was studied by streak assay method and growth of fungal pellicle *in-vitro*. Consortium of MR+PI+LPGPR1 (0.605 g dry weight fungal pellicle/100ml nutrient broth) was found as the best compatible treatment. *In–vivo* the synergistic effect of consortia was studied for improving dry weight of roots, nutrient acquisition, colonization and stress tolerance ability in chickpea. Significant improvement in dry weight of root was observed with MR+PI+LPGPR1 (1.316 g plant^{-1}) in comparison to MR alone treatment (0.980 g plant^{-1}) at 90 days after sowing (DAS). Percentage colonization of *P.indica* improved significantly with consortium MR+PI+LPGPR1 (75.5 and 78.3 %) treatment at 90 DAS. All the treatments significantly improved total soluble sugar content (12.2-26.9 %); amino N content (1.36-1.80 fold) and stress tolerance ability (4-6 fold) over the MR alone treatment. Reducing sugar content significantly improved with MR+PI+LPGPR1 (0.62 and 0.79 mg mL^{-1}) over MR alone (0.42 and 0.58 mg mL^{-1}) treatment in *desi* PBG1 and *kabuli* BG1053 chickpea, respectively. The tripartite combination MR+LPGPR1+PI can be explored as potent biofertilizer for improvment in chickpea productivity.

Keywords: Chickpea, *Mesorhizobium cicer*, Multipartite interactions, *Piriformospora indica*, *Pseudomonas* species

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important draught-tolerant food legume in the world. Chickpea being a symbiotic crop establishes a cooperative association with bacterium *Mesorhizobium* of family Rhizobiacea to fulfill its nitrogen (N) requirements and fixes about 141 Kg ha^{-1} yr^{-1} molecular N (Singh, 2014). The success of rhizobial inoculation on plant roots is limited by several factors, including environmental conditions, number of infective cells applied, presence of competitive native rhizobia and inoculation method (Jida and Assefa, 2012). The native rhizobia often have nutritional competitive advantage over the inoculated strains which fail to ensure effective nodulation and adequate Biological Nitrogen Fixation (BNF). One of the strategies to increase survival and establishment of inoculated *Mesorhizobium* sp. in soil environment is to integrate it with other plant growth promoting rhizobacteria (PGPR). PGPR are known to improve plant growth by direct and indirect mechanisms (Bashan and de-Bashan 2010). Recently, co-inoculation of *Mesorhizobium* sp. with *Pseudomonas* aeruginosa was reported to significantly increase the nodulation, plant growth and yield in chickpea. (Verma *et al*., 2013). *Piriformospora indica*, belongs to the order Sebacinales including mostly endophytic fungi with cryptic biodiversity (Oberwinkler *et al*., 2013). *P. indica* helps in mobilization of insoluble phosphates (Varma *et al.,* 1999) and also provides protection against biotic and abiotic stress including root and leaf pathogens (Johnson *et al.,* 2014), enhances seed production and stimulates active ingredients in plants allowing plants to grow under extreme physical and nutrient conditions (Danesh, 2015). Co-inoculation of *P. indica* with PGPR (*Pseudomonas striata*) was found to have synergistic effect on its population build up and plant dry biomass with respect to single inoculation in chickpea (Meena *et al*., 2010). Recently there is emerging trend on application of binary and multiple mixtures of microbes for improving plant productivity. Beneficial micro-organisms have the potential to reduce the dependence on chemicals, increase resistance to biotic and abiotic stress while still maintaining productivity of crop (Mishra *et al.,* 2014). Therefore, the present study was designed to investigate compatibility of *P. indica* with PGPR and *Mesorhizobium in-vitro* and assess the synergistic effect of this tripartite mixture for plant growth promotion , percentage colonization, nutrient acquisition and stress tolerance in chickpea.

