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COMPENSATION OF DROUGHT STRESS IN WHEAT BY PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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ABSTRACT

A field experiment was conducted at Soil Bacteriology Section, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan in the year 2013-14, to evaluate the potential of PGPR on wheat, facing water stress at tillering and grain filling stages. Inoculum of PGPR having CFU 10^6 mL^{-1} was applied as seed coating at sowing. Water stress was given at two sensitive stages (tillering and grain filling). Fertilizers (100-100-60 kg NPK ha^{-1}) were applied in the form of Urea, single super phosphate and sulphate of potash to all the treatments, following RCBD results revealed that PGPR inoculation produced more grain yield (4.95 t ha^{-1}) in treatment where water stress was given at grain filling stage compared to water stress at tillering (4.51 t ha^{-1}). Number of tillers (582), 1000-grains weight (40.3 g) and spike length (18.4 cm) were more with PGPR where water stress was given at grain filling stage compared to where irrigation was skipped at tillering stage (499, 37.7 g and 18.3 cm). It was concluded that drought is more harmful at tillering than at grain filling stage and stress can be evaded to some extent by inoculation with PGPR.

KEYWORDS: Growth stages; PGPR; water stress; wheat; Pakistan.

INTRODUCTION

Day by day water is becoming a rare commodity for the whole living sphere especially in Pakistan. Its scarcity is especially a serious threat for plants. In the prevailing scenario planning should be made to improve the water use efficiency or to reduce the plant's requirements. Environment of Pakistan is arid and semiarid, therefore, numerous stresses, particularly drought, restrict the yield and efficiency of crops. Its outcome is very serious financial losses in agriculture. Whole plant upto cellular level is affected by drought stress with respect to plant water relation, metabolism of nitrogen and carbon, leads to alter physiology of plant and photosynthetic activity (Benabdellah *et al.,* 2011; Osakabe *et al.,* 2014).

Stressed plants adopt different molecular mechanisms for the adjustment of their resources and healthy growth under unfavorable environmental circumstances (Nishiyama *et al.,* 2013; Ha *et al.,* 2014). Reduced plant under harsh environment of drought is due to decrease in gas exchange through stomata which results in less supply of CO_2 for photosynthetic activity, and thereby poor growth of the plant (Osakabe *et al.,* 2014).

Drought stress impairs the accumulation of dry matter in all organs of the plant. In marigold fresh and dry weight of shoot and flower was decreased under drought condition (Asrar and Elhindi, 2011). Number of leaves, leaf area and turgor is also reduced due to scarcity of water (Farooq *et al.,* 2011). However, scarcity of water does not affect all growth stages of crop uniformly. Some stages can manage water shortage successfully while others show susceptibility to water shortage and may cause distinctive yield losses. Drought stress at any stage may decrease biomass, tillering ability, number of grains per spike and size of the grain. Hence the combine effect of drought stress depends on intensity and duration of stress (Bukhat, 2005). Drought stress at tillering and anthesis stages at the same time caused severe decrease in grain yield as compared to that at tillering or grain filling stage alone. Severe decrease in grain yield was observed when irrigation was skipped at tillering and grain filling stages. However, if drought was imposed only at tillering or grain filling stage, reasonable yield of the crop can be obtained (Kang *et al.,* 2002; Pszcz'okowska *et al.,* 2003).

Plant can tolerate drought by certain adaptations like root extension and thus allowing an efficient uptake of water (Narusaka *et al., 2003*). Plants, response to water deficit is variable at their various developmental stages (Sandhya *et al.,* 2010).

Reduction in number of kernels/ears and kernel weight was observed due to water stress at later stages (Gupta *et al.,* 2001). Several factors affect the plant response to water stress, such as developmental stage, severity

and duration of stress and genetics of cultivar (Beltrano and Marta, 2008). If stress remains for long duration, growth and yield of the crop is seriously impaired. Under such condition, plant has to adopt complicated physicochemical changes to bear the stress. Due to these changes, plant enables to tolerate stress, sustain hormone homeostasis and block the damage due to light (Osakabe *et al.,* 2014). Flowering and pod-fill stages of pea (*Pisum sativum* L.) showed sensitivity to water stress (Sandhya *et al.,* 2010).

