

EFFECT OF CONSORTIUM OF PLANT GROWTH PROMOTING AND COMPOST INHABITING BACTERIA ON PHYSICO-CHEMICAL CHANGES AND DEFENSE RESPONSE OF MAIZE IN FUNGUS INFESTED SOIL

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Biotic stress in the rhizospheric environment induces defense mechanism in plant via signaling between plant and nonpathogenic microbes. In order to evaluate the defense response of maize plant, in relation to beneficial bacteria, a jar experiment was conducted in diseased soil (infected with *Fusarium oxysporum*). Seed was inoculated with consortium of selected isolates of plant growth promoting rhizobacteria (PGPR, Mb4 (*Bacillus* spp.), Mb7 (*Pseudomonas aeruginosa*) and compost inhabiting bacteria (CIB, Cb4 (*Serratia* spp.) and Cb9 (*Pseudomonas* spp.). Inoculum was used as inducer of defense compounds in maize crop. Hoagland half strength solution was applied to meet the water and nutrient requirements of the plants. Treatments were arranged in completely randomized design under axenic condition and were repeated thrice. Results indicated that different combination of Mb4 (*Bacillus* spp.), Mb7 (*Pseudomonas aeruginosa*), Cb4 (*Serratia* spp.) and Cb9 (*Pseudomonas* spp.) showed 100 percent seed germination than un-inoculated control. Significantly higher root/shoot growth were observed in treatments, inoculated with consortium of selected isolates. Survival of maize plants in diseased environment is the result of physicochemical changes in plant. Proline contents were observed up to 4.97 $\mu\text{mol g}^{-1}$ in bacterial inoculated treatment compared to un-inoculated control (1.760 $\mu\text{mol g}^{-1}$). Consortium of above mentioned isolates helped to maintain the chlorophyll 'a' 'b' and higher relative water contents than control. Inoculation with different consortia of selected isolates reduced membrane permeability up to 54.18%. The higher catalase activity was 4.5 $\text{mM H}_2\text{O}_2 \text{min}^{-1} \text{g}^{-1}$, while ascorbate peroxidase activity was 0.57 $\mu\text{M H}_2\text{O}_2 \text{min}^{-1} \text{g}^{-1}$ due to inoculation with bacterial consortia. It is therefore concluded that consortium inoculation of compatible bacterial isolates induced physicochemical changes in stressed plant, which help plant to withstand the biotic stress and promote growth.

Keywords: Antioxidants, disease suppression, *Fusarium oxysporum*, maize, soil pathogens.

INTRODUCTION

Soil borne pathogens are responsible for considerable yield losses in field crops. Different species of *Fusarium* i.e. *F. culmorum* and *F. pseudograminearum* were the combined causal organisms of seedling blight in many cereal crops (Charles *et al.*, 2007).

Plants, facing biotic stress, strongly depend on internal resistance, to fight against pathogens. The internal resistance is activated due to the presence of pathogens in the rhizosphere. Plant immunity depends upon the resistance, developed due to defense responses (Boller and Felix, 2009). The plant- microbe interaction followed the signaling process by the production of reactive oxygen species and ion fluxes (Garcia-Brugger *et al.*, 2006). In response to pathogen stress, salicylic acid and jasmonic acid were produced within hours, resulted in the regulation of defense genes (Browse, 2009; Vlot *et al.*, 2009). Sometimes this reaction resulted in

plant cell death in the particular infected region (hyper sensitive reaction) (Heath, 2000).

Soil-borne plant diseases can be controlled by composts (Noble and Coventry, 2005). Compost was useful against the *Fusarium oxysporum* f. sp. basilici while its ability to control diseases was due to microorganisms, inhabit in it (Reuveni *et al.*, 2002). Composts vary in their physical, chemical and biological composition and accordingly control the soil borne pathogens (Termorshuizen *et al.*, 2006). Compost inhabiting microbes showed varying capacity to suppress damping-off in cucumber, infected by *Pythium ultimum* (Carisse *et al.*, 2003).