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MATERIALS AND METHODS

Procurement of Reference cultures: *Mesorhizobium cicer* (LGR33, MR) used in the present study was procured from Pulses Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana and maintained on Yeast Extract Mannitol Agar (YEMA) medium. Reference strain of PGPR (*Pseudomonas sp.* LK884) was procured from GB Pant University of Agricultural and Technology, Uttarakhand and native strains of PGPR isolated from chickpea rhizosphere *viz. Pseudomonas argentinensis* (LPGPR1) and *Pseudomonas sp.* (LPGPR2) were procured from Pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana and were maintained on King's B medium. Fungus *Piriformospora indica* (PI) was procured from Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar and was subcultured on Potato dextrose agar (PDA) medium and stored at 4ºC in refrigerator.

Compatibility of *P.indica* with different PGPR and *Mesorhizobium:* Compatibility of *P.indica* with different *Pseudomonas* spp. (LPGPR1, LPGPR2 and LK884 (reference)) along with recommended strain of *Mesorhizobium cicer* (LGR33) was examined *in-vitro* by streak assay method (Raja *et al*., 2006) and on the basis of growth of fungal pellicle. A small block of agar (PDA) with fungal growth was cut using sterile cork borer, placed in the centre of fresh nutrient agar plate and kept as control. In streak assay, different *Pseudomonas* strains (LPGPR1, LPGPR2 and LK884) were streaked on one side of fungal bit with LGR33 on the opposite side and allowed to incubate at 25±2°C for 3–5 days. Each treatment had three replications with one control plate with small block of agar with fungal growth. For examining growth of fungal pellicle fresh inoculum of *Mesorhizobium cicer* (LGR33) was prepared by growing in Yeast Extract Mannitol Broth (YEMB) whereas different *Pseudomonas* species (LPGPR1, LPGPR2 and LK884) were grown in Luria broth for 24–48 hrs. *P. indica* was raised in PDA medium. One ml inoculum of each organism was then transferred to 100 ml nutrient broth in separate 250 mL conical flask in triplicate, with different PGPR combinations. The inoculated cultures were incubated at 25±2°C in rotary shaker for 6–8 days. Fungal pellicle was dried in hot air oven at 60°C and weight was recorded.

Cultivation of chickpea: Field experiment was conducted in *rabi* season (2011–12) at Pulses Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The pooled mean of maximum and minimum temperature during crop growth period was 23.9°C and 9.3°C, respectively. Experimental soil was loamy sand having pH (8.2) with low organic carbon (0.12 %) and available N (92.0 kg acre^{-1}), medium available P (18.4 kg acre^{-1}) and high available K (105 kg acre^{-1}). Chickpea seeds of varieties *desi* (PBG1) and *kabuli* (BG1053) were procured from the Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana. Seeds were applied at the rate of 18-20 kg acre^{-1} for *desi* and 35-37 kg acre^{-1} for *kabuli* for sowing. Experiment was designed in factorial randomized block design (FRBD) with 36 plots and 8 rows having row to row distance of 30 cm and plant to plant distance about 10 cm with net plot size 5.4 sq.m. Seeds were inoculated with recommended cultures of MR, PI and different PGPR spp. (LK884, LPGPR1 and LPGPR2) as per treatment. Twenty gram charcoal inoculants were used per kg of chickpea seeds for inoculation in monoculture treatment. In co-inoculation treatments, *Mesorhizobium cicer*, *P.indica* and different PGPR strains were applied to chickpea seeds in ratio of 1:1:1. Before sowing, inoculated seeds were air dried at room temperature under shade and sown with in two hours. Each treatment had three replications. All the agronomic practices were followed for raising chickpea crop.

Dry weight of root: Roots of three randomly selected plants were uprooted at 60 and 90 DAS from each plot and dry weight of root was recorded in g.

Root colonization studies: The observations were recorded on percentage colonization of *P.indica* at 90 and 120 DAS by randomly selecting 5-10 root samples from the chickpea root. Colonization was studied by staining roots with trypan blue (Phillips and Hayman, 1970). The stained root sections were studied under microscope (Leica type 020-518.500) for the presence of hyphae, vesicles and spores. The percentage colonization was calculated for the inoculated plants according to following formula:

% Infection= (no of fungus infection segments/Total no segments examined)100

Collection of root exudates: Frozen root tissues were homogenized at 4 ºC in an ice-chilled mortar in QB buffer (Kumar *et al*., 2009). Crude homogenates were centrifuged at 15 000 *g* for 15 min at 4 ºC, and the supernatant fractions were frozen at -20 ºC. Data on various plant biochemical parameters *viz.* total soluble and reducing sugars, amino N content and antioxidant enzyme activities *viz.* superoxide dismutase (SOD) and catalase (CAT) activities were analyzed at 90 and120 DAS, respectively.