Water stress in plant is related to more discharge of endogenous ethylene (Mayak *et al.,* 2004). Regulation of most of the physiological effects is associated with secretion of endogenous ethylene (Arshad and Frankenberger, 2002; Pirdashti *et al.,* 2003). Peas showed sensitivity under drought condition as a result of continuous flow of ethylene due to water stress. ACC is converted to ammonia and α-ketobutyrate instead of ethylene due to the secretion of ACC-deaminase enzyme by some beneficial microorganisms (Shaharoona *et al.,* 2006). Rhizobacteria, contains ACC-deaminase, has the capability to support the growth of the plant through regulation of ethylene synthesis in the roots (Dodd *et al.,* 2004; Potters *et al.,* 2003) under stressed condition.

To support the plant's capability to bear the stress, the plant growth promoting rhizobacteria play significant role against stress condition (Shaharoona *et al.,* 2006) by changing the target plant's morphology (Belimov *et al.,* 2005). During water shortage PGPR inoculation induces the drought tolerance in the plants (Ilyas and Bano, 2010). Plant showed tolerance to abiotic stress through physical and chemical changes towards abiotic stress by a mechanism called induced systemic tolerance.

The objective of current research was to identify the sensitive stages of wheat crop for drought and role of PGPR under water stress condition with the hypothesis that PGPR may help plant to tolerate the water stress.

MATERIALS AND METHODS

Sampling of rhizosphere

This study was conducted at Soil Bacteriology Section, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan in year 2013-14. Rhizosphere samples were collected with the root of wheat crop at tillering stage from the permanent plot of the selection. Samples were collected from ten different sites, removed the rhizosphere soil from root by brushing and isolation was carried out from each site in five Petri plates (5 repeats).

Isolation of rhizobacteria

Rhizobacteria were isolated from the rhizosphere of wheat following the dilution plate technique in the laboratory of Soil Bacteriology Section, ABRI, AARI, Faisalabad. Isolation was carried out according to Dworkin and Foster (DF), 1958 (Dworkin and Foster, 1958) on minimal salt medium, having ACC (concentration as mentioned in the composition of DF media) as the sole N source. Briefly one gram of each soil sample was thoroughly mixed with 99 ml of sterile distilled water. Series of dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were made and 10^{-6} dilutions were inoculated on DF agar media. The plates were incubated at ± 25°C for three days. Morphologically different colonies were selected, marked and re-streaked until the pure cultures were obtained. Presumptive tests were carried out for identification, following standard methods as outlined in Bergey's Manual of Systematic Bacteriology (Vazquez *et al.,* 2000). Rhizobacteria were screened for plant growth promoting activities in wheat under axenic condition. The selected strains of rhizobacteria were stored at -20°C with 20% glycerol.

Characterization of isolates

Chemical characterization of isolates was carried out by methyl red test, oxidase test, catalase production test, siderophore production, phosphate solubilization, auxin biosynthesis and ACC-deaminase test (Table. 1) as fallows.

- For Methyl Red Test, Dworkin and Foster minimal salt medium was prepared, sterilized and inoculated with isolated rhizobacterial strains of PGPR (CFU 10^6 mL^{-1}) in 10 mL test tube. The flasks were incubated at 28 ± 1°C for 48 h in the orbital shaking incubator at 100 rpm. Methyl red was added @ 1 mL per test tube, positive MR test showed red color while control remained yellow following Voges and Proskauer technique (Mayak and Glick, 2004).
- Oxidase test was performed to determine the presence of oxidase enzyme in bacterial isolates (Steel, 1961). Some of the test culture was inoculated on an oxidase dry slide and observed the change in color to purple/blue after one minute.
- Bacterial colony from fresh culture (24 h old) was placed on a glass slide and one drop of H_2O_2 (30%) was dropped on the colony using micro pipette. Appearance of gas bubbles indicated the presence of catalase enzyme (McFadden, 1980).
- The PGPR isolates were evaluated for siderophore production on the Chrome Azurole S agar (CAS) described by Clark and Bavoil (1994). Chrome Azurole S agar plates were prepared and inoculated with test organism as spots.

