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RESEARCH ARTICLE

Biological seed priming mitigates the effects of water stress in sunflower seedlings

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Abstract The sunflower (*Helianthus annuus* L. cv. PAC 36) seedlings were inoculated with plant growth promoting rhizobacteria (PGPR), *viz*. *Azotobacter chroococcum* (A+), *Bacillus polymyxa* (B+), separately and in combination of the two (AB+). Relative water content and seedling growth were maximum in AB+ seedlings under control. Water stress significantly decreased the RWC, growth and dry mass of non-inoculated seedlings. However, inoculated seedlings maintained higher growth even under water stress. Pigments and protein contents decreased under water stress, but higher amount of the same was observed in stressed AB+ seedlings. Enhanced activity of nitrate reductase was recorded in AB+ seedlings with maximum in control. Water stress significantly decreased the nitrate reductase activity. A significant increase in the activity of superoxide dismutase (SOD) in leaves was recorded under water stress except in B+ with maximum increase in non-inoculated seedlings. Catalase (CAT) activity decreased in stressed non-inoculated seedlings while increased in the leaves of A+ and AB+ seedlings. Almost similar trends were recorded for both leaves and cotyledons. PGPR improved the water status in stressed seedlings and thereby physiological and biochemical parameters and thus ameliorated the severe effects of water stress.

Keywords *Azotobacter chroococcum* · *Bacillus polymyxa* · *Helianthus annuus* · Plant growth promoting rhizobacteria · Water stress

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Introduction

Water stress caused by irregular rains and insufficient ground water is a major constraint for the growth and development of plant. The negative effects at the hand of water stress on growth of plant is due to the loss in evolution of O_2 and decline in CO_2 fixation which further leads to photodamage of the PS II reaction (Biswal 1997a,b) and oxidative stress in cell components. Oxidative stress generates free radicals and toxic derivatives of O_2 such as singlet oxygen and superoxide anions (Smirnoff 1993) and consequent lipid peroxidation during senescence (Ishida et al. 1997). An antioxidative defense system comprising of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) has been shown to be activated in response to a variety of oxidative stress (Móller et al. 2007; Kohler et al. 2008). These enzymes play various complementary roles in the concerted cellular defense, such as direct scavenging of reactive oxygen species (ROS) for detoxification of the cell (Rubio et al. 2002).

Plant-growth promoting rhizobacteria (PGPR) are a group of bacteria which are associated with roots of many plants and increase the plant growth. PGPR such as *Azotobacter* and *Bacillus* species are being more widely used in agriculture as biofertilizers because of their ability to fix nitrogen and to solubilize phosphorus respectively (Mahfouz and Sharaf-Eldin 2007). They also promote the growth of plants by their efficient role by producing growth promoting substances such as indole acetic acid (IAA), gibberillic acid (GA), cytokinin and vitamins and several other hormones (Gomes et al. 2001). Protective role of PGPR against various stresses, *viz*. water stress (Arshad et al. 2007 and Timmusk et al. 1999b), salinity stress (Saravanakumar et al. 2007), water logging stress (Grichko and Glick 2001), temperature stress (Barka et al. 2006) and heavy metal stress (Arshad et al. 2007) are well documented.

Sunflower (*Helianthus annuus* L.) is an important economic crop. *H. annuus* cv. PAC 36 is one of the most commonly grown cultivars in North India. In the present study the sunflower seeds were inoculated with *Azotobacter chroococcum* and *Bacillus polymyxa* separately and also in combination. Different biochemical parameters were assayed to observe the protective nature of inoculants in enhancing the adaptability of plants during water deficit.

