Seed priming with plant growth-promoting rhizobacteria alleviate salinity stress in wheat plant

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Abstract

The objective of this paper was to study the effects of two strains of isolated plant growth-promoting rhizobacteria (PGPR) on physiological and biochemical changes of wheat leaves under salinity conditions. The sterilized seeds were soaked in distilled water (control) and Nutrient Broth liquid medium of Enterobacter cloacae and Bacillus cereus (inoculated treatment) for 2 hours. After 10 days of growth, seedlings were selected for salinity stress treatments (0, 100, and 200 mM NaCl). After 10 days of starting salinity treatments, plants were harvested. Results showed that wheat plant growth, chlorophyll content and catalase activity were reduced under salinity condition; however, PGPRs pre-treatments improved them under stress condition. Salinity significantly increased phenolic, carotenoids, proline and sugars content and peroxidase activity. Moreover, inoculation of seeds by PGPRs increased them under salinity. Lipid peroxidation increased but ascorbate peroxidase activity decreased as a response of saline stress and PGPRs pretreatment of seeds reduced them. Salinity increased sodium content, and PGPRs pretreatments decreased sodium absorption under salt stress. Moreover, in this study, Enterobacter cloacae inoculation of seeds increased wheat plants magnesium and potassium content in control condition as well as under salt stress. Data of experiment showed that the priming with PGPRs which tested in the present study especially E. cloacae can promote plant growth and salt tolerance.

Keywords: Antioxidant enzymes; ions content; osmolytes; plant growth-promoting rhizobacteria; salinity

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Introduction

Exposure of plants to excess salt causes alterations in a wide range of physiological, biochemical, and molecular processes (Shams and Farzami Sepehr, 2018). Also, salinity causes imbalance of ions, ion toxicity-induced metabolism, production of reactive oxygen species and hyperosmotic stress which induced water deficit (Parida and Das, 2005; Orcutt and Nilsen, 2000).

Naturally, Plants interact with different microbes that are in soil (Dobbelaere et al., 2003). A wide range of bacteria are vital components of rhizosphere which are a group of microorganisms which colonize the plant roots. Beneficial rhizobacteria, often known as plant growth-promoting rhizobacteria (PGPR), affect plant

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growth either directly or indirectly through numerous mechanisms of action. PGPR can increase yield and stress tolerance (El-Ghany et al., 2015). Besides nitrogen fixation, the proposed mechanisms of plant growth promotion of bacteria include improving soil structure, solubilizing mineral soil nutrient, producing vitamins and plant growth regulators, as well as impacting many cellular, metabolic and biosynthetic processes (Santi et al., 2013). Therefore, the PGPR can be useful for soil fertility as a biofertilizer which reduce the need for expensive chemical fertilizers in agricultural systems and consequently they would provide economic benefits to farmers. Also they improve and preserve natural resources (Kannaiyan et al., 2004). Recently, numerous studies have been published that explain assessment of PGPR in modifying abiotic stress in various plants (Jha and Subramanian, 2013; Kohler et al., 2009; Singh and Jha, 2017). Today, PGPR are used worldwide as bioinoculant to promote plant production under unfavorable conditions, like heat (Shaik et al., 2011), salinity (El-Ghany et al., 2015) and etc.

Wheat (*Triticum aestivum* L.) is one of the most important food crops of the world, and its productivity directly affects human survival and quality of life. It is cultivated over a wide range of environments, because of wide adaptation to diverse environmental conditions. In Iran, 6.2 million hectares are under wheat cultivation, of which 33% is irrigated and 67% is rain fed, the irrigated wheat growing areas (2 million hectares) are located mostly in southern, central and eastern of Iran (Sardouie-Nasab et al., 2014). Improving salt tolerance of bread wheat and increasing its productivity are the major objectives of researcher's programs. Seed germination and seedling growth of plants are sensitive stage to salinity stress. The objective of this paper is to study the effects of seed priming with two strains of isolated plant growth-promoting rhizobacteria (PGPR) on accumulation of osmoprotectants, phenolic and mineral content, as well as enzymatic antioxidant activity in wheat leaves under salinity conditions.