Some beneficial bacteria (PGPR) have the ability to enhance the defensive capacity of plants against soil borne pathogens (Poza *et al.*, 2002). They can inhibit the growth and activity of soil borne pathogen like fungi, oomycetes, bacteria and nematodes (Jos *et al.*, 2009). These microbes indirectly defended the plant by inducing systemic resistance

(Srivastava *et al.*, 2010). The PGPR and CIB help plant to release elicitors in response to the presence of pathogens in the rhizosphere. Elicitation is used to induce the expression of genes responsible for the amalgamation of antimicrobial compounds (Sekar and Kandavel, 2010). They exert beneficial effects in terms of enhanced plant growth and decreased the sensitivity of plant to diseases (Compant *et al.*, 2005). They suppressed the pathogens by producing antibiotic compounds (Deshwal *et al.*, 2003) and compete by producing compounds such as siderophore (Arora *et al.*, 2001). They contribute to osmotic adjustment by protecting the structure of macromolecule and membrane through the production of proline, sucrose, and other organic sugars (Prado *et al.*, 2000). Proline is also play role in metabolism by providing nitrogen and acting as reducing agent (Meloni *et al.*, 2001). Bacterial inoculation provides relief to the plant by enhancing chlorophyll contents and stomatal conductance under stress (Vivas *et al.*, 2003). The aim of current study was to scrutinize compatible consortium of bacterial isolates, capable to induce defense compounds in stressed plant against *Fusarium oxysporum*. And to study the production of defense compounds and physiological changes for better understanding of biocontrol process.

MATERIALS AND METHODS

Collection and cross check of pathogen: The pathogen (*Fusarium oxysporum*) was collected from department of Plant Pathology, Ayub Agricultural Research Institute, Faisalabad. Pathogen was isolated by tissue segment method (Rangaswami, 1958) and identified by using colony morphology and microscopic features (Singh *et al.*, 1991). Pathogenicity was proved by live plant and cut stem method (Barnett and Hunter (1975). For cross check of pathogen, the fresh broth culture of mycelial suspension was incubated for 6 days. Three weeks old seedlings of maize, grown in sterilized soil were inoculated with the mycelial suspension of the fungal isolates. Jars were observed for symptoms of the disease. The pathogen was re-isolated from the infected plants and compared with the initial isolates.

Isolation of PGPR: Maize rhizosphere samples were collected from different locations at the Ayub Agriculture Research Institute, Faisalabad, Pakistan. Isolation of PGPR was performed in the Soil Bacteriology Section, Agricultural Biotechnology Research Institute Faisalabad, using Luria-Bertany (LB) agar medium (Luria and Burrous, 1995). The rhizosphere was dug out with intact root. The samples were placed in plastic bags and stored at 4°C in refrigerator. Ten grams of rhizosphere soil placed in conical flask and 90 mL sterile distilled water was added. The flask was shaken for 10 min on a rotary shaker. One milliliter of suspension was added to a 10 mL tube and shaken for 2 min. Serial dilutions with distilled water was performed up to 10⁻⁷ dilution. An aliquot (0.1 mL) of this suspension was spread on the plates

of LB agar medium. Plates were incubated for 3 days at 28°C. Bacterial colonies were streaked to other LB agar plates for purification and the plates incubated at 28°C for 3 days. Typical bacterial colonies were observed over the streaks and well isolated single colonies were picked up and re-streaked to fresh LB agar plates and incubated. The technique was perpetuated thrice and cultures were made single colony type. The isolated strains were coded and stored.

Isolation of compost inhabiting bacteria: Compost inhabiting bacteria were isolated using standard dilution-plating procedure as described by Pugliese *et al.* (2008). One gram of the compost samples was placed in 99 ml of sterile distilled water in a conical flask, shaken vigorously on a vortex mixer for 10 min and then serially diluted with distilled water (10⁻², 10⁻⁴, 10⁻⁶). An aliquot (0.5 mL) of these suspensions were spread on plates of Luria-Bertany (LB) agar medium. Inoculated plates were incubated at 28°C for 48 h and were sub-cultured on LB agar medium until pure cultures were established. The isolated strains were stored at -40 °C.

Consortium of PGPR and CIB: Selected isolates of PGPR (Mb4 (*Bacillus* spp.), Mb7 (*Pseudomonas aeruginosa*), and CIB (Cb4 (*Serratia* spp.) and Cb9 (*Pseudomonas* spp.) were used for consortium preparation and evaluation.