Analysis of plant biochemical parameters: Root exudates from different treatments were collected at 90 DAS and studied for total soluble and reducing sugars and amino nitrogen content. Total soluble sugars (TSS) of root exudates from different treatments were assayed spectrophotometrically at 490 nm (DuBois *et al*., 1956) using 5 % phenol reagent followed by concentrated sulphuric acid (H_2SO_4). For analyzing reducing sugars, root exudates from different treatments (0.1 mL) were subjected to reaction with Nelson's arsenomolybdate reagent (Somogyi, 1952). The absorbance of clear solution was read at 620 nm

using UV-Vis spectrophotometer. Amino N content of root exudates of different treatments was determined by using ninhydrin reagent at 570 nm (Moore and Stein, 1948).

Antioxidant enzyme activities: Antioxidant enzymes activities viz. Catalase (CAT) and Superoxide dismutase (SOD) activities of root exudates from different treatments were recorded at 120 DAS.

Catalase (CAT) activity: The catalase activity of root exudates was calculated on the basis of micromoles of hydrogen peroxide (H_2O_2) oxidized per minute per g fresh weight of root tissue extracted with phosphate buffer (Aebi, 1983).

Superoxide dismutase (SOD) activity: For SOD activity, the root exudates were treated with Tris-HCl buffer, EDTA and pyragallol solution (Marklund and Marklund, 1974). A unit of enzyme activity had been defined as amount of enzyme causing 50% inhibition of auto-oxidation of pyragallol observed in blank.

Statistical analysis: The data was analyzed using analysis of variance (ANOVA). Critical difference (CD) at 5% percent level was calculated. Further mean separation of treatment effects was accomplished using Tukey's Honestly Significant Difference Test. All the data analysis was carried out using SAS software (Slaughter and Delwiche, 2010) using Factorial Randomized Block Design.

RESULTS AND DISCUSSION

Compatibility of *P. indica* with different PGPR (LPGPR1, LPGPR2 and LK884) along with *Mesorhizobium cicer* (MR) in streak assay method revealed that *P. indica* and *Mesorhizobium* showed better

compatibility with LPGPR1 and LK884 in comparison to LPGPR2 (Plate 1). In the present study, synergistic effect of *Mesorhizobium cicer*, *P.indica* and different PGPR enhanced the growth of fungal pellicle (*P.indica*). Maximum dry weight of fungal pellicle was observed with the consortium of MR+PI+LPGPR1 (0.605 g dry weight fungal pellicle 100mL^{-1} nutrient broth) followed by MR+PI+LK884 (0.591 g dry weight fungal pellicle 100 mL^{-1} nutrient broth) and MR+PI+LPGPR2 (0.578 g dry weight fungal pellicle 100 mL^{-1} nutrient broth) as compared to the *P.indica* alone (0.515 g dry weight fungal pellicle 100 mL^{-1} nutrient broth) (Fig.1). Indole acetic acid (IAA) is best known for its role in plant signal transduction (Ghosh *et al*., 2013). *Mesorhizobium cicer* and LPGPR1 used in the present study are known to produce IAA (Kaur and Sharma, 2013). However, this hormone can act as a signal molecule in bacteria and fungi (Leveau and Preston, 2008) and was also found to induce adhesion and filamentation of *Saccharomyces cerevisia* (Prusty *et al*., 2004). Therefore, IAA produced by PGPR and *Mesorhizobium cicer* might have induced filamentation and increased dry weight of the fungal pellicle.

Table 1. Synergistic effect of different multiple inoculations on dry weight, total soluble sugar, reducing sugars and amino nitrogen content in chickpea.