Materials and methods

Growth, inoculation and stress condition

The seeds of sunflower (*Helianthus annuus* L. cv. PAC 36) were surface sterilized with 30% ethanol and divided into four sets for different treatments. Nitrogen fixing *Azotobacter chroccoccum* (A) and phosphate solubilizing *Bacillus polymyxa* (B) were taken as biofertilizers. The seeds were inoculated separately (A+, B+) and also in combination (AB+) in heavy bacterial suspension (containing>10^8 cells/mL) by soaking them for 20 min. The inoculated seeds were air dried in shade for 30 min. The inoculated and the non-inoculated seeds were sown in pots filled with well manured sandy loam soil (1:4) in the experimental plot in the Department of Botany, University of Allahabad, Allahabad (24°47' and 50°47' N latitude; 81°91' and 82°21'E longitude; 78 m above sea level) in the month of April, 2007 (Temperature: 32±5 °C, Relative humidity: 60±5 %). Irrigation was done as and when required. Fifteen days old seedlings of uniform size with the first pair of fully expanded leaves were selected for further experimentation. Each set of treatments was subdivided into two sets. One set was subjected to water stress by withholding water supply for five days while other set was regularly irrigated and was treated as control. The stressed plants were rewatered and recovery was recorded after 24 h. Cotyledons provide a major proportion of assimilate, which is required for growth and establishment of seedling during the developing stage of the first pair of leaves and hence, the senescing cotyledons and first fully expanded leaves from different treatments were sampled for biochemical analyses.

Relative water content

For the measurement of relative water content (RWC) leaves and cotyledons samples were cut into discs of uniform size, weighed for a fresh weight (FW) and then they were immediately floated on distilled water at 25 °C in darkness. The cotyledons being thick and fleshy deteriorated when immersed in water for more than 4 h. The turgid weight (TW) of discs of cotyledons was taken after 4 h and that of leaf discs after 12 h. The discs were dried in oven at 80 °C for 48 h for the dry weight (DW). The RWC was calculated following Bars and Weatherley (1962): RWC (%)=(FW-DW)/ (TW-DW) × 100.

Measurement of pigments and protein contents

The pigments of leaves and cotyledons *viz*. chlorophyll a, chlorophyll b and carotenoids were extracted with 80 % acetone and quantified following Lichtenthaler (1987). Protein content was determined following Lowry et al. (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

Extraction and assay of enzymes

Nitrate reductase (NR) (EC 1.7.99.4) activity was assayed by modified procedure of Jaworski (1971) based on incubation of fresh tissue (0.25 g) in 4.5 mL medium containing 100 mM phosphate buffer, 3 % KNO_3 and 5 % propanol. 0.4 ml aliquot was treated with 0.3 ml 3 % sulphanilamide in 3 N HCL and 0.3 ml 0.02 % N-1 naphthyl ethylenediamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. NR activity was calculated with a standard curve prepared from $NaNO_2$.

Superoxide dismutase (EC 1.15.11) activity was determined by the nitroblue tetrazolium (NBT) phototochemical assay method following Beyer and Fridovich (1987). 0.2 g fresh leaf tissue was homogenized in 1 % polyvinyl pyrrolidone (PVP) prepared in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 15,000 g for 30 min at 4 °C. The reaction mixture contained 0.5 ml clear supernatant, 2 ml 0.15 mM ethylene diaminetetra aceticacid (EDTA), 20 mM of methionine, 0.12 mM NBT and 0.5 ml 11.96 μM riboflavin, 0.5 mL PVP and the activity determined colorimetrically against blank at 560 nm. One unit of SOD activity was defined as the amount of enzyme that was required to cause 50 % inhibition of the reduction of NBT.

Catalase (EC 1.11.1.6) was assayed according to the method of Sinha (1972). 0.2 g fresh leaf tissue was homogenized in 100 mM potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 g for 30 min at 4 °C. The reaction mixture contained enzyme extract, 1.25 ml 0.2 M H_2O_2, potassium phosphate buffer. The reaction mixture was mixed with potassium dichromate acetic acid reagent for absorbance at 570 nm.

Statistical analysis

Treatments were arranged in a randomized block design with three replications. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS (Ver.10; SPSS Inc., Chicago, IL, USA). Appropriate standard deviation of means (±SD) was calculated for presentation with tables and graphs. The treatment means were analyzed by Duncan's multiple range test (DMRT) at $P<0.05$.

Results

Relative water content (RWC) and plant growth

RWC decreased under water stress Table 1. Minimum decrease in RWC was recorded in cotyledons and in the leaves of AB+ plants. Maximum loss was exhibited by non-inoculated plants. Inoculated plants exhibited improved water status during stress. Recovery on watering was more pronounced in plants with inoculants (Tables 2 and 3). Maximum growth of seedlings was recorded in AB+ under control condition as evinced from highest dry mass of seedlings. All inoculated seedlings had higher DM as compared to non-inoculated ones under control as well as under water stress. Seedlings inoculated with bacteria exhibited higher root length even under water stress, except A+ under control. Non-inoculated seedlings exhibited a decrease of 10.61 % in root length under water stress. Maximum shoot length was recorded in AB+ seedlings under control. Interestingly, no significant difference in SL was observed between stressed B+, AB+ and control non inoculated seedlings. Difference in dry mass, root and shoot length was not significantly different between stressed and stress recovered seedlings (Table 1).