Materials and Methods

Triticum sp. L. culture conditions and inoculums preparation

The bacterial (*Enterobacter cloacae* (BT) and *Bacillus cereus* (BW)) inoculum was produced by transferring one loop full of each strain to 100 ml of Nutrient Broth liquid medium in a 250 ml Erlenmeyer flask incubated at 28 °C on a rotary shaker for 24 h. Certified seeds of wheat (*Triticum aestivum* L. cv. Moghan as a sensitive cultivar) were achieved from Research and Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman. Seeds of wheat were surface sterilized with 70% ethanol for five min, followed by washing with sterile distilled Water. The sterilized seeds were soaked in distilled water for 2 hours in case of un-inoculated control. The rest of sterilized seeds soaked in Nutrient Broth liquid medium of BT and BW for 2 hours in case of inoculated. Seeds (five seeds per pot) were sown in plastic pots (14 cm diameter and 12 cm deep) and was kept in a greenhouse. The soil stayed air-dried and sieved (< 2mm). The soil contained 70% sand, 5% silt, and 25% clay; it had pH (1:2) (soil: water, v: v) 7.2 and electrical conductivity (1:2) (soil: water, v:v) 0.43 ds m^{-1} (Hanlon, 2015). For preparation of sterile soil, field soil was autoclaved for 20 min at 120 °C. After 10 days of seedlings growth, uniformly developed seedlings were selected for salinity stress treatments. The salinity stress was imposed on the irrigation water. Water irrigation salinity were in three levels of 0 (control), 100, and 200 mM of sodium chloride. The seedlings were exposed to salinity for five times, every other day. The pots were irrigated with 100 ml of each solution every 10 days. In addition, 30% leaching were considered in order to avoid the accumulation of salt in the soil pots.

After 10 days of starting salinity treatments, the roots and leaves of the plants were sampled separately and immediately frozen in liquid nitrogen and stored at -20 °C for future analysis. In briefly plants are divided to 9 groups as follow: 1: Untreated plants as control, 2: seeds of plants were soaked in *E. cloacae* bacterial cell suspension, 3: seeds of plants were soaked in B. cereus bacterial cell suspension, 4: seeds of plant were soaked with distilled water and then plants were treated with 100 mM NaCl solution, 5: seeds

of plant were soaked with distilled water and then plants were treated with 200 mM NaCl solution, 6: seed of plants were soaked in *E. cloacae* bacterial cell suspension and then plants were treated with 100 mM NaCl 7: seed of plants were soaked in B. cereus bacterial cell suspension and then plants were treated with 100mM NaCl, 8: seed of plants were soaked in *E. cloacae* bacterial cell suspension and then plants were treated with 200 mM NaCl 7: seed of plants were soaked in *B. cereus* bacterial cell suspension and then plants were treated with 200mM NaCl.

Shoot length

The distance from the soil surface to leaf tip was considered as shoot length.

Photosynthetic pigments content

Chlorophylls and carotenoids were extracted by 80% acetone and assessed according to Lichtenthaler (1987).

Lipid peroxidation content

Malondialdehyde and other aldehydes content was estimated by using Heath and Packer (1968) and Meir et al. (1992) method.

Enzymes extraction and assays

Three hundred milligrams of leaf fresh weigh were homogenized in an ice-cold mortar using 3 mL of 50 mM potassium phosphate buffer. After centrifugation at 17000×g for 20 min, the supernatant was used for the determination of enzymes' activities and protein content.

Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard. GPX (EC 1.11.1.7) activity was measured in a reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.3% (v/v) H_2O_2, 1% (v/v) guaiacol and the enzyme extract by a method described by Plewa et al. (1991). One unit (U) of GPX activity was defined as the amount of enzyme that produced 1 µmol of tetraguaiacol per minute. The enzyme activity was expressed in U per milligram protein. CAT (EC 1.11.1.6) activity was determined according to the method of Dhindsa et al. (1981).