Jar experiment: Jar experiment was conducted in growth room of Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad. Sterilized broth medium of LB (Tryptone 10 g L⁻¹, Yeast extract 5 g L⁻¹, Sodium chloride 5 g L⁻¹ at pH 7.2 at 37°C) was inoculated with selected isolates (Mb4, Mb7, Cb4 and Cb9) and placed at 25 ± 1°C in a shaking incubator. Consortium was prepared by combining these isolates in all possible combinations, using LB medium and tested their compatibility for disease control and growth promotion in maize crop. Jars were filled with sterilized silt at the rate of 500 g pot⁻¹ and treated with fungus. Before sowing, surface disinfected seed was inoculated by peat based inocula while control was un-inoculated but treated with slurry (peat, sugar and LB broth culture). All the treatments were placed according to completely randomized design and replicated thrice. Hoagland half strength solution (Hoagland, 1950) was applied to fulfill the requirements of water and nutrients when required. Data regarding germination percentage was recorded 48 hours after sowing by counting the germinated seed out of total seed sown. Disease incidence was recorded after fifteen days and calculated using following formula described by Vincent (1947),

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{total number of plants}} \times 100$$

Fresh leaves (2 g) were sampled from each treatment, stored at 4°C and analyzed for physicochemical changes and defense related enzymes.

Physicochemical changes: Relative water contents in fresh leaves were determined by following method given by Mayak *et al.* (2004). Briefly 0.5 g fresh leaves were sampled, weighed (FW) and placed in distilled water for full turgidity at 4°C. After 48 hr, weight of fully turgid leaves was recorded. Then leaves were air dried followed by drying in an oven and noted the weight of oven dried leaves. Relative water contents were calculated via following formula (Whetherley, 1950).

$$\text{Relative water contents (RWC)} = \frac{\text{FW} - \text{DW}}{\text{FTW} - \text{DW}} \times 100$$

FW is the fresh weight, DW, dry weight and FTW is the fully turgid weight. Dry weight is the oven dry weight of sample for 24 h, while the FTW was determined after it was held in 100% humidity at 4°C for two days.

Relative membrane permeability was determined by cutting uniform leaf discs with a sharp cork borer from several plants. Leaf discs were transferred to flasks having 20 mL of sterilized distilled water. The flasks shake for 10s and measured the electrical conductivity (EC0) by conductivity meter. Kept the flasks over night at 4°C and examined the EC1. The flasks were then autoclaved at 121°C for 20 min to measure EC2. Relative membrane permeability was calculated by using the formula,

$$\text{RMP (\%)} = \frac{\text{EC1} - \text{EC0}}{\text{EC2} - \text{EC0}} \times 100$$

Chlorophyll contents were measured by the method given by Arnon (1949). Fresh leaf sample of 0.5 g from each treatment was crushed in 80% acetone (v/v) and filtered. Samples were run to take absorbance in spectrophotometer at 663 and 645 nm for chlorophyll a and b, respectively, and calculated as

Chl 'a' (mg/g fresh weight) =

$$[12.7 (\text{O.D } 663) - 2.69 (\text{O.D } 645) \times V/1000 \times W]$$

Chl 'b' (mg/g fresh weight) =

$$[22.9 (\text{O.D } 645) - 4.68 (\text{O.D } 663) \times V/1000 \times W]$$

Where V is the volume of sample, W is weight of fresh tissue, $0.0480 + 0.114 (\text{O.D } 663) - 0.0638 (\text{O.D } 645)$.

Defense enzymes: Proline accumulation was determined using the method of Bates *et al.* (1973). One gram fresh leaf was homogenized with 3% sulfosalicylic acid and after 72 h the proline was released; the homogenate was centrifuged at 16000 rpm for 20 min. The supernatant was treated with glacial acetic acid and ninhydrin, kept at 96°C for 1 hour in water bath and cooled immediately by using ice bath to stop the reaction. The mixture was extracted with toluene and measured the absorbance at 520 nm by spectrophotometer. Standards were prepared with toluene having concentration of 0-5 ppm. Standard curve was used to determine the proline concentration and expressed as $\mu\text{mol g}^{-1}$.

Catalase is responsible for the decomposition of H_2O_2 expressed by decrease in absorbance at 240 nm spectrophotometrically. Leaf sample was extracted with

potassium phosphate buffer (50 mM). Required sample was prepared by taking 3 mL test mixture, 2 mL diluted leaf extract (diluted 200 times with 50 mM potassium phosphate buffer) and 10 mM H_2O_2 . The extinction co-efficient of H_2O_2 ($40 \text{ mM}^{-1} \text{ cm}^{-1}$ at 240 nm) was used to calculate the enzyme activity and expressed in millimoles of H_2O_2 per minute per gram fresh weight (Aebi, 1984).