Pigments and Protein

The plants inoculated with the bacteria, *viz*. A+, B+ and AB+ showed marked increase in chlorophyll and carotenoid contents. Lesser amount of chlorophyll and carotenoids was observed in senescing cotyledons as compared with their respective first fully expanded leaf irrespective of water regime. Under control condition maximum amount of pigment was observed in AB+ seedlings, while minimum was observed in non-inoculated ones. Water stress caused a decrease of pigments in all treatments as compared with their respective control. The cotyledons and leaves of inoculated seedlings under stress exhibited higher amount of total chlorophyll as compared with non- inoculated stressed seedlings with maximum in AB+ ones. Maximum amount of carotenoids was recorded in AB+ seedlings among the stressed plants. In comparison to the non-inoculated seedlings higher amount of total chlorophyll and carotenoids was maintained on re-watering of stressed inoculated ones. Leaves and senescing cotyledons exhibited almost similar pattern under stress and recovery treatments. Water deficit decreased the protein content in both. In control AB+ plants exhibited maximum respond index 79.39 % in cotyledons and 119.86 % in leaves. The leaves of inoculated seedlings maintained higher amount of protein content with maximum in AB+ ones as compared with that of non- inoculated one in their respective treatments. Cotyledons of AB+ seedlings exhibited higher amount of protein in all the treatments (Tables 1 and 2).

Enzymes

Nitrate reductase activity (NRA) in cotyledon and first fully expanded leaf was studied in plants with and without bacteria with different water regimes. Irrigated inoculated seedlings exhibited increase in NRA with maximum in AB+. Inoculated stressed seedlings exhibited higher NR activity as compared with stressed non inoculated seedlings. Similar

Table 1 Effect of water stress on growth parameters of inoculated and non-inoculated sunflower seedlings

Treatments	Inoculation	Seedling length (cm)			Dry mass of seedling (mg seed^{-1})		
		Shoot	Root	Total length	Shoot	Root	Total DM
Control	–	12.37±0.12d	8.20±0.10c	20.57±0.15e	408.33±30.14e	145.90±11.00c	554.23±19.24e
	A+	14.03±0.15c	8.73±0.12bc	22.77±0.06c	531.67±16.07b	155.78±3.02c	687.44±13.49b
	B+	15.33±0.15b	9.47±0.31b	24.80±0.26b	689.73±36.51a	165.71±4.21b	855.44±40.59a
	AB+	16.57±0.31a	10.39±0.39a	26.96±0.29a	697.13±6.59a	177.40±6.47a	874.53±9.11a
Stress	–	10.27±0.12f	7.33±1.01d	17.60±0.92 h	355.00±15.00f	110.48±5.26d	465.48±19.52f
	A+	11.50±0.10e	8.13±0.32c	19.53±0.21f	419.67±15.50de	145.13±5.00c	564.79±13.58de
	B+	12.55±0.51d	9.17±0.35b	21.71±0.69d	460.67±20.03 cd	146.71±5.81c	607.37±15.55 cd
	AB+	12.57±0.12d	9.43±0.38b	22.00±0.46 cd	485.00±40.93c	148.93±3.68c	633.93±43.10c
Recovery	–	10.77±0.60f	7.86±1.05de	18.63±0.89 g	355.96±15.06f	111.33±5.39d	467.30±19.68f
	A+	11.40±0.20e	8.65±0.40 cd	20.05±0.41ef	421.10±15.11de	145.91±4.88c	567.01±13.31de
	B+	12.55±0.51d	9.87±0.12ab	22.41±0.40 cd	462.14±20.34 cd	146.71±5.81c	608.84±15.75 cd
	AB+	12.57±0.12d	9.93±0.06ab	22.50±0.10 cd	486.37±40.47c	149.69±3.58c	636.05±42.73c