The assay mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 100 µL of the enzyme extract. The decline in absorbance at 240 nm was recorded (Ɛ=40 mM-$1cm^{-1}$). The enzyme activity was expressed in U per milligram protein (1 µM of H2O2 reduction min^{-1} mg^{-1} protein). APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 150 µL of enzyme extract. The absorbance was read as decrease at 290 nm against the blank (Ɛ=2.8 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in U per milligram protein.

Phenolic content

Fifty milligrams of leaves fresh weight were ground and dissolved in 1 mL of 80 % ethanol. The homogenate was kept at 25 °C for 24 h in the dark. Then it was brought to a volume of 5 mL with ethanol and centrifuged at 2000 g for 10 min. Phenolic content was analyzed by the method of Gao et al. (2006). The absorption was recorded at 765 nm by spectrophotometry. In order to measure total flavonoids, nine hundred milligrams of leaves were ground and dissolved in 3 mL of 80% methanol. Then it was brought to a volume of 3 mL with methanol and centrifuged at 2000 g for 10 min. Total flavonoid content was determined according to the aluminum chloride colorimetric method (Chang, 2002). The absorbance was monitored at 412 nm.

Anthocyanin content was determined according to Wagner (1979) method. The leaf was crushed in 10 mL acidified methanol [methanol:HCl (99:1, v:v)]. The tissues were soaked and incubated at room temperature for 24 h in the dark. The extracts were then centrifuged at 4000 g for 10 min at 25 °C. The absorption of the supernatant was read by spectrophotometer at 550 nm.

Proline content

Proline was determined following Bates et al. (1975).

Total carbohydrate content

Leaves extract was taken in 25 ml test tubes and 6 ml anthrone reagent (150 mg of anthrone in 72 % H_2SO_4) was added, and then heated in boiling water bath for 10 min. The test tubes were ice cooled for 10 min and incubated for 20 min at 25 °C. Absorbance was read at 625 nm (Fales, 1951).

Ions content

Oven - dried samples of leaves were powdered for estimation of ions. One g of dried sample was digested by using 10 ml of nitric acid. Ions concentrations were determined by ICP method (PerkinElmer Optima 7000 DV, USA).

Statistical analysis

All experiments, including plant treatment, growth and the assessment of biochemical parameters, were performed by a factorial arrangement, based on complete randomized design (CRD). All experiments comprised four replications (pots) per treatment. Data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the SPSS 23 software (IBM, Armonk, NY, USA). Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 ($P < 0.05$) were considered statistically significant. The results were shown as mean ± SEM (standard error of the mean).

Results

As shown in Table1, salinity stress (100 and 200 mM) decreased wheat growth. Pretreatment of seeds with BT bacteria significantly increased plant height in comparison with control plants. The results showed that the application of BT in seed priming, significantly increased dry weight under non saline and saline conditions, while BW priming of wheat seeds increased dry weight only under 200 mM salt stress. It was observed that Chl a, b and total chlorophylls decreased in plants which were under 200 mM NaCl when compared with control plants. PGPRs pretreatment of seeds significantly increased chlorophylls in these plants in comparison with no pretreated plants. PGPRs pretreatment of seeds also improved chlorophylls content in plants, which were under 100 and 200 mM salinity, compared to non-pretreated plants. In the present research, carotenoids content increased in plants under salinity. Pretreatment of seeds with PGPR increased the carotenoids of plants in both saline and control conditions as shown in Table 1.

Our results showed that the MDA and other aldehydes content increased significantly in plants that were under salinity stress (100 and 200 mM) and PGPRs pretreatment of seeds (BT and BW) caused a decrease in MDA and other aldehydes contents at 200 mM salinity.