Ascorbate peroxidase (APX) activity was determined by measuring the rate of decrease in absorbance of ascorbate at 290 nm. APX activity was assessed by a modified method of Nakano and Asada, 1981. Leaf was grinded with potassium phosphate to get extract and 1mL test mixture plus 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM H_2O_2 , 0.5 mM ascorbate and 10 μL of crude leaf extract. H_2O_2 was added in the end to start the reaction and decrease in absorbance was noted for 3 min. The enzyme activity was calculated by using extinction co-efficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ for reduced ascorbate.

Total soluble sugar was determined by the procedure, described by Morris, 1948. Standard solution of glucose was prepared with concentration of 1-5 ppm. One mL of aqueous sample and four mL ice-cold anthrone reagents was added in all the test tubes, vortexed and placed at 100°C for exactly 10 min. Samples were rapidly cooled by placing the tubes into an ice bath. Absorbance was recorded for blank, standards and samples at 630 nm after shaking well. Total sugar contents were calculated with reference to the sugar(s) selected as standard, from the standard curve. Absorbance was recorded for blank was subtracted from samples and standards.

Statistical analysis: Data for all the treatments were subjected to analysis of variance in completely randomized design (Steel *et al.*, 1997). The significance of differences among treatments was determined using LSD test and significant difference between means was determined according to Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

Effect of PGPR and CIB on growth parameters: Axenic condition was executed in jars to study the effect of different consortium of PGPR and CIB on growth and defense response of maize crop. Different combinations of Mb4, Mb7, Cb4, and Cb9 showed the significant increase in growth parameters than other combinations of selected strains. Germination percentage is expressed by Fig.1, while data regarding root/shoot length, root/shoot weight, disease incidence and disease suppression is given in Table 1. Different combinations of above mentioned strains gave better seed germination (100%), higher root length (up to 29.35 cm), shoot length (up to 31.29 cm), root weight (up to 1.59 g plant⁻¹) and shoot weight (up to 2.83 g plant⁻¹) compared to their respective control.

Table 1. Effect of consortium of PGPR and CIB on growth of maize under axenic condition.

Treatments	Root length (cm)	Shoot length (cm)	Root weight (g plant ⁻¹)	Shoot weight (g plant ⁻¹)	Disease incidence (%)
Control	20.5c *	20.33 d	1.18 d	1.60 e	88.9 a
Mb4+Cb1	29.04 a	29.32 ab	1.46 bc	2.31 cd	22.2 d
Mb4+ Cb4	29.07 a	31.29 a	1.58 a	2.70 ab	11.1 e
Mb4+Cb8	28.32 a	29.48 ab	1.47 b	2.40 c	33.3 c
Mb4+Cb9	27.83 a	30.19 ab	1.58 a	2.65 b	11.1 e
Mb5+Cb1	27.09 a	25.88 bc	1.44 bc	2.39 c	33.3 c
Mb5+ Cb4	26.18 a	30.34 ab	1.45 bc	2.30 cd	44.4 b
Mb5+Cb8	28.88 a	26.99 abc	1.45 bc	2.41 c	22.2 d
Mb5+Cb9	27.39 a	28.37 ab	1.41 c	2.32 cd	22.2 d
Mb6+Cb1	26.15 a	28.40 ab	1.44 bc	2.19 d	44.4 b
Mb6+ Cb4	25.25 ab	22.95 cd	1.58 a	2.31 cd	33.3 c
Mb6+Cb8	29.33 a	28.60 ab	1.46 bc	2.35 c	22.2 d
Mb6+Cb9	21.07 bc	26.34 abc	1.45 bc	2.33 cd	33.3 c
Mb7+Cb1	28.22 a	29.38 ab	1.47 b	2.37 c	22.2 d
Mb7+ Cb4	29.21 a	30.23 ab	1.58 a	2.75 ab	11.1 e
Mb7+Cb8	27.41 a	29.26 ab	1.45 bc	2.29 cd	22.2 d
Mb7+Cb9	29.35 a	30.34 a	1.59 a	2.83 a	11.1 e
LSD	2.296	4.66	0.059	0.145	5.236

*Means sharing same letter(s) in a column do not differ significantly at $p < 0.05$. Data is the average of three repeats

Disease incidence was calculated after counting the diseased plants out of total plants in each treatment (Table 1). Control exhibited 88.9% disease incidence while range of disease incidence in inoculated treatments varied from 11.1 to 44.4%. These consortiums also suppress disease up to 88.9%. Least control of disease among inoculated treatments was with Mb5 + Cb1 and Mb6 + Cb1 (55.6%) but higher than control (11.1%).

