Water and Salt Stress Alleviation in Wheat Induced by Rhizosphere Bacteria with Multi-functional Traits

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Abstract

Twenty-seven (W1-W27), twenty-three (IP1-IP23) and nine (S_1-S_5, N_1, N_2, N_3 and N_4) bacterial isolates from wheat (*Triticum aestivum*), blady grass (*Imperata cylindrica*) and Bermuda grass (*Cynodon dactylon*) rhizosphere which were found to be highly salt-tolerant were further tested for PGPR characteristics *in vitro*. Those bacterial isolates showing positive responses *in vitro* were identified by morphological, biochemical and 16SrDNA sequencing to be *Ochrobactrum pseudogregnonense* (IP8), *Bacillus safensis* (W10), *Bacillus cereus* (S_4). *In vivo* studies on salinity using *B.cereus* (S_4), N_2 and N_4 revealed that all three isolates could promote growth of wheat plants significantly in terms of increased length of leaf as well as root and shoot biomass. The isolates could also elicit antioxidant responses against salt stress in wheat, as evidenced by increased activities of anti-oxidative enzymes at different hours of treatment. *B. safensis* and *O. pseudogregnonense* which were found to be drought tolerant could promote growth in six varieties of wheat tested in terms of increase in root and shoot biomass, height of plants, yield, as well as increase in chlorophyll content. Besides, the wheat plants could withstand water stress more efficiently in presence of the bacteria as indicated by delay of appearance of wilting symptoms increases in RWC of treated water stressed plants, and elevated antioxidant responses which were evident as elevated activities of enzymes such as catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase as well as increased accumulation of antioxidants such as carotenoids and ascorbate. Results clearly indicate that the ability of wheat plants to withstand water stress is enhanced by application of these bacteria which also function as plant growth promoting rhizobacteria. Thus, osmo-tolerant PGPR strains could be used in field condition in order to mitigate salinity and water stress in crop plants

1. Introduction

Wheat is one of the most important cultivated cereals of the world. In different parts of India, productivity is affected by drought stress conditions. Besides direct water stress, increase in salinity also lowers osmotic potential leading to decreased water availability. Certain soil bacteria can help the plants to avoid or partially overcome a variety of environmental stresses. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth, (1978) include those bacteria, which, on inoculation into the soil, colonize the roots of plants and enhance plant growth. PGPR-elicited induced systemic tolerance (IST) help the growth of plants under abiotic stresses by producing various antioxidants, which result in the degradation of reactive oxygen species (ROS) (Figueredo et al., 2008).

The present study aims to isolate bacteria from rhizosphere of wheat as well another facultative halophyte, to select those bacteria with ability to grow in high salt medium and evaluate the different traits of such bacteria to utilize them as plant growth promoters as well as for alleviation of water stress. Besides, the study also aims to determine the biochemical mechanisms of induced systemic tolerance in wheat varieties.

2. Materials and Methods

2.1. Plant material

Six varieties of wheat (*Triticum aestivum* L.), namely Gayetri (GY), Mohan Wonder (MW), KW51, Ghandhari (GN), Kedar (KD) and PBW343 were collected and their viabilities were initially checked.

2.2. Collection of soil samples and isolation of salt-tolerant bacteria

Soil samples were collected from the rhizosphere of *Triticum aestivum*, *Imperata cylindrical* and *Cynodon dactylon*. For isolation of salt-tolerant bacteria, nutrient agar (NA) supplemented with 10% NaCl was used as a selective medium. These isolates were also grown in NA supplemented with 4% and 8% NaCl. Mannitol Salt Agar (MSA) containing 7.5% NaCl (Hitchens and McCarron, 1995) to confirm the salt tolerance ability of the isolates.

2.3. In vitro PGPR traits

Phosphate solubilization (Pikovskaya, 1948), siderophore (Schwyn and Neiland, 1987), IAA (Dobbelaere et al., 1999) and ACC deaminase (Honma and Shimomura, 1978) production were assayed.

