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

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ABSTRACT

Agriculture is facing scarcity of water worldwide. Scientists are busy in searching such techniques which may overcome the problem to some extent. Keeping in view this contest an experiment was conducted at the field area of Soil Bacteriology Section, Agricultural Biotechnology Research Institute (ABRI), Ayub Agricultural Research Institute (AARI), Faisalabad in year 2013-14, to evaluate the potential of PGPR on wheat, facing drought stress at booting and milking stages. Inoculum of PGPR having colony forming unit (CUF) 10^6 mL^{-1} was applied as seed coating at sowing. Drought stress was given at two sensitive stages (tillering and grain filling). Fertilizers (100-100-60 kg NPK ha^{-1}) were applied as Urea, single super phosphate and sulphate of potash to all the treatments, following randomized complete block design. Results revealed that PGPR inoculation produced more grain yield 4.95 t ha^{-1} where water stress was given at milking stage compared to 4.51 t ha^{-1} having water stress at booting stage. Number of tillers (582), thousand 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 499, 37.7g and 18.3 cm respectively where drought was given at booting stage. So, it is concluded that drought is more harmful at booting stage than at milking stage and stress can be evade to some extent by inoculation with PGPR.

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

INTRODUCTION

Day by day water is becoming a rare commodity for living sphere. 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 up to 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 (Beltrano *et al.,* 2008; 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 growth and development of crop under 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).

However, drought stress does not affect all growth stages of crop uniformly. Some growth stages can manage drought successfully compared to others show susceptibility to water shortage and may cause distinctive yield losses. Drought stress at any stage may affect all growth parameters of wheat crop. Therefore combine effect of drought stress depends on strength and period of stress (Bukhat, 2005). Drought stress at booting and milking stages at the same time caused severe decrease in ultimate yield as compared to booting or milking stage. Severe decrease in wheat yield was observed when water was not applied at booting or milking stages. However, if water was not applied at 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). Crop response to water deficit is variable at different growth stages (Sandhya *et al.,* 2010; Gupta *et al.,* 2001). Several factors affect the plant response to water stress, such as growth stage, brutality and time period of stress and heredity of cultivar (Beltrano and Marta, 2008). If stress remains for prolong time, 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 (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 generation 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 plant through ethylene regulation in the roots (Dodd *et al.,* 2004; Potters *et al.,* 2007) 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 aim of present study was to identify sensitive stages of the 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

Rhizosphere samples were collected from wheat crop roots at tillering stage present in the permanent plot of Soil Bacteriology Section, Agri. Biotechnology Research Institute, AARI, Faisalabad, Pakistan. Samples were collected from ten different sites, remove the rhizosphere soil from root by brushing and isolation was carried out from each site in five petri plates with five repeats).

Isolation of rhizobacterial strains

For the isolation of bacteria rhizosphere of wheat was collected and isolated the bacteria by dilution plate technique in the laboratory of Soil Bacteriology Section, ABRI, AARI, Faisalabad. Isolation was carried out according to Dworkin and Foster (DF) (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 dissolved in 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 incubation of plates were carried out at ± 25°C for three days. Physically dissimilar colonies were marked and re-inoculated till the uncontaminated colonies were obtained. Presumptive tests were carried out for identification, ensuing standard methods as outlined in Bergey's Manual of Systematic Bacteriology (Vazquez *et al.,* 2000). Bacteria were selected for plant growth promoting activities in wheat under controlled condition. The designated strains of bacteria were stored at -20°C with 20% glycerol.

Categorization of bacterial strains

Categorization of bacterial isolates were done by different tests as fallows **(Table. 1).**

- 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 strains were stored at 27 ± 1°C for two days in incubator which have 100 rpm of orbital shaking. Methyl red was added at the rate of 1 mL per test tube, positive MR test showed red color while control remained yellow following Voges and Proskauer technique (Mayak *et al.,* 2004).
- Oxidase test was carried out to conclude the presence of oxidase enzyme in bacterial isolates (Steel, 1961). Some of the test culture was inoculated on dry slide (oxidase) and observed the change in color to purple/ blue after 60 seconds.
- A colony of bacteria from freshly prepared culture (One day before) was placed on a glass slide and one droplet of Hydrogen peroxide (30%) was poured on the bacterial culture with the help of micro pipette. Bubbling was appeared, showed the incidence of the enzyme (catalase) (McFadden, 1980).
- Bacterial strains were tested for the production of siderophore on the specific media according to the method given by Clark and Bavoil (Clark and Bavoil, 1994). Specific media (Chrome Azurole S agar) was inoculated with bacterial strains as spots. Five days Incubation was given to the inoculated plates at 29-30 °C for one week. Yellowish radiance were appeared around the bacterial colony due to the formation of siderophore.
- A qualitative method was used to determine the phosphorus solubilizing activity (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.