Effect of PGPR and CIB on physicochemical changes in plant: Data regarding membrane permeability, chlorophyll and relative water contents is given in Table 2. Microbial consortia with Mb4, Mb7, Cb4, and Cb9 showed decreased trend in membrane permeability compared to control. The lowest membrane permeability was observed with Mb4 + Cb9 (54.18%) which was statistically at par with Mb4 + Cb4 (55.78%) and Mb7 + Cb4 (55.78%).

Table 2. Effect of consortium of PGPR and CIB on membrane permeability, chlorophyll and relative water contents.

Treatments	¹ MP (%)	² Chl a (mg/g FW)	² Chl b (mg/g FW)	³ RWC (%)
Control	75.91a*	0.908 m	1.099 p	57.49 g
Mb4+Cb1	70.02bc	1.175 i	1.897 o	60.43efg
Mb4+ Cb4	55.78i	1.767 a	2.351 d	78.23a
Mb4+Cb8	64.08fg	1.134 j	2.001kl	62.01c-g
Mb4+Cb9	54.18 i	1.677 b	2.499 b	76.78 a
Mb5+Cb1	66.09cd	1.176 i	1.977 n	66.0bcd
Mb5+ Cb4	65.89efg	1.277 f	2.005 k	69.85b
Mb5+Cb8	68.18ef	1.286 e	2.114 i	76.45a
Mb5+Cb9	65.92efg	1.134 j	2.012 j	65.41b-e
Mb6+Cb1	62.07gh	1.117 l	2.132 h	67.30bc
Mb6+ Cb4	72.51ab	1.123k	2.156 g	60.58d-g
Mb6+Cb8	71.55ab	1.243g	2.231 e	58.79fg
Mb6+Cb9	61.71 d	1.333d	2.225 f	63.46c-f
Mb7+Cb1	69.20 e	1.432c	1.998 l	59.62fg
Mb7+ Cb4	55.78i	1.766a	2.513 a	77.56 a
Mb7+Cb8	61.90gh	1.222h	1.985 m	60.54d-g
Mb7+Cb9	58.63 hi	1.676b	2.480 c	78.56 a
LSD	5.010	0.0049	0.0044	5.5082

*Means sharing same letter(s) in a column do not differ significantly at $p < 0.05$. Data is the average of three repeats; ¹MP: membrane permeability; ²Chl: chlorophyll; ³RWC: Relative water contents.

Chlorophyll contents varied considerably with inoculated treatments. Chlorophyll b was elevated in all the treatments than chlorophyll a but both the values were significantly higher than control.

Relative water contents ranged from 60.43% (Mb4 + Cb1) to 78.56% (Mb7 + Cb9). The higher value (78.56%) was statistically at par with Mb4 + Cb4, Mb7 + Cb4, Mb4 + Cb9 and Mb5 + Cb8 and higher than control (57.49%).

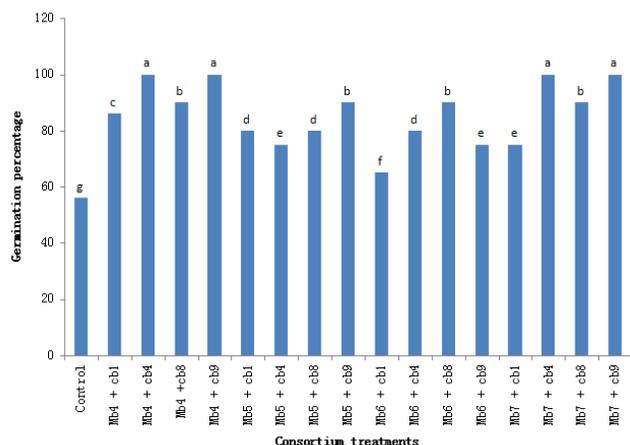


Figure 1. Effect of consortium of PGPR and CIB on seed germination of maize.