2.4. 16 S rDNA sequence

The 16SrDNA sequences obtained from PCR products were subjected to BLAST analysis and aligned with ex-type isolates sequences from NCBI GenBank for identification.

2.5. Selection of the bacterial strains for in vivo plant growth promotion

Of the fifty-nine isolates, five strains-S_4, N_2, N_4, W10 and IP8 which tested positive in all *in vitro* PGPR were selected for *in vivo* studies.

2.6. Determination of plant growth promoting activity

The isolates (10^{8-9} cfu ml^{-1}) were applied to the rhizosphere of six varieties of wheat plant after 15 days, once a week. Growth promotion was recorded in terms of increase in root and shoot biomass.

2.7. Induction of saline stress in wheat plant

Aqueous suspensions of bacterial isolates (10^{8-9} CFU ml^{-1}) were applied as a soil drench to the rhizosphere of *Triticum asetivum*. Salinity was imparted to the plants by adding 200ml of 200 mM NaCl solution to the rhizosphere of the plants after 15 days of growth thrice at an interval of one day. The bacterial isolates were also applied after 15 days, once a week along with the application of NaCl.

2.8. Testing of drought tolerance of bacteria

The two isolates- W10 and IP8 were tested for their tolerance to water stress *in vitro* as described by Sandhya et al., (2011). Addition of 25% PEG 6000 gave a water potential of-0.73 MPa and the ability of any bacterium to grow in such a medium was considered as drought tolerant.

2.9. Induction of drought stress in plants

For soil application of bacteria, prior to imposition of drought stress, aqueous solution of bacteria at a concentration of 1×10^8cfu ml^{-1} were applied twice as soil drench in all varieties. Drought stress was then induced in one-month-old plants by withholding water completely for the required period. Sampling was carried out after 3, 6 and 9 days of each period of drought stress. Morphological changes and the relative water content (RWC) of leaves were determined as described by Farooqui et al., (2000).

2.10. Biochemical analyses

Activities of peroxidase (POX, Chakraborty et al.,1993), catalase (CAT, Chance and Machly, 1955), ascorbate peroxidase (APOX, Asada and Takahashi, 1987), superoxide dismutase (SOD, Dhindsa et al., 1981), glutathione reductase (GR, Lee and Lee, 2000), chitinase (CHT, Boller and Mauch, 1988), β-1,3-glucanase (GLU, Pan et al., 1991) and phenylalanine ammonia lyase (PAL, Chakraborty et al., 1993) were assayed.

3. Results and Discussion

3.1. Identification of the selected isolates

Three isolates-S_4, N_2 and N_4 from *C. dactylon*, two isolates-W10 from wheat and IP8 from *I. cylindrica* showed positive result in all tests such as phosphate solubilization, IAA and ACC deaminase production, siderophore and chitinase production. S_4 was identified as *Bacillus cereus* which was further confirmed (No. MTCC 10655) by Microbial Technology Institute, Chandigarh. On the basis of 16S rDNA sequencing, BLAST analysis revealed IP8 to have 99% homology with *Ochrobactrum pseudogregnonense* and W10 98% homology *Bacillus safensis*. The sequences were deposited in NCBI with accession numbers JX 660688 and JX 660689 respectively. *B. safensis* and *O. pseudogregnonense* could grow under water stressed conditions, but among the two, *O. pseudogregnonense* could grow better at-0.73 MPa.

3.2. In vivo plant growth promotion

O.pseudogregnonense increased growth more significantly than *B.safensis* in four of the varieties. *B. cereus*, N_2 and N_4 also significantly increased growth of wheat plants (var: Gayetri) in terms of increased fresh and dry shoot biomass (Tables 1&2). It was reported by Principe et al., (2007) that *Bacillus* and *Ochrobactrum* strains were isolated from saline soils and these had biocontrol and plant growth promoting characteristics.