Treatments	Dry weight of root plant^{-1} (g)			Total soluble sugars (mg mL^{-1})			Reducing sugars (mg mL^{-1})			Amino N content (mg mL^{-1})		
	PBG1	BG1053	Mean	PBG1	BG1053	Mean	PBG1	BG1053	Mean	PBG1	BG1053	Mean
Control	(0.092) 0.898	(0.118) 0.850	(0.105) 0.874	1.13	1.37	1.25	0.23	0.26	0.25	0.142	0.132	0.137
MR	(0.108) 0.991	(0.138) 0.975	(0.123) 0.980	1.45	1.67	1.56	0.42	0.58	0.50	0.164	0.151	0.158
PI	(0.125) 1.206	(0.141) 1.256	(0.133) 1.231	1.25	1.46	1.36	0.46	0.56	0.51	0.176	0.175	0.176
MR+PI+LPGPR1	(0.137) 1.248	(0.165) 1.383	(0.151) 1.316	1.72	2.24	1.98	0.62	0.79	0.70	0.303	0.267	0.285
MR+PI+LPGPR2	(0.127) 1.205	(0.145) 1.262	(0.136) 1.234	1.57	1.92	1.75	0.50	0.57	0.54	0.200	0.229	0.215
MR+PI+LK884	(0.131) 1.223	(0.154) 1.327	(0.143) 1.275	1.66	2.17	1.92	0.54	0.7	0.62	0.253	0.251	0.252
Mean	(0.120) 0.979	(0.144) 1.176	(0.132) 1.077	1.46	1.81	1.63	0.46	0.58	0.52	0.206	0.201	0.204
CD 5%	Variety (V): 0.004 (0.114) Treatment (T): 0.007 (0.198) V×T: NS (0.280)			Variety (V): 0.03 Treatment (T): 0.06 V×T: 0.08			Variety (V): 0.04 Treatment (T): 0.07 V×T: NS			Variety (V): 0.006 Treatment (T): 0.009 V×T: .014		

*The data for all the parameters represents values at 90 days after sowing (DAS) and that in parenthesis for dry weight of roots represents values at 60 DAS. It has been analyzed using Analysis of Variance (ANOVA) and critical difference (CD) was calculated at 5% significance level. All the values are average of three replicates.

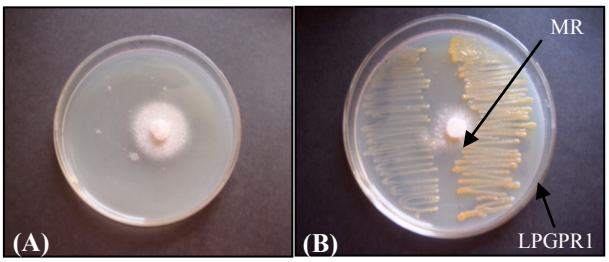

Plate 1. *Compatibility of P.indica with Mesorhizobium cicer along with P. argentinensis (LPGPR1). (A) Control (P.indica); (B) MR+PI+LPGPR1.*

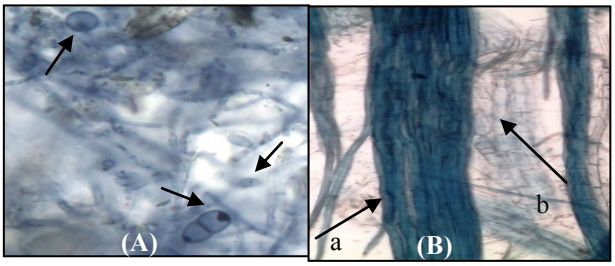

Plate 2. *Intracellular root colonization of P. indica in chickpea (A) Chlamydospores (black arrows) (B) Hyphae of P.indica (black arrow a) and root cells (black arrow b)*