Effect of PGPR and CIB on defense compounds: Data regarding proline contents, total soluble sugar, catalase and ascorbate peroxidase activity is given in Table 3. In response to fungal stress, proline is synthesized in various plant parts

which provide relief to the stressed plant. More proline contents were observed by inoculation with different consortium of selected strains compared to control. The best consortium with respect to above mentioned enzymes were Mb7 + Cb4, Mb7 + Cb9 and Mb4 + Cb9. They showed maximum proline contents (up to 4.97 $\mu\text{mol g}^{-1}$), higher catalase activity (4.5 $\text{mM H}_2\text{O}_2 \text{min}^{-1} \text{g}^{-1}$) more ascorbate peroxidase activity (0.57 $\mu\text{M H}_2\text{O}_2 \text{min}^{-1} \text{mg}^{-1} \text{protein}$) and total soluble sugar (up to 10 $\text{mg g}^{-1} \text{FW}$).

DISCUSSION

Plants expose to various biotic and abiotic stresses in natural environment. To survive under such circumstances, plant has to develop a defense system in response to external signaling. Phytohormone may be the part of signaling pathways that enables plants to bear stresses. Secondary metabolites support the plant against biotic stress via signaling cross talk with requisite rhizospheric microbes. Beneficial microflora (PGPR, CIB), particularly fluorescent pseudomonads are the strong candidates to act as biocontrol agent. They produce antifungal secondary metabolites and show efficient root colonization (Erdogan and Benlioglu, 2010). They are natural enemies of the pathogens and produced antifungal metabolites at the infected site, leading to the better growth and yield of crop in diseased environment.

Efficient germination is the basic criteria for growth and yield of the crop. Seed inoculation with bacterial consortia significantly enhanced the germination of seed. Germination percentage varied with different bacterial consortia however

Table 3. Effect of consortium of PGPR and CIB on defense response of maize.

Treatments	Proline $\mu\text{mol/g FW}$	Catalase $\text{mM H}_2\text{O}_2/\text{min/g protein}$	Ascorbate peroxidase $\mu\text{M H}_2\text{O}_2/\text{min/mg protein}$	Total soluble sugars mg/g Fw
Control	1.76 i*	1.3 d	0.25 l	5.6 e
Mb4+Cb1	2.19 gh	3.4 abc	0.36 k	6.7de
Mb4+ Cb4	4.14 b	4.5 a	0.55 ab	9.9 a
Mb4+Cb8	2.11 h	3.2 abc	0.46 efg	8.6 abc
Mb4+Cb9	4.94 a	4.2 a	0.56 a	10.0 a
Mb5+Cb1	2.14 h	1.9 cd	0.49 de	8.8 ab
Mb5+ Cb4	3.13 e	2.0 bcd	0.44 fgh	7.9 bcd
Mb5+Cb8	2.38 fg	3.3 abc	0.50 cde	6.9 cde
Mb5+Cb9	3.43 d	3.5 abc	0.47 def	8.8 ab
Mb6+Cb1	3.45 d	3.9 a	0.43 f-i	9.7 a
Mb6+ Cb4	3.48 d	3.3 abc	0.39 ijk	8.7 ab
Mb6+Cb8	4.22 b	3.6 ab	0.42 g-j	6.9 cde
Mb6+Cb9	3.67 c	4.0 a	0.38 jk	9.5 ab
Mb7+Cb1	3.73 c	3.4 abc	0.41 hij	8.7 ab
Mb7+ Cb4	4.97 a	4.4 a	0.54 abc	9.7 a
Mb7+Cb8	2.54 f	3.8 a	0.51 bcd	5.8 e
Mb7+Cb9	4.79 a	4.1 a	0.57a	9.6 ab
LSD	0.232	1.610	0.046	1.800

*Means sharing same letter(s) in a column do not differ significantly at $p < 0.05$. Data is the average of three repeats.

all the inoculated treatments showed significantly higher germination rate than the control. It is justified by previous finding that seed treatment with antagonistic PGPR enriched the seed worth (Umesha, 2006). They have the ability to increase seed germination and plant growth, improving seedling emergence, bear the external stress and protecting plants from disease (Lugtenberg *et al.*, 2002). Moreover, PGPR (*Pseudomonas fluorescens*) amplified the seed germination (Srivastava *et al.*, 2010) and vigor of seedlings (Abo-Elyousr and El-Hendawy, 2008) by colonizing the root of cereals (maize and wheat) may be due to secretion of auxins, gibberellins and cytokinins (Rosas *et al.*, 2009; Yao *et al.*, 2010). Furthermore PGPR inoculation improved the seed germination in pearl millet (Niranjan *et al.*, 2004), sunflower and wheat (Shaukat *et al.*, 2006 a,b). It might be due to the synthesis of growth hormones which perhaps activated the specific enzymes activity, involved in prompt germination and intensification of starch assimilation.