Mean±(SD) values followed by the same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) $n=3$
–non-inoculated, *A+* inoculated with *A. chroococcum*, *B+* inoculated with *B. polymyxa*, *AB+* inoculated with both bacteria

Table 2 Effect of water stress on relative water content (RWC), pigments and protein contents of cotyledons of inoculated and non-inoculated sunflower seedlings

Treatments	Inoculation	Cotyledon					
		RWC (%)	Pigments (μg/mgFW)				Protein (mg/g FW)
			Chl a	Chl b	Total chl	Carotenoids	
Control	−	96.52±3.61ab	0.61±0.08c	0.16±0.04c	0.76±0.07def	0.03±0.02f	21.89±1.81de
	A+	97.02±1.58ab	0.77±0.08ab	0.22±0.03abc	0.99±0.07b	0.08±0.03ef	32.35±2.48b
	B+	93.87±2.94ab	0.64±0.06c	0.21±0.02bc	0.85±0.05 cd	0.14±0.03 cd	23.77±1.83 cd
	AB+	97.43±1.46a	0.88±0.05a	0.24±0.05ab	1.12±0.04a	0.24±0.04b	39.27±4.46a
Stress	−	67.33±1.53f	0.41±0.03e	0.22±0.03abc	0.64±0.03 g	0.18±0.04c	17.19±2.58 fg
	A+	87.08±1.87 cd	0.57±0.06 cd	0.25±0.04ab	0.81±0.04cde	0.30±0.03a	14.73±2.14gh
	B+	82.11±2.00e	0.45±0.06de	0.24±0.04ab	0.70±0.05efg	0.27±0.03ab	11.75±0.87hi
	AB+	88.06±2.01 cd	0.64±0.05c	0.28±0.04a	0.92±0.06bc	0.31±0.02a	26.36±3.46c
Recovery	−	84.54±1.91de	0.47±0.06de	0.17±0.03c	0.64±0.05 fg	0.12±0.03de	9.87±0.65i
	A+	88.65±1.16c	0.66±0.06bc	0.14±0.03d	0.80±0.05cde	0.18±0.02c	19.33±1.75ef
	B+	93.33±1.53b	0.72±0.46de	0.19±0.03bc	0.91±0.90bc	0.17±0.03 cd	14.76±1.26gh
	AB+	95.28±2.58ab	0.80±0.16a	0.03±0.04d	0.83±0.14 cd	0.15±0.03 cd	20.31±2.25def

Mean±(SD) values followed by the same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) $n=3$
−non-inoculated, *A*+ inoculated with *A. chroococcum*, *B*+ inoculated with *B. polymyxa*, *AB*+ inoculated with both bacteria

trend was followed in recovery treatments. Though the water deficit reduced the NRA, but the decrease was not significantly different in leaves of A+ and B+. Lowest NRA was recorded in seedlings without bacteria and the same was maintained in stress and recovery treatments. Highest NRA was found in AB+ plants in all the treatments. Leaf NRA was always greater than cotyledon NRA in respective treatments.

Water stress enhanced the SOD activity except in cotyledons of non-inoculated seedlings. Under stress treatment leaves of non-inoculated plants showed 1.8 fold increase in

Table 3 Effect of water stress on relative water content (RWC), pigments and protein contents of leaves of inoculated and non-inoculated sunflower seedlings

Treatments	Inoculation	Leaf					
		RWC (%)	Pigments (μg/mgFW)				Protein (mg/g FW)
			Chl a	Chl b	Total chl	Carotenoids	
Control	−	82.64±2.25c	2.55±0.23d	0.47±0.06c	3.03±0.22d	0.83±0.02def	47.53±3.37f
	A+	88.32±1.85ab	3.30±0.17bc	0.56±0.03b	3.86±0.15b	0.91±0.01def	86.54±3.41b
	B+	89.13±1.39a	3.61±0.28ab	0.71±0.03a	4.32±0.27a	0.97±0.03cde	64.40±1.92d
	AB+	89.43±0.61a	3.77±0.29a	0.65±0.04a	4.42±0.26a	1.24±0.12bc	104.50±3.40a
Stress	−	49.26±1.24f	1.57±0.25e	0.35±0.02de	1.92±0.23f	0.84±0.03def	21.51±1.71i
	A+	52.42±2.39f	2.29±0.19d	0.38±0.04d	2.67±0.17e	0.77±0.03ef	41.98±2.42 g
	B+	62.33±2.45e	2.50±0.13d	0.51±0.03bc	3.01±0.10d	1.01±0.11cde	57.21±2.06e
	AB+	70.80±2.07d	3.18±0.11c	0.34±0.03def	3.52±0.11c	1.60±0.43a	74.50±3.22c
Recovery	−	74.28±2.46d	1.14±0.11f	0.14±0.04 g	1.28±0.09 g	0.44±0.03 g	33.62±2.59 h
	A+	82.74±3.83c	1.88±0.15e	0.31±0.02ef	1.93±0.22f	1.10±0.09 cd	44.13±2.00 fg
	B+	83.65±2.91c	1.58±0.14e	0.35±0.03de	1.92±0.12f	0.63±0.04 fg	45.93±3.59 fg
	AB+	84.83±2.68c	1.64±0.24e	0.28±0.03f	2.19±0.13f	1.39±0.31ab	55.55±3.97e