Incubated the inoculated plates at 30° C for 5 days. Development of yellow-orange halo around the colony showed the production of siderophore.

- The phosphorus solubilizing activity was determined according to the qualitative method described by Mehta (Mehta and Nautiyal, 2001). The growth and solubilization diameter were determined after incubation at 28 ± 2°C for seven days. On the bases of diameter of clearing halo zones, solubilization index (SI) was calculated using the following formula.

$$SI = \frac{Colony\ diameter + halo\ zone\ diameter}{Colony\ diameter}$$

- In vitro auxin production by isolated PGPR was determined by using the protocol described by Sarwar *et al.* (1992). The Auxin biosynthesis potential was determined as Indole-3- acetic acid (IAA) equivalents using Salkowski's reagent (2 mL of 0.5M $FeCl_3$ +98 mL of 35% $HClO_4$) and run the samples in spectrophotometer at 535nm wavelength. Samples were run after the standards and IAA equivalents were calculated with the help of standard curve (Table 1).
- ACC-deaminase activity was determined by monitoring the amount of *α*-ketobutyrate as described by Honma and Shimomura, 1978.

Field experiment

Inoculum preparation was completed by growing the selected strains of PGPR in 250 mL Erlenmeyer flask having 100 mL DF broth. The inoculum was incubated at 28±1° C in the orbital shaking incubator at 100 rpm for three days. To attain uniform cell density (10^8– 10^9 CFU mL^{-1}), an optical density of 0.5 recorded at a wavelength of 535 nm was achieved, using spectrophotometer. The surface disinfected seeds of wheat was inoculated by mixing with peat based slurry containing 3-days old inoculum of respective strain (10^8– 10^9 CFU mL^{-1}) and sugar solution (10%) whereas, seeds for control were treated with peat containing sterilized broth of DF and sugar solution. Inoculated seeds were air dried before sowing.

Field study was conducted with sandy clay loam soil having pH 7.9, EC_e 1.50 d Sm^{-1}, nitrogen 0.028% and available phosphorus 7.3 mg kg^{-1}. Three fourth of recommended dose of fertilizers was applied to all the treatments. There were six treatments as detailed below:-

T_1 = ¾ of recommended NP (Full irrigation), T_2 = ¾ of recommended NP (irrigation skip at tilliring stage), T_3 = ¾ of recommended NP (irrigation skip at grain filling stage), T_4 = T_1+ PGPR inoculation (full irrigation), T_5 = T_2 + PGPR inoculation (irrigation skip at tilliring stage), T_6 = T_3 + PGPR inoculation (irrigation skip at grain filling stage).The experiment was laid out in randomized complete block design with three replications. Plant health was monitored throughout the growing period. Data regarding grain yield, biomass, number of tillers, plant height, spike length, number of grains, 1000 grain weight, nitrogen and phosphorus contents of grain and soil were recorded after harvesting the crop. Nitrogen was determined according to Kjeldhal method (Bremner and Mulvaney, 1982) while soil P by modified Olsen method (Olsen and Sommers, 1982). Data were subjected to statistical analysis following RCBD according to standard procedures (Steel *et al.,*1997). The difference among the treatment means were compared by applying the Duncan's multiple range tests (Duncan, 1955) using software statistix 8.1. Meteorological data war recorded at the observatory of Plant Physiology Section, Ayub Agricultural Research Institute, Faisalabad (Table 4).

Table 1. Characterization of PGPR

Isolated strains	**MR test	Oxidase test	Catalase test	Siderophore Production	PO_4 Solubilization Index	***IAA production (µg mL^{-1})	*ACC-deaminase nmolg^{-1} biomass h^{-1}
PGPR 1	+	+	+	+	+	9.0 a	171.22 b
PGPR 2	-	+	-	-	-	1.2 c	59.35 f
PGPR 3	-	-	+	-	-	1.6 c	64.33 e
PGPR 4	+	+	+	+	+	9.3 a	174.3 a
PGPR 5	+	+	+	+	+	7.6 b	170.3 b
PGPR 6	+	-	-	-	-	1.6 c	65.32 e
PGPR 7	-	-	-	-	-	1.4 c	96.66 d
PGPR 8	+	+	+	+	+	7.3 b	163.5 c
PGPR 9	+	+	+	+	+	8.4 ab	165.12 c
PGPR 10	+	-	+	-	-	1.3 c	63.22 e
LSD						1.1194	3.8463