The data in Table 1 recorded significant improvement in dry weight of root at 60 and 90 DAS with multiple inoculations of *Mesorhizobium cicer*, *P.indica* and different PGPR as compared to *Mesorhizobium cicer* alone treatment. There was significant (P<0.05) difference between the varieties for dry weight of root; however interaction between variety and treatment was significant at 90 DAS. Mean of both the varieties at 60 DAS revealed that multiple inoculation of MR+PI+LPGPR1 showed maximum increase in root dry weight (0.151 g plant^{-1}) (Meena *et al*., 2010) followed by MR+PI+LK884 (0.143 gplant^{-1})and MR+PI+PGPR2 (0.136 g plant^{-1}). At 90 DAS significant increase in dry weight of root was observed in MR+PI+LPGPR1 (1.316 g plant^{-1}) followed by MR+PI+LK884 (1.275 g plant^{-1}) and MR+PI+PGPR2 (1.234 g plant^{-1}) treatments (P<0.05). These results are corroborated with the earlier findings reporting that co-inoculation of the endophytic fungus *P.indica* with the phosphate-solubilising bacterium *P.striata* signifi-cantly increased dry plant biomass in comparison to treatment with single inoculation and non-inoculated control in chickpea (Meena *et al*., 2010). Moreover, co -inoculation of the PGPR (*Pseudomonas* and *Bacillus*) with effective *Rhizobium* strains of chickpea resulted in a significant improvement in the growth parameters (Parmar and Dadarwal, 1999). It was further supported by investigation in *Arabidopsis* where *P. indica* formed intracellular structures in the epidermal root cells and caused changes in the root growth, leading to branched root systems (Sirrenberg *et al*., 2007). Since the fungus also releases auxin in the culture medium and thus affecting root growth which is responsible for, or at least contributes to, the beneficial interaction between the two symbionts (Oelmuller *et al*., 2009).

P.indica successfully colonizes roots of chickpea plants with hyphal growth of chlamydospores (Plate 2) showing balanced symbiotic relationship (Murphy et al 2013). Data on percent colonization (Figs. 2 a and b) revealed that all the multiple inoculations *P.indica* treatments varied significantly over control and MR alone treatment (90 and 120 DAS). At 90 DAS significantly higher colonization percentage was found in MR+PI+LPGPR1 (75.5 and 78.3 %) followed by MR+PI+LK884 (70.7 and 73.3%) and MR+PI+LPGPR2 (57.7and 55.7%) in *desi* PBG1 and *kabuli* BG1053 chickpea, respectively (P<0.05). In uninoculated control plots native fungal (*Glomus* sp.) colonization was 38.3 and 30.3%, whereas in MR alone treatment 43 and 40.3 % colonization recorded in *desi* PBG1 and *kabuli* BG1053 chickpea, respectively. This colonization revealed the infective potential of both the native mycorrhiza present in the legume rhizosphere along with inoculated *P.indica*. At 120 DAS maximum colonization percentage was found in MR+PI+PGPR1 (60.7 % and 61.7 %) followed by MR+PI+LK884 (ranged between 58.3 % and 60.3%). A positive influence of root colonization with *P.indica* treatments on vegetative growth and development in chickpea plants was observed in present study. Our study is well in line with Bagde *et al.*, (2010) for *Spilanthes calva* and *Withania somnifera* where *P.indica* treated plants showed pronounced root colonization and growth relative to non-inoculated control . Similarly, it was demonstrated that root colonization of *Adhota vasica* by *P.indica* increased with time from 53 to 95 % (Rai *et al*., 2001). There was remarkable enhancement in the growth rate of plant inoculated with *P.indica* over the control so it is reasonable to assume that beneficial interaction between host plant and *P.indica* is dependent on the size and surface of the root, which can be colonized by fungus (Oelmuller *et al*., 2009). However, this was in disagreement with the earlier work where root colonization of the *Arabidopsis* (tf12) mutant having dwarf phenotype and small root surface is strongly reduced due to reduced auxin and glucosinolate level (Bennett *et al*., 2005). Colonization is a time dependent phenomenon