Mean±(SD) values followed by the same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) $n=3$
−non-inoculated, *A*+, inoculated with *A. chroococcum*, *B*+ inoculated with *B. polymyxa*, *AB*+ inoculated with both bacteria

SOD activity, which was greater than that of inoculated ones, i.e., 1.2, 1.3 and 1.04 folds in A+, AB+ and B+ respectively. A significant decrease in SOD activity was observed in plants relieved from stress. Senescing cotyledons exhibited successive decrease in SOD activity under stress and recovery treatments in non-inoculated seedlings.

Maximum activity of CAT was recorded in leaves of A+ plants followed by cotyledons of non-inoculated ones under control. In stress condition CAT activity decreased in non inoculated seedlings but increased in the leaves of A+ and AB+ plants. However no significant difference was recorded in B+ seedlings. Inoculated plants always maintained higher CAT activity as compared with the non-inoculated stressed plants (Figs. 1 and 2).

Discussion

Reduction of plant growth is one of the most common effects of water stress (Anjum et al. 2008 and Marulanda et al. 2009). The inoculation of plants with PGPR helped mitigating the effect of water stress. Timmusk et al. (1999b) also reported that inoculation with *B. polymyxa* enhanced drought tolerance. They suggested that PGPR helped in formation of a biofilm around the roots which enhanced the drought tolerance. According to Kohler et al. (2008) inoculation helped maintaining higher level of proline, which served as osmolyte. In our opinion proline might have helped maintaining RWC. Maintenance of RWC has been considered to be a drought resistance mechanism (Grashoff and Ververke 1991). Inoculation helped in maintaining RWC in stressed seedlings at the level of control. The bacteria enhanced the water use efficiency of plants by increasing water absorptive surface of the seedlings (Zhang and Zhang 2001). There are reports that root and shoot growth stimulatory activity of bacteria may be attributed to the production of growth regulators and/ or better availability of minerals or due to combination of their diverse activities (Glick et al. 1999). According to Smirnoff (1993) decrease in chlorophyll is an index of water stress. The decrease in chlorophyll contents might be due to inhibition of biosynthesis or degradation of chlorophyll contents in the plants (Iturbe-Oxmaetxe et al. 1998 and Rahman et al. 2002, 2004). Increase in carotenoids, accessory pigments which absorb photon and transfer excitation energy to reaction centers *via* chlorophyll, protect chlorophyll from photo-oxidation. Carotenoids also serve as antioxidants against free radicals and photochemical damage (Mishra et al. 2006). PGPR helped accumulation of pigments in stressed seedlings. Increased seedling growth and accumulation of dry matter is due to maintenance of photosynthetic efficiency due to PGPR. Photosynthetic efficiency of inoculated seedlings was improved due to increase in pigment contents in the leaves of inoculated stressed seedlings as compared with non inoculated stressed ones. Similar results of pigment accumulation were obtained by Arkhipova et al. (2005) in lettuce plants inoculated with *Bacillus subtilis*.