$$S1 = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

- In vitro auxin production by isolated PGPR was determined by using the protocol described by Sarwar (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 535 nm wavelength. Samples were run after the standards and IAA equivalents were deliberated from standard curve (Table 1).

ACC-deaminase activity was determined by monitoring the amount of α-ketobutyrate as described by Honma and Shimomura (Honma and Shimomura, 1978).

Table 1. Categorization of bacterial strains

Isolated strains	Methyl red test	Oxidase test	Catalase test	Siderophore Production	PO_4 Solubilization Index	Indole-3-Acetic Acid (µg mL^{-1})	*ACC-deaminase nmolg^{-1} biomass h^{-1}
PGPR a	+	+	+	+	+	9.0a	171 b
PGPR b	-	+	-	-	-	1.2 c	59 f
PGPR c	-	-	+	-	-	1.6 c	64 e
PGPR d	+	+	+	+	+	9.3 a	174 a
PGPR e	+	+	+	+	+	7.6 b	170 b
PGPR f	+	-	-	-	-	1.6 c	65 e
PGPR g	-	-	-	-	-	1.4 c	96 d
PGPR h	+	+	+	+	+	7.3 b	163 c
PGPR i	+	+	+	+	+	8.4 ab	165 c
PGPR j	+	-	+	-	-	1.3 c	63 e
LSD						1.1194	3.8463
* ACC= 1-aminocyclopropane-1-carboxylic acid,							

Field experiment

Broth culture of required bacterial strains were prepared and stored at requisite temperature (28±1ºC) for 72 hours. To manage the cell density (10^8 –10^9 CFU mL^{-1}) at uniform level, an optical density of 0.5 was achieved (recorded at a wavelength of 535 nm, on spectrophotometer. The surface disinfected wheat seed was mixed with inoculum using peat based slurry containing 72 hours old bacterial inoculum of respective strain (10^8–10^9 CFU mL^{-1}) and sugar solution (10%) whereas, the control was treated with peat containing uncontaminated culture of DF and sugar solution. Inoculated seeds were air dried before sowing.

Field study was carried out in sandy clay loam soil having pH 7.9, EC_e 1.50 dSm^{-1}, nitrogen 0.028% and available phosphorus 7.3 mg kg^{-1} at Soil Bacteriology Section, Agri. Biotechnology Research Institute, AARI, Faisalabad during the year 2013-14. Three fourth (¾) of suggested quantity of fertilizers was put in all the treatments. There were six treatments as follows 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 (RCBD) with three replications. Plant health was monitored throughout the growing period. Data regarding physical and chemical (nitrogen and phosphorus) parameters of grain and soil were recorded after harvesting the crop. Nitrogen was determined according to Kjeldhal method (Bremner and Mulvaney, 1982), while soil P was determined by 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 Least Significant difference (LSD) tests (Fisher, 1949) using software statistix 8.1. Meteorological data was recorded at the observatory of Plant Physiology Section of Ayub Agricultural Research Institute, Faisalabad (Tab 2).

Table 2. Meteorological data documented at the observatory of plant physiology Ayub Agricultural Research Institute, Faisalabad (during growing season of wheat)

Months from sowing to harvest	Air Temp (ºC)		Differ 1&2	Rel. humid %		Pan evaporation		Rain fall (mm)	Wind velocity (km h^{-1})		Dew	Cloudy		Frosty nights	Sun shine hours		Fog
	Max	Min		8am	5 pm	8am	5 pm		8 am	5 pm		Days	Nights		H	M	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Mean November	27.3	11.3	16.0	85.5	48.5	1.0	1.5	T	0.3	1.0	29	-	1	-	06	23	5
Mean December	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
Mean January	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
Mean February	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
Mean March	28.8	13.6	15.3	75.9	47.0	1.2	2.0	1.9	1.6	2.4	28	-	3	-	08	00	-
Mean April	34.6	19.7	14.7	54.2	32.3	2.0	3.3	19.0	1.9	2.8	19	-	10	-	08	22	-
Mean 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	-

RESULTS AND DISCUSSION

Bacterial strains were 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 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 current experiment PGPR were selected having requisite characteristics. Healthy plants have the better capability to bear the stresses than weak plant. It was also observed by Yuming (Yuming *et al.,* 2003) that plants, inoculated with PGPR, showed healthy growth which result 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 finding that ACC-deaminase characteristic of beneficial bacteria (PGPR) is evidently associated with better growth of the inoculated plants under water stress condition (Dodd *et al.,* 2004; Mayak *et al.,* 2004). 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 (Richardson, 2001).