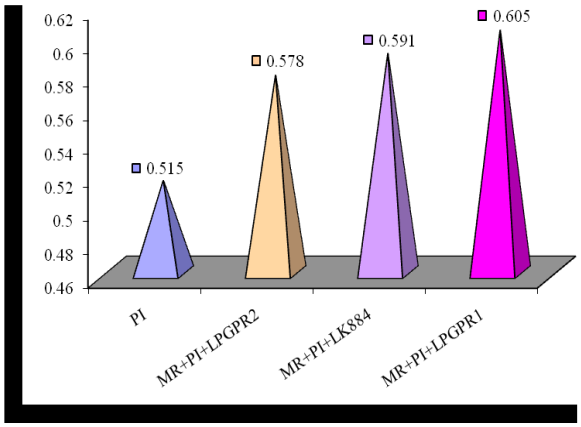

Fig.1. *Synergistic effect of different multiple inoculations on dry weight of fungal pellicle in chickpea.*

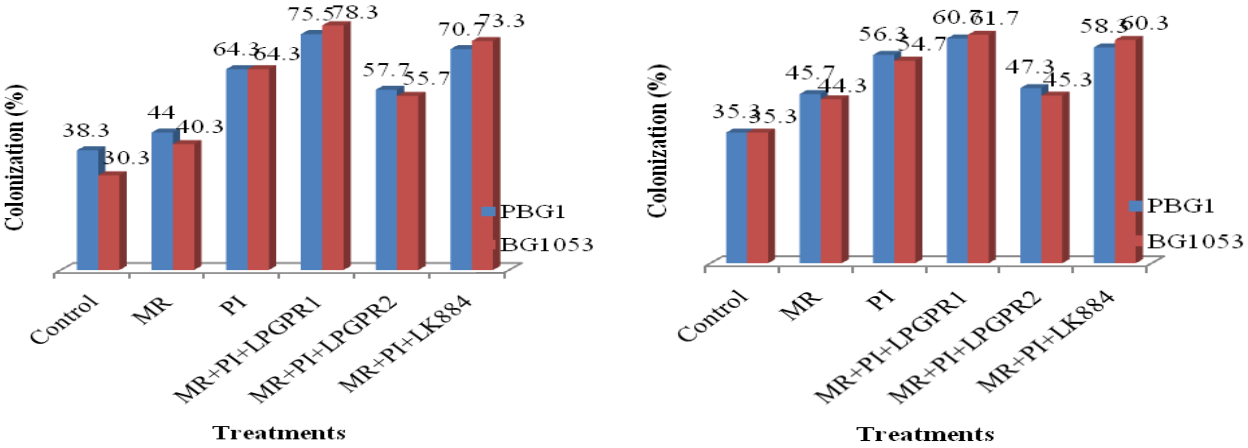

Fig. 2. *Synergistic effect of different multiple inoculations on P. indica colonization (90 and 120 DAS).*

affected by several factors as light, moisture, host genotype, age of host plant, nutrient deficiency (Badri and Vivanco, 2009). However, in present study *P.indica* colonization further showed decreasing trend towards crop maturity stage. It was related to limited carbohydrate availability, as a consequence of a decline in photosynthesis due to mobilization of the reserves towards the pod formation and also due to qualitative and quantitative changes in the root exudate towards the root senescence (Haichar *et al*., 2014). However, colonization in MR alone showed improvement over the control. *Mesorhizobium cicer* could have acted synergistically on the growth of native mycorrhiza. Our results are well supported by the study that inoculation with *Rhizobium* significantly increased root colonization by native mycorrhizal fungi and spore numbers in soil in lentil (Lakshman and Kadam, 2011). There was increase in the percent colonization in all the multiple inoculations and also the PI alone as compared to the MR alone treatment. Moreover, the colonization percentage was significantly higher in MR+PI+LPGPR1 followed by MR+PI+LK884 over the PI alone treatment at 90 and 120 DAS (P<0.05) . It is well documented that highest mycorrhizal colonization percentage was found in plants inoculated with the AMF and *B.coagulans* suggested a possible mechanism of PGPR due to hydrolytic enzymes which caused the cortical cells to dilate, provided a larger intercellular surface area for AMF to penetrate and colonize more easily (Mamatha *et al*., 2002). This study well corroborates with our results. Similarly, PGPR- *Azospirillum brasilense* with production of IAA stimulated mycorrhizal colonization of *Glomus deserticola* in maize and enhanced spore germination and mycelial growth, which in turn increased the chance of contact between fungal hyphae and plant roots (Sharma *et al*., 2008).