3.3. Effect of B. cereus, N_2, N_4 isolates and NaCl on defense and antioxidative enzymes

All the 3 tested defense enzymes- CHT, GLU and PAL showed an increase in activities following bacterial application and salinity stress (Figure 2). All antioxidative enzymes- POX, CAT, SOD, GR and APOX showed similar enhancements though there were variations in the quantum of increase based on the bacterial isolate, duration and concentration of salt solution.

Table 1: Effect of application of W10 (*B. safensis*) and IP 8 (*O. pseudogregnonense*) on root and shoot dry mass of different varieties of wheat plants

Wheat varieties	Treatments	Root dry wt. plant (g^{-1})	Shoot dry wt. plant (g^{-1})
Gayetri	Control	0.90±0.03	1.30±0.04
	B. safensis	1.30±0.02	1.96±0.04
	O. pseuodgronense	2.37±0.04	2.62±0.01
Mohan-wonder	Control	0.98±0.05	1.59±0.09
	B. safensis	1.43±0.02	2.45±0.04
	O. pseuodgronense	1.84±0.01	3.37±0.05
KW 51	Control	1.10±0.07	1.35±0.03
	B. safensis	1.88±0.06	2.67±0.06
	O. pseuodgronense	2.32±0.09	3.64±0.02
Ghand-hari	Control	0.88±0.01	1.58±0.02
	B. safensis	1.40±0.02	4.43±0.05
	O. pseuodgronense	1.32±0.04	2.94±0.06
Kedar	Control	0.94±0.05	1.55±0.07
	B. safensis	2.21±0.08	2.59±0.05
	O. pseuodgronense	1.88±0.03	2.32±0.01
PBW 343	Control	0.85±0.06	1.37±0.04
	B. safensis	1.67±0.08	2.53±0.09
	O. pseuodgronense	1.84±0.09	2.66±0.03
CD (p=0.05)	Treatments	0.43	0.68
	Varieties	0.60	0.96

Average of 3 replicate sets with10 plants in each. ±= Standard error; Difference between control and treated significant at p=0.01 in all varieties as determined by Student's 't' test

Table 2: Effect of application of *B. cereus* (S_4), N_2 and N_4 on fresh and dry shoot wts. (g) of *T. aestivum* (var: Gayetri- GY)

Treatments	Shoot Fresh Mass (g)	Shoot Dry Mass (g)
Control	3.64±0.07	1.59±0.01
N_2	5.78±0.02	2.45±0.01
N_4	9.15±0.01	3.37±0.06
S_4 (*B. cereus*)	8.72±0.05	4.43±0.07
P. polymyxa	7.24±0.09	2.94±0.09
O. anthropi	7.03±0.02	2.59±0.04
B. megaterium	5.59±0.03	2.12±0.06

Average of 3 replicate sets with 10 plants in each; ±= Standard error, Difference between control and treated significant at p=0.01 in all varieties as determined by Student's 't' test

Figures 1&3). It is thus clear that these bacteria enhance antioxidative responses in wheat leading to It has been shown that genera such as *Bacillus* and *Pseudomonas* tend to be pre-dominant in saline soils (Tank and Saraf, 2010).