Bacterial inoculation increased the root mass and area which help plant to absorb water and nutrients more efficiently. As a result of which plant became physically strong to tolerate biotic and abiotic stresses. In our finding root mass was higher with consortium inoculation than control. Increased biomass of root by inoculation with bacterial consortium was also observed previously by Gholami *et al.* (2009) and Fatima *et al.* (2009) in wheat. Higher biomass of root by inoculation was also recorded by Pacôme *et al.* (2013) in maize crop.

Our study exhibited that seed inoculation with all bacterial consortia showed an increase in plant height. Early germination and efficient absorption of water and nutrients due to well established root system leads to better growth of shoot. These results are corroborated with previous finding that plant height and leaf area was increased in different crops due to PGPR inoculation (Siddiqui and shaukat, 2002). It is attributed to capability of PGPR to promote plant growth and productivity through synthesizing phytohormone, increasing the nutrient availability, antagonizing phytopathogens and decreasing metal toxicity (Bharathi *et al.*, 2004). Results also showed an increase in shoot dry weight in response to all inoculated treatments over control. The beneficial effect of seed inoculation with bacterial consortia on shoot dry weight and yield of maize was also reported by Shaharoon *et al.* (2006). Such an improvement might be due to the ability of bacteria to fix nitrogen, solubilize the phosphate and production of growth regulators (Salantur *et al.*, 2006).

Combined application of PGPR and CIB was the most effective biocontrol strategy and provided the disease suppression against pathogen (*Fusarium oxysporum*). It might be due to early germination, efficient root development and competition for food and space. Root colonization is the pre requisite for pathogen to cause seedling blight in plant. CIB and PGPR are the aggressive

colonizers compared to fungus thus diminished the possibility of disease initiation. Our results confirmed the previous finding that plant growth promoting rhizobacteria can be used as biofertilizer and biocontrol agents (Manikandan *et al.*, 2010). It was also studied by Morsy *et al.* (2009) that the dual inoculation with *Triticum viride* and *Bacillus subtilis* was effective against *Fusarium oxysporum* than individual treatment. In diseased soil, beneficial bacteria help plant to promote growth by suppressing the disease through antibiosis, by inducing systemic resistance, producing toxins, bio-surfactants, lytic enzymes and competing for food and niches (Erdogan and Benlioglu, 2010). Beneficial microbes may also protect the plant against pathogens by increasing uptake of nutrient, change in the root architecture and instigation of plant defense system (Wehner *et al.*, 2010). Antifungal activity of PGPR was particularly attributed to the production of antifungal metabolites against *Fusarium oxysporum* f.sp. lycopersici (Karkachi *et al.*, 2010). The increased growth of maize crop by inoculation with bacterial consortium was probably due to the additive effect of PGPR and CIB for disease suppressive potential under diseased environment.

Membrane permeability/electrolyte leakage increased under stress. PGPR and CIB inoculation decreased it by strengthening the plant due to nutrient availability. It may be due to interaction between plant and beneficial microbes, which improved the nutrient uptake, increase resistance against soil-borne pathogens and reduce the effect of heavy metals and drought stress (Gosling *et al.*, 2006).

Chlorophyll contents decreased due to fungal stress however significantly higher chlorophyll contents were observed by inoculation with bacterial consortia. Chloroplasts are membrane bound organelles and its constancy depends upon membrane stability. Increased membrane permeability under stressed condition did not keep them intact and results in chlorophyll reduction (Ashraf *et al.*, 2005). Chlorophyll contents decreased due to mobilization of thylakoid proteolipids and thus decrease the photosynthesis (Thomas *et al.*, 2000). Chlorophyll a, b and a/b significantly decreased under stress condition (Moussa, 2006; Iqbal *et al.*, 2006).