* ACC= 1-aminocyclopropane-1-carboxylic acid, ***IAA = Indole-3-Acetic Acid, ** Methyl Red test

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR 4) was selected on the basis of good auxin production (9.3 µg mL^{-1}), ACC-deaminase activity (174.3 deaminase nmol

g^{-1} biomass h^{-1}) and positive response to MR-test, oxidase test, catalase test, siderophore production and PO_4- solubilization. PGPR having above mentioned characteristics helps the plant to bear the stresses such as drought. Abiotic stresses like drought are included in severe issues, related to plant growth, development and production (Hamayun *et al.,* 2010). PGPR provide resistance against stress thus enhance the plant growth. In the present study, PGPR were selected on the basis of above mentioned characteristics. Healthy plants have the better capability to bear the stresses than weak plant. It was also observed by Yuming *et al.* (2003) that plants, inoculated with PGPR, showed healthy growth which resulted in more nutrient uptake and gave better stand to crop. Good ACC-deaminase producers provide the plant with better competency to survive under drought stress condition. It was justified by previous findings of Dodd *et al.* (2004) and Mayak *et al.* (2004) ACC-deaminase characteristic of beneficial bacteria (PGPR) is evidently associated with better growth of the inoculated plants under water stress condition. Siderophore producing microbes chelate the Fe and other micronutrients and make it available for the plants, thus make the plant strong enough to withstand the stresses. The P-solubilization by PGPR is also a common phenomenon which increases nutrient availability to host plants as described by Richardson (2001).

Data regarding number of tillers, grain yield, 1000-grain weight and spike length of wheat as affected by drought, with and without inoculum, is given in (Table 2).

The results revealed that tillering stage of the crop was more sensitive to water stress than the grain filling stage. Ultimate yield of the crop depends on number and health of the tillers. PGPR inoculation with full irrigations gave mover number of tillers (595 m^{-2}) which did not differ statistically from inoculated treatment, where drought stress was imposed at grain filling stage (582 m^{-2}). The inoculated treatment facing drought at tillering stage produced 499 m^{-2} tillers which was 16.6% less than the treatment where drought imposed at grain filling stage. PGPR inoculation produced higher grain yield of (4.956 t ha^{-1}) at un-inoculated and full irrigation which is comparable with the inoculated treatment where irrigation was skipped at grain filling stage (4.933 t ha^{-1}). Growth and yield parameters are positively influenced by inoculation with PGPR. Number of tillers, grain yield, 1000-grain weight and total biomass increased by PGPR inoculation. These findings are in-line with previous work that PGPR inoculation cause significant increase in yield parameters of wheat (Afzal and Bano, 2008) and lentil (Naseem *et al.,* 2012). Reason behind is that PGPR increase the efficiency of plant to use water under water stressed conditions, thus enhance the vegetative growth of the crops (Shaharoona *et al.,* 2006; Zahir *et al.,* 2008). The survival of plants is justified by the previous work that inoculated plants showed tolerance to drought stress and 83% of the inoculated plants survived than non-inoculated plants (Asrar and Elhindi, 2011). Grain yield with PGPR, where water stress was applied at tillering, was increased upto 4.6% than its respective control while it was 4.7 % more than control the sufficient water in leaves under water stress due

Table 2. Effect of drought on yield parameters of wheat with and without PGPR inoculation

Treatments	Grain yield (t ha^{-1})	No. of tillers (m^{-2})	1000 grain weigh (g)	Spike length (cm)	Biomass (Kg ha^{-1})
T_1 ¾ of recommended NP (Full irrigation)	4.933 ab	568 b	36.9bc	18.0ab	9708c
T_2 ¾ of recommended NP (One irrigation skip at tillering stage)	4166 d	470e	35.3 bc	17.6ab	8467e
T_3 ¾ of recommended NP (One irrigation skip at grain filling stage)	4.733 bc	548 c	33.2 c	17.4b	9032d
T_1+PGPR inoculation	5.233 a	595 a	42.7 a	18.5a	10170a
T_2 + PGPR Inoculation	4.516 cd	499 d	40.3 ab	18.4a	8592e
T_3 + PGPR Inoculation	4.956 ab	582 a	37.6 abc	18.3ab	9922b
LSD	0.465	6.509	5.417	1.08	213.9