Variation in total soluble sugar, reducing sugar and amino N content was recorded in *desi* and *kabuli* chickpea varieties (Table 1). It is well documented that root exudation rates also vary with developmental stage of plant and between genotypes within single species (Inceoglu *et al*., 2010 and Haichar *et al*., 2014). The data in table 1 depicts that all the treatments significantly improved total soluble sugars as compared to uninoculated control treatment at 90 DAS (P<0.05). On the basis of pooled mean, MR+PI+LPGPR1 treatment significantly improved total soluble sugar content in the range of 12.2-26.9 % over the *Mesorhizobium cicer* alone treatment followed by MR+PI+LK884 and MR+PI+LPGPR2. Variety BG1053 recorded significantly high amount of total soluble sugars as compared to variety PBG 1. Significant improvement in the reducing sugars was recorded with MR+PI+LPGPR1 (0.62 and 0.79mg

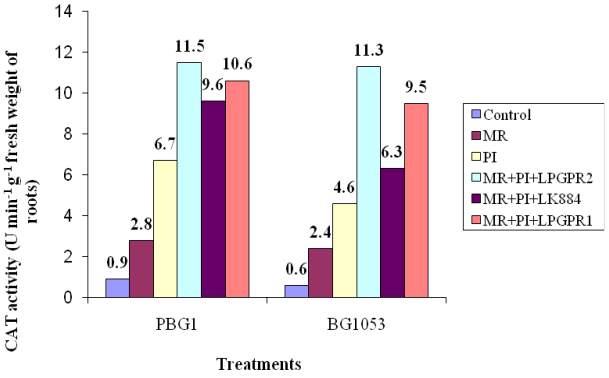

Fig. 3. *Synergistic effect of different multiple inoculations on CAT activity.*

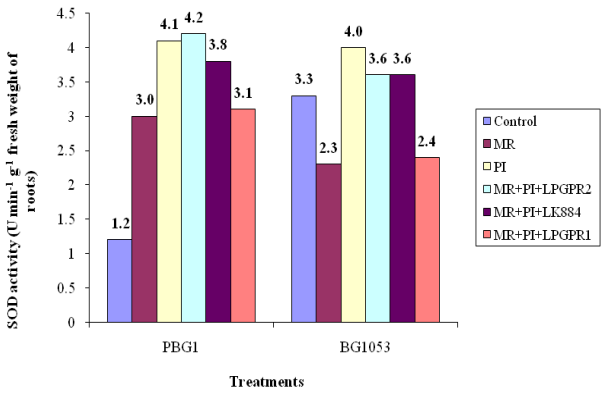

Fig. 4. *Synergistic effect of different mutiple inoculations on SOD activity.*

mL^{-1}) and MR+PI+LK884 (0.54 and 0.70mg mL^{-1}) treatments while the difference was non-significant with MR+PI+LPGPR2 treatment. Chickpea variety *kabuli* BG1053 yielded significantly high reducing sugars as compared to *desi* PBG1. On the basis of mean of two varieties, all the multiple inoculation treatments showed significant improvement in amino N content ranging from 1.36-1.80 fold. Multiple inoculants have brought considerable change in the root exudates content. In single inoculation less amount of total sugars, reducing sugars and amino nitrogen content was detected. Total sugars, reducing sugars and amino nitrogen content in the consortium applied treatments were higher than in individual inoculant applied treatments. High root exudation in multiple inoculant treatments might have positively correlated with root growth which stimulated the actively growing root system to secrete more root exudates (Garcia *et al*., 2001). It is also well documented that the total soluble sugars, reducing sugars and amino nitrogen content were higher with use of bio-inoculant consortium of *Azospirillum lipoferum* with PGPR (*Pseudomonas fluorescens* and *Bacillus megaterium*) in the rice root exudates than in individual inoculant applied treatments (Raja *et al*., 2006).