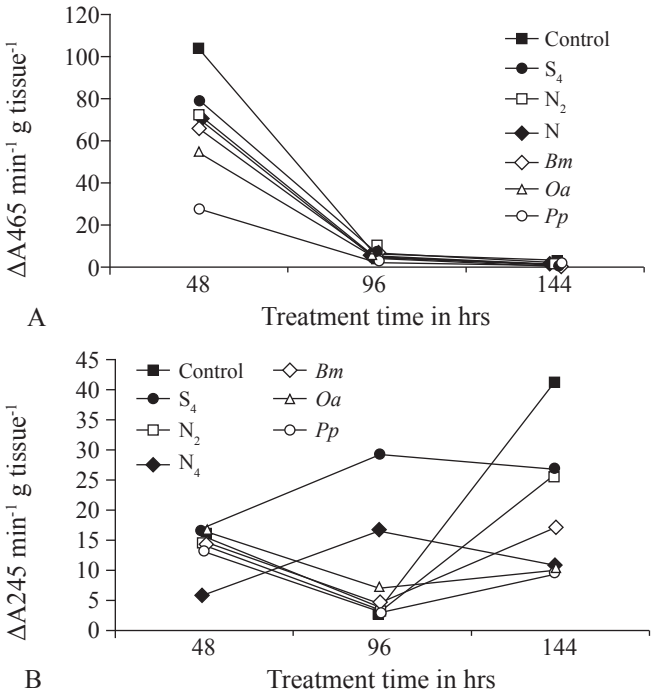

Figure 1: Activities of POX (A) and CAT (B) in wheat plants following treatment with bacterial isolates along with 200 mM NaCl.

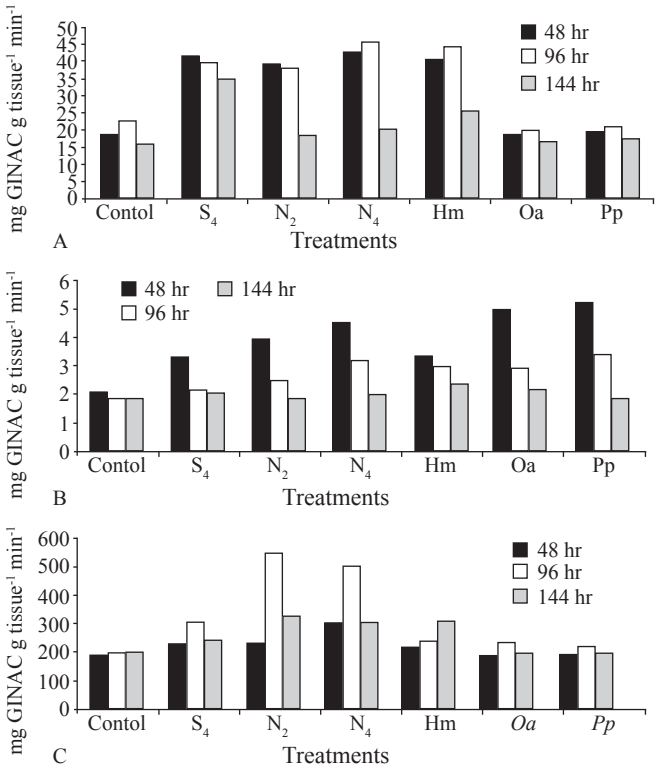

Figure 2: Activities of CHT (A), GLU (B) and PAL (C) enzymes in *T. aestivum* (var Gayetri-GY) following application of *B. cereus*, N_2, N_4 and 200 mM NaCl.