Data regarding tillers yield, grain weight and other physical parameters of wheat were suffered by drought, with and without inoculum, is given in Table 3. Data showed that tillering stage of the crop was more sensitive to drought than the milking stage. Ultimate yield of the crop depends on number and health of the tillers. PGPR inoculation with full irrigations gave number of tillers 595 m^{-2} which was not different statistically from the inoculated treatment, where water deficiency was forced at grain filling stage (582 m^{-2}). The number of tiller was found 499 m^{-2} in the treatment which faced drought at tillering stage and it 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 drought was executed at grain filling stage (4.933 t ha^{-1}). Physical parameters (Growth and yield) were influenced by inoculation with PGPR in positive manner. Number of tillers, grain yield, 1000 grains weight and total biomass increased by PGPR inoculation. Our 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 enhances 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 the 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 up to 4.6% than its respective control while it was 4.7% more than control 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 grains weight was more with PGPR when stress was applied at tillering than the treatment, where plant faced drought at grain filling stage. Spike length was18.5 cm 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. Significantly higher biomass yield (9.92 t ha^{-1}) was observed in the treatment where irrigation was not provided at milking stage than treatment where irrigation was missed at tillering (8.59 t ha^{-1}). Decreased biomass up to 15.4% was observed when the crop

Table 3. Effect of drought on yield parameters of wheat with and without PGPR Average of three replications

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)	4.933ab	568 b	36.9bc	18.0ab	9708c
T_2 ¾ of recommended NP (One irrigation skip*)	4166d	470e	35.3bc	17.6ab	8467e
T_3 ¾ of recommended NP (One irrigation skip**)	4.733bc	548 c	33.2c	17.4b	9032d
T_1+PGPR inoculation	5.233a	595 a	42.7a	18.5a	10170a
T_2 + PGPR Inoculation	4.516cd	499 d	40.3ab	18.4a	8592e
T_3 + PGPR Inoculation	4.956ab	582 a	37.6abc	18.3ab	9922b
LSD	0.465	6.509	5.417	1.08	213.9
* Irrigation skip at tillering stage ** Irrigation skip at milking stage					

Means sharing similar letter(s) in a column do not differ significantly at $p<0.05$ according to LSD

was subjected to drought at tillering stage compared to that where drought was imposed during grain filling. Tolerance against drought is related to multi-gene action passing through a pathway of complex signal transduction (Narusaka *et al.,* 2003). The PGPR help to maintain the sufficient water in leaves under water stress due 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 (Sandhya *et al.,* 2010) observed 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 exopolysaccharides 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 the 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 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, they produce organic acids which lower the soil pH, and solubilizing the fixed 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 bearing drought at tillering. Our 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.

Table 4. Influence of drought on chemical composition of wheat with and without PGPR Average of three replications

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*)	0.028	8.75d	1.73b	0.35cd
¾ of recommended NP (One irrigation**)	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
*Irrigation skip at tillering ** Irrigation skip at Milking stage				

Means sharing similar letter(s) in a column do not differ significantly at $p<0.05$ according to LSD

CONCLUSION

Drought is a serious threat for optimum crop growth and development but it can be overcome if it is properly managed. If irrigation water is less in quantity then it should be applied at booting and milking stage of wheat. Tillering stage is more sensitive to drought than the grain filling stage. PGPR help the plants 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.

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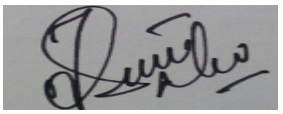
S. No	Author name	Contribution	Signature
1	Naseem Akhtar	Conducted research trial and prepared writeup	
2	Aneela Riaz	Isolated Bacterial strains purified and prepared inoculums for the experiment	
3	Muhammad Aftab	Helped in Analysis of research samples