Significant variation in relative water contents were observed due to bacterial inoculation but all the inoculated treatments had significantly higher water contents than control. *Fusarium* spp affected the plant by entering into the vascular tissues and blocked the uptake of water and nutrients (Agrios *et al.*, 2005). In the defence response of citrus against *Alternaria* infection, increased rate of respiration was observed (Timmer *et al.*, 2003). PGPR and CIB colonize the root and prohibited the fungus to enter the vascular tissues of the plant thus maintain relative water contents in the aerial parts of the plant.

Proline acts as antioxidant and scavenged reactive oxygen species (ROS). Proline, glycine betaine and sugars were solutes, accumulated in plant parts under stressed condition

(Qasim *et al.*, 2003). Proline concentration varies from 5% under normal condition to 80% under stressed condition in many plants (Szabados and Savoure, 2009). Proline helps plant to maintain cytosolic pH, boost up the bustle of diverse enzymes, act as molecular chaperones and regulates the intercellular redox reaction (Verbruggen and Hermans, 2008). Several plant species, inoculated with PGPR revealed high concentration of proline under stressed condition (Kohler *et al.* 2009; Vardharajula *et al.*, 2011). Proline and other compatible solutes were synthesized using 41 moles of ATP but help plant to survive and recover under stressed condition (Munns and Tester, 2008).

Catalase and ascorbate peroxidase activity increased, by co-inoculation with bacterial consortia. Under stressed condition concentration of H₂O₂ increased in the plant leaves and root. Catalase act as sink for H₂O₂ and help plant to survive under stressed condition. H₂O₂ is a toxic agent and act as signaling molecule to regulate the expression of genes related to antioxidants and defense proteins in cells (Hung *et al.*, 2005). The stressed condition of plant is associated with up or down regulation of hundreds of defense related genes (Cheeseman, 2007). Defense proteins, such as peroxidase (POX) are induced in response to pathogen invasion in different plants (Ramamoorthy *et al.*, 2002). The increased activity of peroxidase is a valuable indicator for localized and systemic acquired resistance in diseased plants (El-Khallal, 2007). Systemic resistance is induced by PGPR against *Fusarium oxysporum* due to the accumulation of peroxidase and other defense related proteins in banana (Thangavelu *et al.*, 2003; Saravanan *et al.*, 2004). Cellular compartments including chloroplast were high in antioxidants such as ascorbic acid and glutathione and provide defense against oxidative stress (Miller *et al.*, 2010). Increase in soluble sugar contents was observed in diseased plants. It is justified by the fact that plant stopped assimilatory metabolism (photosynthesis) when started respiration and other functions, necessary for defense (Berger *et al.*, 2007). Degradation of starch in diseased parts of the plant resulted in starch depletion and higher sugar contents (Patakas and Noitsak, 2001), thus increased the soluble sugars: starch ratio. The tolerance mechanism in stressed plant may be linked to accumulation of osmo-protectants such as soluble sugars. Accumulation of soluble sugars was related to water deficit in plants (Hoekstra and Buitink, 2001). *Fusarium* attack blocked the vascular bundles of immature stems due to mycelium growth and results in wilting (Agrios *et al.*, 2005). Phytopathogens secreted carbohydrate results in an increase of monosaccharide and decrease of disaccharide contents in diseased leaves (Qin *et al.*, 2007; Abdalla and El-Khoshiban, 2007). Escalation in the soluble and reducing sugar was observed in diseased plants by Ijaz *et al.* (2011) and Chung (2012). Consortium of compatible bacterial isolates help plant to grow successfully in biotic stress by inducing

physicochemical changes and producing defense compounds in stressed plant

Conclusion: Under stressed condition, synergistic plant-bacterial association plays a vital role in supporting the plant growth and health. Some species of *Fusarium* cause seedling blight in maize crop. In order to provide maximum benefit to the crop, efficient root colonization by beneficial bacteria is of paramount importance. They also protect the plant from pathogens by triggering the production of defense compounds in the plant. It is suggested that inoculation of maize with the consortium of selected isolates of PGPR and CIB having above mentioned characteristics, act as effective biocontrol agent against *Fusarium oxysporum*. Different consortium of PGPR and CIB isolates for disease suppression via production of defense hormone and growth promotion is a good device to select compatible isolates of PGPR and CIB in biocontrol technology. Accumulation of defense enzymes such as proline, catalase, ascorbate peroxidase and total soluble sugars induced resistance in plant against *Fusarium oxysporum*.

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