Data in fig. 3 depicted effect of different tripartite inoculations on catalase activity at 120 DAS. Difference for the catalase activity (CAT) between chickpea varieties, among different treatments and interaction between varieties and treatments over control was significant (P<0.05). On the basis of mean of two chickpea varieties, CAT activity in PI alone treatment was 5.7 U min^{-1} g^{-1} fresh weight of roots which was higher than MR alone treatment (2.6 min^{-1} g^{-1} fresh weight of roots). Further, all the multiple inoculated treatments improved stress tolerance ability ranged from 4-6 fold over the *Mesorhizobium cicer* alone treatment. Catalase activity was significantly high in MR+PI+LPGPR1 treatment (11.5 U min^{-1} g^{-1} fresh weight of roots in *desi* PBG1 and 11. 3 min^{-1} g^{-1} fresh weight of roots in *kabuli* BG1053 U min^{-1} g^{-1} fresh weight of roots). Improved CAT activity in PI alone and multiple inoculant treatment in present study can be explained as *P.indica* symbiosis promoted plant growth during abiotic stress. Similarly, 77-fold increase in CAT activity in *R.trifolii* during water stress has been reported earlier (Goyal *et al*., 1986). Similarly CAT activity reduced draught stress in chickpea with *Mesorhizobium* inoculation (Esfahani *et al*., 2010) and used it as biochemical marker of BNF (Mhadhbi *et al*., 2004). One of the earlier studies also revealed that draught stress induced a range of physiological and biochemical responses in plants such as stomatal closure and repression of photosynthesis (Varma *et al*., 1998). As at 120 DAS the maximum temperature was 35.1°C in the month of April and all the *P.indica* treated plants induced higher CAT activity and might have promoted draught tolerance. Similarly, higher CAT activity was recorded in root and shoot parts in Chinese cabbage (Sun *et al*., 2010), maize (Kumar *et al*., 2009) and barley (Baltruschat *et al*., 2008). The root-colonizing fungal mutualist *P.indica* was discovered in association with the roots of woody shrubs in the Indian Thar desert (Varma *et al*., 1999) suggesting that the fungus may confer fitness benefits under draught-stress conditions. Antioxidant enzymes are known to play an important role in *P.indica* symbiosis conferring abiotic stress tolerance (Sartipnia *et al*., 2013). It was also studied that *P. indica* conveys better tolerance to various abiotic and biotic stresses as well as improved fitness to host plant through colonization (Qiang *et al*., 2014).

Data for superoxide dismutase (SOD) activity for different tripartite inoculations at 120 DAS (Fig.4) revealed significant difference with all the treatments except control (P<0.05). SOD activity in *P.indica* alone treatment (4.1 U min^{-1} g^{-1} fresh weight of roots) was found higher than MR alone treatment (2.7 U min^{-1} g^{-1} fresh weight of roots) on the basis of mean of both chickpea varieties. Further all the multiple inoculant treatments showed improved stress tolerance ability over the *Mesorhizobium cicer* alone treatment. Maximum SOD activity was found in MR+PI+LPGPR1 treatment which was 4.2 and 3.6 U min^{-1} g^{-1} fresh weight of roots in *kabuli* BG1053and *desi* PBG1, respectively. Increased level of SOD (antioxidant enzymes) in PI alone and multiple inoculation treatments during the colonization period might have resulted in detoxification of ROS and played a protective role in interaction of plant and fungi (Singh *et al*., 2011). Our findings are well in line with Kumar *et al*.(2009) who reported enhanced SOD activity in maize roots colonized with *P. indica*. In multiple inoculant treatment root endophytes might have acted as a biological mediator allowing symbiotic plants to activate stress response system more efficiently than uninoculated control plants (Bharadwaj *et al*., 2008).

Conclusion

This study indicated the compatibility of *Mesorhizobium cicer* and *P.indica* with different PGPR *in-vitro* as well as *in-vivo* revealed positive effects on chickpea through amino N-content, total soluble and reducing sugars and *P. indica* colonization. Bioaugmentaion of tripartite inoculant (LPGPR1 with MR and PI) can be explored as potent biofertilizer in chickpea.

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