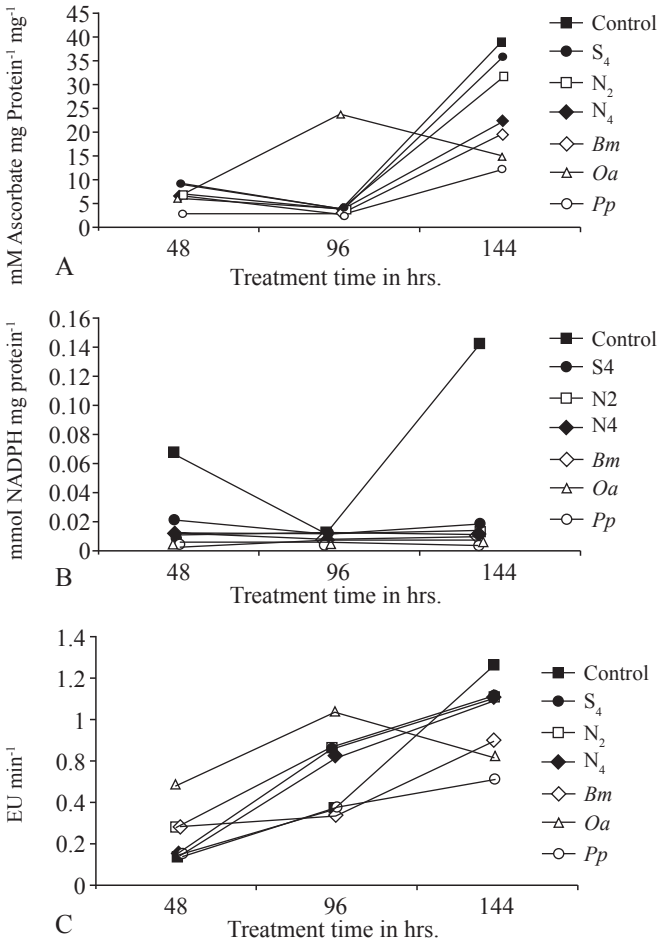

Figure 3: Activities of APOX(A), GR (B) and SOD (C) in wheat plants following treatment with bacterial isolates and 200 mM NaCl.

3.4. Antioxidative enzymes and antioxidants

Significant variations were observed in the activities of the five antioxidative enzymes-i.e. SOD, POX, APOX, CAT and GR tested. In GY, MW, KW 51 varieties, inoculation of bacteria to the soil prior to induction of drought led to enhancement in activities of all the five enzymes in comparison to un-inoculated control. In the varieties where SOD and CAT declined from the early period of drought, bacterial treatment led to increase in activities during different periods of drought. In case of other varieties also the same trend was observed (Tables 3 and 4). It has been reported in several instances that water or salt stress tolerance in plants is related to maintaining of higher antioxidative status for prolonged period (Foyer and Nocter, 2003).

Table 3: Activities of anti-oxidative enzymes in leaves of wheat plants treated with two bacterial isolates followed by different periods of water stress

Wheat varieties	Days of stress	POX activity (A_{460} min^{-1} $gtissue^{-1}$)			APOX (A_{290} min^{-1} $gtissue^{-1}$)		
		C	W10	IP8	C	W10	IP8
GY	0	0.36	0.43	0.47	0.12	0.73	0.71
	3	0.41	0.62	0.67	0.18	0.35	0.55
	6	0.35	0.55	0.77	0.16	0.32	0.31
	9	0.21	0.52	0.44	0.11	0.33	0.29
MW	0	0.14	0.25	0.35	0.11	0.52	0.26
	3	0.42	0.66	0.59	0.06	0.37	0.43
	6	0.34	0.54	0.55	0.19	0.29	0.32
	9	0.21	0.65	0.66	0.17	0.23	0.25
K51	0	0.22	0.56	0.77	0.12	0.21	0.22
	3	0.36	0.45	0.53	0.16	0.27	0.21
	6	0.73	0.99	1.12	0.18	0.42	0.41
	9	1.30	1.70	1.54	0.22	0.49	0.35
GN	0	0.19	0.33	0.49	0.14	0.34	0.45
	3	0.29	0.76	0.69	0.19	0.35	0.44
	6	0.53	0.90	0.97	0.21	0.45	0.57
	9	0.88	1.22	1.30	0.27	0.49	0.55
KD	0	0.13	0.43	0.54	0.09	0.33	0.23
	3	0.27	0.77	0.79	0.23	0.57	0.88
	6	0.78	1.29	1.24	0.28	0.43	0.39
	9	1.63	1.98	1.92	0.33	0.54	0.59
PBW 343	0	0.15	0.71	0.70	0.11	0.77	0.74
	3	0.31	0.65	0.78	0.17	0.38	0.59
	6	0.65	1.44	1.39	0.19	0.39	0.66
	9	1.39	1.56	1.92	0.28	0.77	0.74