Means sharing similar letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's multiple range tests.

where water stress was applied at grain filling stage. Thousand grain weight was also affected where the drought was forced at sensitive stages (tillering and grain filling). Thousand grain weight was more (42.7 g) with PGPR when stress was applied at tillering than the treatment, where plant faced drought at grain filling stage (40.3 g). Spike length was18.5cm with full irrigation which was not statistically different from the treatment where irrigation was skipped at tillering stage (18.4 cm). It was 18.3 cm where drought was given at grain filling stage. Biomass was the ultimate effect of physical growth of the plant.

Significantly higher biomass yield (9.92 t ha^{-1}) was observed in the treatment where irrigation was not

to decreased stomatal conductance which enhances the water use efficiency and finely the development and yield of plant (Benabdellah *et al.,* 2011; Mayak *et al.,* 2004). Sandhya *et al. (*2010) observed that the plants, inoculated with PGPR, tolerated stress four days more as compared to un-inoculated control. PGPR may be helpful when plants are under stress for a prolong period Dodd *et al.* (2004). The drought tolerance potential of PGPR refers to their capability of producing exo-polysachrides in stressed environment which produce mucilage in sheath of the cells (Alami *et al.,* 2000).

Data regarding soil and grains composition is presented in Table 3.

Higher soil N and Olsen P was observed by inoculation with PGPR than control under normal and water stress condition. Maximum soil N (0.032%) was determined in inoculated treatment, under normal condition as well as in treatment where irrigation was not applied at milking stage. Inoculation exhibited maximum available P (14 and 13.95 mg kg^{-1}) where drought stress was applied at milking and tillering stage, respectively, which differed significantly from their respective control (12.97 and 12.75 mg kg^{-1}). Nitrogen percentage in grains did not differ significantly in all treatments except in treatments, inoculated with PGPR (1.86 %) under full irrigation supply. Highest P-uptake (0.45%) was also observed in PGPR inoculated treatments, irrigated as per requirement of the plant. Phosphorus contents did not vary significantly whether the drought

Table 3. Effect of drought on chemical composition of wheat with and without PGPR inoculation

Treatments	Soil N (%)	Soil P (ppm)	Grain N (%)	Grain P (%)
¾ of recommended NP (Full irrigation)	0.031	9.31c	1.78b	0.43ab
¾ of recommended NP (One irrigation skip at tillering stage)	0.028	8.75d	1.73b	0.35cd
¾ of recommended NP (One irrigation skip at grain filling stage)	0.029	8.97d	1.78b	0.35cd
T_1 +PGPR Inoculation	0.032	10.89a	1.86a	0.45a
T_2 +PGPR Inoculation	0.030	9.95b	1.76b	0.38bc
T_3 +PGPR Inoculation	0.032	10.00b	1.78b	0.39bc
LSD	NS	1.2	0.0543	0.0428

Means sharing similar letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's multiple range tests.

applied at tillering or grain filling stage. However, these were less than fully irrigated condition. PGPR help plant to take nutrient from the soil by changing pH of the rhizosphere, solubilizing phosphate and producing growth hormones. Availability of nutrients increased the uptake by plants. Nitrogen concentration of inoculated grains remains statistically equal for both the drought stages (tillering and grain filling) but less than the condition of full irrigation. Available P was significantly affected by inoculation with PGPR as they produce organic acids which lower the soil pH, and solubilizing the precipitated P and make it available to plants (Egamberdiyeva *et al.,* 2004; Naseem *et al.,* 2013). Grain concentration for P varies with inoculation. Both the inoculated drought conditions were statistically at par (0.38 and 0.39%) with each other and more than their respective controls (0.35 and 0.35%). Many researchers reported increased P content of seeds by phosphate solubilizing microorganisms (Hussain *et al.,* 2012; Son *et al.,* 2006). PGPR significantly increased the uptake of both N and P by grain and straw of lentil (Kumar and Chandra, 2008). Soil P was 10.0 ppm where irrigation was not applied at grain filling stage which was statistically similar (9.95 ppm) to the treatment when crop had drought at tillering. These results upheld the previous findings that symbiotic relationship with legumes and non-symbiotic with non-legumes is a source of N for plant (Carvalho *et al.,* 2010). Thus beneficial bacteria help plant to withstand water stress by making certain changes in the physiology of the plant.