In germinating fatty seeds lipids are converted to sugars *via* ß-oxidation and central metabolic pool. In the seeds of sunflower, when stored reserve food (lipid) was exhausted during germination and seedling growth, the cotyledons became photosynthetic. Higher photosynthetic efficiency maintained by the cotyledons helped in the establishment and growth of seedlings at early stages (Ampofo et al. 1976). After the first pair of leaves was fully developed, the photosynthetic efficiency of cotyledons declined and they began to senesce. The PGPR delayed the senescing of cotyledons which

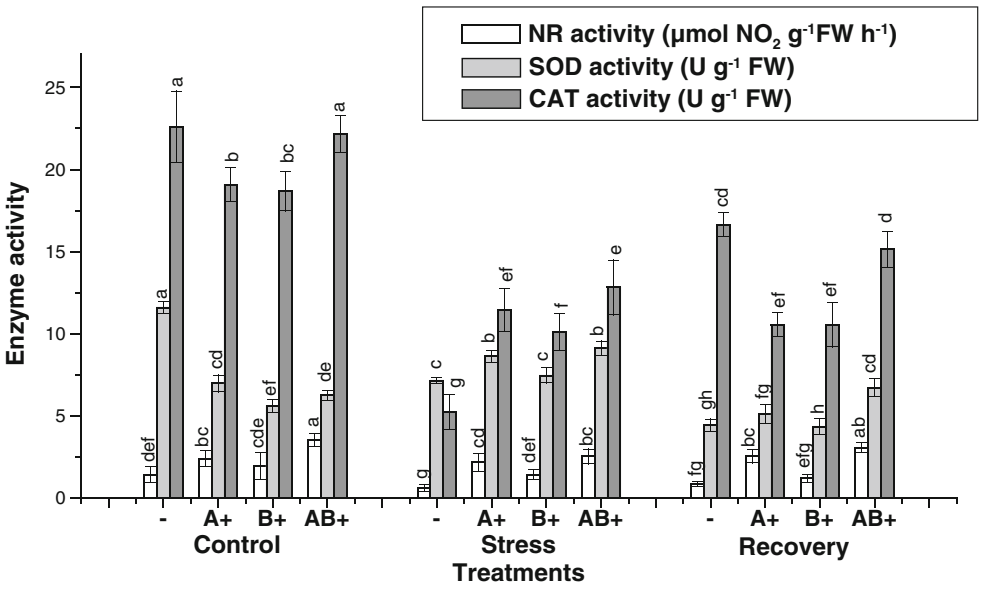

Fig. 1 Effect of water stress on enzyme activities in the cotyledons of seedlings of sunflower. Mean±(SD) values followed by the same *letters within each column* are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) $n=3$

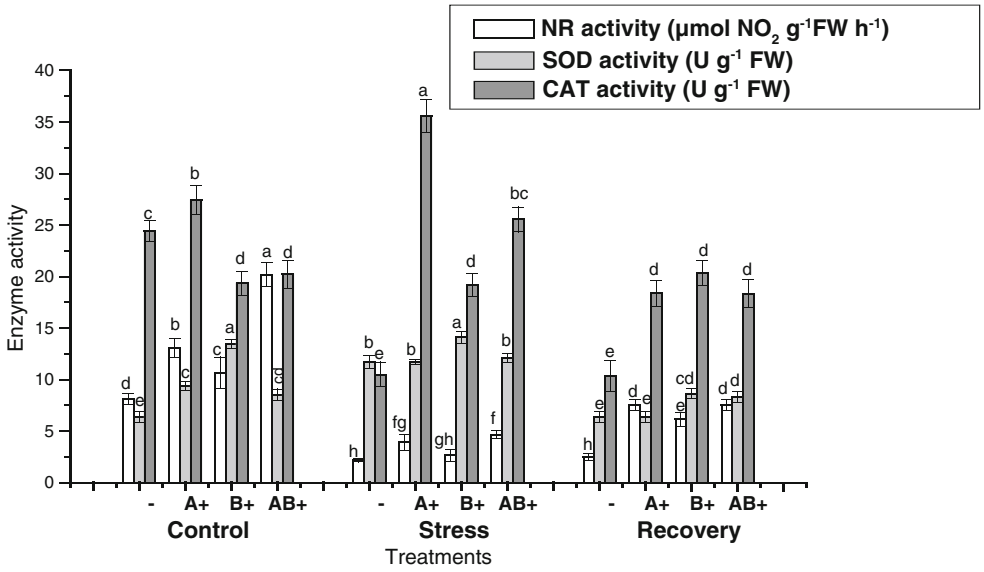

Fig. 2 Effect of water stress on enzyme activities in the leaves of seedlings of sunflower. Mean ±(SD) values followed by the same *letters within each column* are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) $n=3$