Average of three replicates. All treatments were significant at p=0.05 as determined by ANOVA

Table 4: Activities of anti-oxidative enzymes in leaves of wheat plants treated with two bacterial isolates followed by different periods of water stress

Wheat varieties	Days of stress	SOD activity (EU mg^{-1} protein)			GR activity (µM $NADPH^{+}$ oxidized min^{-1} mg^{-1} protein)			CAT activity (A_{245} $min^{-1}g^{-1}$ tissue)		
		C	W10	IP8	C	W10	IP8	C	W10	IP8
GY	0	0.052	0.090	0.092	1.26	1.86	3.75	1.6	1.05	0.63
	3	0.036	0.088	0.089	1.99	2.55	2.79	0.75	3.12	3.38
	6	0.026	0.062	0.050	1.52	2.30	2.00	0.55	3.20	4.20
	9	0.025	0.042	0.041	1.00	2.60	4.70	0.25	2.50	3.37

Continue

	0	0.070	0.090	0.080	0.99	3.48	1.25	2.92	5.70	5.37
MW	3	0.050	0.092	0.070	2.04	2.28	3.24	1.55	3.60	6.90
	6	0.040	0.082	0.074	2.20	3.60	3.65	0.80	3.50	5.50
	9	0.020	0.070	0.062	2.55	3.00	2.88	0.37	1.50	1.12
	0	0.057	0.089	0.090	0.85	1.08	1.50	0.77	1.37	0.75
K51	3	0.030	0.098	0.099	0.90	2.16	2.78	0.50	1.10	1.50
	6	0.020	0.100	0.112	2.40	4.80	6.00	0.40	1.40	2.80
	9	0.015	0.026	0.039	1.25	3.10	4.50	0.37	2.25	3.37
	0	0.036	0.180	0.189	1.38	2.89	3.82	0.80	1.55	2.43
	3	0.062	0.150	0.180	2.08	3.45	3.77	1.30	2.30	2.70
GN	6	0.032	0.120	0.220	3.34	4.45	4.77	0.40	2.30	1.50
	9	0.013	0.092	0.090	2.55	4.32	4.21	1.10	2.30	2.56
	0	0.044	0.065	0.088	0.89	1.56	1.59	0.90	2.30	2.78
KD	3	0.066	0.120	0.130	0.99	3.23	3.67	0.50	4.55	6.55
	6	0.023	0.037	0.045	2.50	4.55	4.56	0.41	3.30	3.55
	9	0.010	0.035	0.059	2.88	3.20	3.23	0.78	3.10	4.30
	0	0.040	0.098	0.090	1.22	1.76	1.83	1.12	4.30	4.55
PBW343	3	0.080	0.210	0.180	1.88	3.99	4.23	0.98	5.56	5.21
	6	0.040	0.120	0.097	3.45	5.22	5.88	0.90	6.50	6.32
	9	0.020	0.240	0.086	4.45	6.78	7.45	0.50	2.77	4.45

Average of three replicates, All treatments were significant at p=0.05 as determined by ANOVA.

4. Conclusion

It is quite clear from our studies that all the five isolates were not only salt-tolerant, but also had plant growth promoting attributes. Three isolates- S_4 (identified as *Bacillus cereus*), N_2 and N_4 from the rhizosphere of Bermuda grass more or less increased the growth of wheat plants under saline stress conditions. Other two isolates from the rhizosphere of *Triticum aestivum* and *Imperata cylindrica*, identified as *Bacillus safensis* and *Ochrobactrum pseudogregnonense* also promoted plant growth and alleviated water stress. Alleviation of water stress is suggested to be due to enhancement of antioxidants and proline. The use of such salt as well as drought tolerant PGPR strains may be a boon to agriculture since urbanization and industrialization are fast depleting our cultivable lands. These microorganisms can also contribute to sustainable agriculture under adverse conditions

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