CONCLUSION

Drought is a serious stress for crop growth but it can be overcome if it is properly managed. If irrigation water is less in quantity then it should be applied at tillering and grain filling stage of wheat. Tillering stage is more sensitive to drought than the grain filling stage. PGPR help the plant to grow successfully under water stress condition by producing ACC-deaminase. They make the plant physically strong by Auxin biosynthesis and nutrients availability through P-solubilization and N fixation, to withstand the stress (drought). In the studies of microbial application the quantities of amendment, applied to the soil, should be less than recommended dose because microorganisms perform well under nutrient deficient conditions. Performance of strains should be checked under controlled and ambient conditions because neither approach is realistic without the interaction of the plants, rhizospheric and environmental conditions.

Table 4. Meteorological data mean recorded at the observatory of Plant Physiology Ayub Agricultural Research Institute, Faisalabad (during growing season of Wheat)

Months from sowing to harvest	Air Temp (°C)		Difference of 1 & 2	Relative humidty %		Pan evaporation		Rain fall (mm)	Wind velocity (km h^{-1})		Dew	Cloudy		Frosty nights	Sun shine hours		Fog
	Max	Min		8am	5pm	8am	5pm		8am	5pm		Days	Nights		H	M	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Nov	27.3	11.3	16.0	85.5	48.5	1.0	1.5	T	0.3	1.0	29	-	1	-	06	23	5
Dec	20.6	6.6	14.0	87.9	58.9	0.5	1.2	16.4	0.6	0.9	29	3	2	2	05	18	10
Jan	18.7	4.4	14.4	87.2	51.8	0.4	0.9	3.2	0.9	1.2	24	2	7	10	04	57	7
Feb	21.0	9.1	11.9	87.6	59.8	0.6	1.2	54.3	1.3	2.4	19	4	9	-	05	01	6
Mar	28.8	13.6	15.3	75.9	47.0	1.2	2.0	1.9	1.6	2.4	28	-	3	-	08	00	-
Apr	34.6	19.7	14.7	54.2	32.3	2.0	3.3	19.0	1.9	2.8	19	-	10	-	08	22	-
May	40.8	23.7	17.1	39.5	22.2	3.0	4.4	1.4	2.4	2.6	13.0	-	5	-	10	16	-

REFERENCES

Afzal, A. and A. Bano. 2008. *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum* L.). Int. J. Agric. Biol. 10(1): 85-88.

Alami, Y., W.A. Achouak, C. Marol and T. Heulin. 2000. Rhizosphere soil aggregation and plant growth promotion of sunflower by an exo-polysaccharide producing *Rhizobium spp*. strain isolated from sunflower roots. Appl. Environ. Microbiol. 66(8): 3393-3398.

Asrar A.W.A. and K.M. Elhindi. 2011. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. Saudi J. Biol. Sci. 18(1): 93-98.

Arshad, M. and W. T. Frankenberger, Jr. 2002. Ethylene: Agricultural Sources and Applications. Kluwer Academic Publishers, New York.

Belimov, A.A., N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bulitta and B.R. Glick. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L.). Soil Biol. Biochem. 37(2): 241-250.

Beltrano, J. and G.R. Marta. 2008. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and re-watering by the arbuscular mycorrhizal fungus *Glomus claroideum*: Effect on growth and cell membrane stability. Braz. J. Pl. Physiol. 20(1): 29-37.

Benabdellah, K., Y. Abbas, M. Abourouh, R. Aroca and R. Azcón. 2011. Influence of two bacterial isolates from degraded and non-degraded soils and arbuscular mycorrhizae fungi isolated from semi-arid zone on the growth of *Trifolium repens* under drought conditions. Mechanisms related to bacterial effectiveness. Eur. J. Soil Biol. 47(5): 303-309.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen Total. p. 595-624. *In:* Page, A-L (ed.), Methods of Soil Analysis. Agron. No. 9, Part 2: Chemical and microbiological properties, 2nd Ed., Am. Soc. Agron., Madison, WI, USA.