increased the photosynthetic area of developing seedlings. This delay in senescence of cotyledons might be mediated by cytokinins (Wingler et al. 1998). Several workers have reported the production of cytokinins by PGPR (Salamone et al. 2001; Timmusk et al. 1999a and Gomes et al. 2001). The decrease in protein in plants under water stress might be due to inhibition of biosynthesis of protein (Rahman et al. 2002, 2004) and/or degradation of proteins (Møller et al. 2007) or due to oxidation of protein caused by reactive oxygen species (ROS) (Iturbe-Ormaetxe et al. 1998). The plants inoculated with bacteria maintained higher amount of protein in stress as well as in recovery treatments as compared with the non-bacterial stressed plants. Improved water status of inoculated seedlings helped in maintaining metabolic activities.

NRA was sensitive to water stress. NRA declined on induction of water stress. However, it regained as stress was released (Hsiao 1973). Several complementary factors and reactions are responsible for decline of NRA under water stress. According to Huber et al. (1996) the reduction of photosynthesis under water deficit was correlated with the reduction of NRA. The effect of water stress on NRA could be minimized in the presence of high nutrient supply. Smirnoff et al. (1985) reported that NRA was highest in the plants growing with ample nitrogen supply irrespective of water regime. However, our results clearly revealed sensitivity of NRA to WS. High nutrient and water availability due to PGPR might be the reason of higher NRA in inoculated seedlings as NR is a substrate inducible enzyme (Crawford 1995). Direct nitrogen supply enhanced the NRA which accumulated in plants in the presence of N-fixative agent, *A. chroococcum*. The increased supply of NO_3^- in inoculated plants helped in the acclimation of stressed plants by producing osmolytes like soluble sugars and amino acids which were synthesized by using photochemical energy (Smirnoff et al. 1985).

According to Radin (1984) leaves of P- deficient plants accumulated more ABA in stressed plants, which resulted in stomatal closure. Increased supply of phosphate due to phosphate solubilizing activity of PGPR have ameliorated the effects of water stress by influencing the increased stomatal conductance (Radin 1984) which enhanced the photosynthesis efficiency. High amount of photosynthates, i.e., sucrose and phosphorus level contributed in osmotic adjustment in stressed plants as reported by Ackerson (1981).

In order to limit oxidative damage under stress condition, plants have developed a series of detoxifying systems which caused the breakdown of highly toxic ROS. SOD activity increased in plants under water stress prevented the loss of cellular homeostasis as well as oxidative damage of membrane, lipids, protein and nucleic acids (Srivalli et al. 2003) which were caused by production of ROS. SOD converts one form of ROS (singlet oxygen) to another equally toxic H_2O_2, which is further detoxified by the coordinated action of H_2O_2 scavenging enzymes, *viz*. catalase (CAT), peroxidase (POX), glutathione reductase (GR) (Foyer et al. 1997). However in present study, CAT activity decreased in stress and recovery treatments in cotyledons and leaves except in the leaves of A+ and AB+ plants where increase was recorded. The CAT activity was always higher in stressed plants with inoculants as compared with that of non-inoculated stressed plants. Thus, it was evident that bacteria ameliorated the effect of water stress and enhanced the adaptive capability of inoculated stressed plants. According to Ünyayar et al. (2005) CAT activity was increased in tolerant *Lycopersicon peruvianum* and decreased in sensitive *L. esculentum* under drought stress. In oily seeds of *H. annuus* L. CAT played an important role in

early growth and establishment of seedlings by detoxifying H_2O_2 which was produced in water deficit (Bailly et al. 2001; Jiang and Zhang 2002).

The present investigation showed that water stress severely affected the metabolism of plant and inhibited the growth of sunflower seedlings. The PGPR delayed the senescing of cotyledons resulting in increased photosynthetic area which maintained the health of seedlings and helped the plants to mitigate the effects of water stress since early developmental stages. Recovery on watering was more pronounced in inoculated plants. PGPR helped in maintaining higher RWC, strong anti-oxidative defence system and activity of nitrate reductase in stressed seedlings, which resulted in more accumulation of dry matter and increased seedling growth.

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