Bukhat, N.M. 2005. Studies in Yield and Yield Associated Traits of Wheat (*Triticum aestivum* L.) Genotypes under Drought Conditions. M.Sc Thesis Department of Agronomy. Sindh Agriculture University, Tandojam, Pakistan.

Carvalho, F.M., R. Souza, F.G. Barcellos, M. Hungria, A.T.R. Vasconcelos. 2010. Genomic and evolutionary comparisons of diazotrophic and pathogenic bacteria of the order rhizobiales. BMC Microbiol. 27(12) 10:37.

Clark, V.L. and P.M. Bavoil. 1994. Methods in Enzymology 235(A), Academic Press, London. 315-372.

Dodd, I.C., A.A. Belimov, W.Y. Sobeih, V. I. Safronova, D. Grierson and W. J. Davies. 2004. Will modifying plant ethylene status improve plant productivity in water-limited environments? Proc. 4th International Crop Science Congress, Brisbane, Australia, 26 September-1 October 2004.

Duncan, D.B. 1955. Multiple range and multiple F-test. Biometrics 11(1): 1-42.

Dworkin, M. and J. W. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75(5): 592-603.

Egamberdiyeva, D., D. Juraeva, S. Poberejskaya, O. Myachina, P. Teryuhova, L. Seydalieva and A. Aliev. 2004. Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria. p. 58-66. *In:* Jordan, D. and D. Caldwell (eds.). 26th Southern Conservation Tillage Conference for Sustainable Agriculture, June 8-9, 2004. Raleigh, North Carolina. North Carolina Agricultural Research Service.

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique. 2011. Heat Stress in Wheat during Reproductive and Grain-Filling Phases. Critic. Rev. Plant Sci. 30 (6): 491-507.

Gupta, N.K., S. Gupta, A. Kumar. 2001. Effect of water stress on physiological attributes and their

relationship with growth and yield of wheat cultivars at different stages. J. Agron. 186(1): 55-62.

Ha, C.V., M.A. Leyva-Gonzalez, Y. Osakabe, U. T. Tran, R. Nishiyama, Y. Watan-abe, *et al.,* 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proc. Natl. Acad. Sci. U.S.A. 111(2): 851-856.

Hamayun, M., S.A. Khan, Z.K. Shinwari, A.L. Khan, N. Ahmed and I.J. Lee. 2010. Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. Pak. J. Bot. 42(2): 977-986.

Honma, M. and T. Shimomura. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agri. Biol. Chem. 42 (1): 825-1 831.

Hussain, A, A. Ali and I. R. Noorka. 2012. Effect of phosphorus with and without *Rhizobium* inoculation on nitrogen and phosphorus concentration and uptake by mung bean (*Vigna radiata* l.). J. Agric. Res. 50(1): 49-57.

Ilyas, N. and A. Bano. 2010. *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. Biol. Fert. Soil. 46(4): 393-406.

Kang, J.Y., H.I. Choi, M.Y. Im and S.Y. Kim. 2002. Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell. 14(2): 343–357.

Kumar, R. and R. Chandra. 2008. Influence of PGPR and PSB on *Rhizobium leguminosarum* Bv. viciae strain competition and symbiotic performance in lentil. Orld J. Agri. Sci. 4 (3): 297-301.

Mayak, S., T. Tirosh and B. R. Glick. 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. Pl. Sci. 166(2): 525–530.

McFadden. 1980. Biochemical tests for identification of Medical bacteria. Williams and Wilkins, Baltimore, USA: 51-54.

Mehta, S. and C. S. Nautiyal. 2001. An efficient method for qualitative screening of phosphate solubilizing bacteria. Curr. Microbiol. 43(1): 51-56.

Narusaka, Y., K. Nakashima, Z.K. Shinwari, Y. Sakuma, T. Furihata, H. Abe, M. Narusaka, K. Shinozaki and K.Y. Shinozaki. 2003. Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high salinity stresses. The Plant J. 34(2): 137-149.

Naseem, A. I. Arshad, M. A. Shakir, M. A. Qureshi, J. Sehrish and L. Ali. 2013. Co-inoculation with *Rhizobium* and *Bacillus* spp. to improve the phosphorus availability and yield of wheat (*Triticum aestivum* L.). J. Anim. Pl. Sci. 23(1): 190-197.

Naseem, A. M.A. Qureshi, A.Iqbal, M. J. Ahmad and K.H. Khan. 2012. Influence of Azotobacter and IAA on symbiotic performance of *Rhizobium* and yield parameters of lentil. J. Agric. Res. 50(3): 361-372.

Nishiyama, R., Watanabe,Y., Leyva-Gonzalez, M.A.,Van Ha,C., Fujita,Y., Tanaka, M. et al. 2013. Arabidopsis AHP2, AHP3, and AHP5 histidine phos photransferproteins function as redundant negative regulators of drought stress response. Proc. Natl. Acad. Sci. U.S.A. 110(12): 4840-4845.

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. *In:* Method of Soil Analysis, Chemical and Microbiological Properties. Part 2. (2): 403-430. Amer. Soc. Agron. Madison, WI, USA.

Osakabe, Y., K. Osakabe, K. Shinozaki and L. P. Tran. 2014. Response of plants to water stress. Frontiers in plant science. 5(86): 1-8.

Pirdashti, H., Z. Sarvestani Tahmasebi, G.H. Nematzadeh and A. Ismail. 2003. Effect of water stress on seed germination and seedling growth of rice (*Oryza sativa* L.) genotypes. Pak. J. Agron. 2(4): 217-222.

Potters, G., T.P. Pasternak, Y. Guisez, K.J. Palme and M.A.K. Jansen. 2007. Stress-induced morphogenic responses, growing out of trouble? Trends Plant Sci. 12(3): 98-105.

Pszcz´okowska, A., J. Olszewski, K. Plodzien, T. Kulik, G. Fordo´oski and K. Zuk-Goaszewska. 2003. Effect of the water stress on the productivity of selected genotypes of pea (*Pisum sativum* L.) and yellow lupin (*Lupinus luteus* L.). Electron. J. Pol. Agr. Univ. 6(1): 1-8.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Pl. Physiol. 28(9): 897-906.

Sandhya, V., S.K.Z. Ali, M. Grover, G. Reddy and B. Venkateswarlu. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Pl. Growth Regul. 62(1): 21-30.

Sarwar, M., D. A. Martens, M. Arshad and W. T. Frankenberger Jr. 1992. Tryptophan dependent biosynthesis of auxins in soil. Plant and Soil 147(2): 207-215.

Shaharoona, B., M. Arshad, and Z. A. Zahir. 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiate* L.). Lett. Appl. Microbiol. 42(2): 155–159.

Son, T. T. N., C. N. Diep and T. T. M. Giang. 2006. Effect of bradyrhizobia and phosphate solubilizing

bacteria application on soybean in rotational system in the Mekong delta. Omonrice. 14(2): 48-57.

Steel, K.J. 1961. The oxidase reaction as a toxic tool. J. Gen. Appl. Microbiol. 25(2): 297-306.

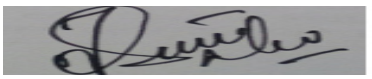
Steel R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics- A Biometrical Approach. 3rd Edition, McGraw- Hill Book International Co., Singapore. 204-227.

Vazquez, P., G. Holguin, M. E. Puente, A. Lopez-Cortes and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves growing in a semiarid coastal lagoon. Biol. Fertil. Soils 30(1): 460-468.

Yuming, B., Z. Xiaomin and D. L. Smith. 2003. Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci. 43(3): 1774-1778.

Zahir, Z. A., A. Munir, H.N. Asghar, M. Arshad and B. Shaharoona. 2008. Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. J. Microbiol. Biotechnol. 18(5): 958-963.

CONTRIBUTION OF AUTHORS

S. No.	Author name	Contribution	Signature
1.	Naseem Akhtar	Conducted research trial and wrote manuscript	
2.	Aneela Riaz	Isolated Bacterial strains purified and prepared inoculums for the experiment	
3.	Muhammad Aftab	Helped in analysis of research samples