It was found that salinity did not change proteins content of wheat plants. However, BT and BW pretreatments of seeds leads to increase of protein content in control group and wheat plants that were under 100 mM salinity. In the present study, pretreatment of seeds with BT and BW did not affect GPX activity, although increased CAT and decreased APX activity in control groups. Results showed that, salinity significantly increased GPX activity while decreased the activity of CAT and APX. In plants which were under salinity stress, BT and BW inoculation of seeds cause enhancement of CAT activity. Seeds inoculation with BT and BW declined GPX and APX activity in wheat plants that were under 100 mM salinity, but increased the activity of these enzymes under 200 mM salinity (Table 2). As shown in Table3, phenolic content increased in wheat plants which were under 100 mM salinity. In control plants, BT pretreatment of seeds decreased phenolic content. In plants that where under 100 mM salinity, pretreatment of seeds with both strains of the bacteria decreased the phenolic compounds but in plants which were under 200 mM salinity stress, BT strain enhanced phenolic contents.

In this study, it was not any significant change in total flavonoids and anthocyanins contents of wheat that were under salinity and inoculation of seeds with BW leads to enhancement of wheat flavonoids and anthocyanins contents under both non saline and saline conditions. Moreover, the results indicated that BT pretreatment of seeds in wheat plants that where under 100 mM salinity enhanced flavonoids and anthocyanins contents.

Table 1.
Effect of PGPRs pre-treatment on dry weight, height and photosynthetic pigments of wheat plants under control and salinity stress. Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ($p \leq 0.05$).

Treatment	Dry Weight (g)	Height (Cm)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Carotenoids (µg/g FW)
Control	0.104bc±0.006	18.6b±1,15	2.04ab±0.06	0.67b±0.02	2.71bc±0.07	0.37c±0.01
E. cloacae (BT)	0.137a±0.003	20.8a±1.58	2.07ab±0.07	0.80a±0.02	2.87ab±0.08	0.54a±0.02
B. cereus (BW)	0.106b±0.007	18.75b±1.38	2.10ab±0.06	0.82a±0.02	2.93a±0.08	0.55a±0.01
100 mM NaCl	0.098bc±0.003	16.85c±0.80	1.92bc±0.03	0.66bc±0.01	2.58cd±0.03	0.44b±0.01
200 mM NaCl	0.066d±0.001	12.36d±1.47	1.83c±0.05	0.61c±0.01	2.44d±0.04	0.45b±0.02
100 mM NaCl + BT	0.126a±0.001	17.77b±0.76c	2.20a±0.05	0.80a±0.01	3a±0.03	0.56a±0.02
200 mM NaCl + BT	0.090c±0.002	13.05d±1.44	2.18a±0.03	0.86a±0.01	2.98a±0.02	0.54a±0.01
100 mM NaCl + BW	0.094bc±0.003	17.35bc±0.82	2.18a±0.03	0.78a±0.01	2.97a±0.04	0.53a±0.01
200 mM NaCl + BW	0.093bc±0.003	12.85d±1.42	2.22a±0.03	0.79a±0.01	3.01a±0.04	0.55a±0.01

Table 2.
Effect of PGPRs pre-treatment on dry weight, height and photosynthetic pigments of wheat plants under control and salinity stress. Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ($p \leq 0.05$).

Treatment	MDA Content (µmol/g FW)	Other Aldehydes Content (µmol/g FW)	Proteins Content (mgFW)	CAT Activity (U/mg Protein)	GPX Activity (U/mg Protein)	APX Activity (U/mg Protein)
Control	0.057e±0.004	1.9d±0.06	4.89c±0.14	27.25de±1.24	5.01e±0.09	10.36b±0.20
E. cloacae (BT)	0.038f±0.002	1.69d±0.03	8.95a±0.28	41.9bc±2.5	3.84e±0.11	6.23e±0.41
B. cereus (BW)	0.048ef±0.003	1.52d±0.04	9.1a±0.25	47.63a±0.98	3.77e±0.10	5.65e±0.21
100 mM NaCl	0.109cd±0.003	3.79c±0.10	4.94c±0.26	22.5ef±0.71	11.02d±0.47	9.73bc±0.24
200 mM NaCl	0.164a±0.004	6.8a±0.11	5.37c±0.43	15.77g±0.35	13.5c±0.62	8.19cd±0.47
100 mM NaCl + BT	0.107cd±0.004	3.64c±0.13	6.84b±0.19	37.33c±2.12	10.17d±0.36	7.01de±0.23
200 mM NaCl + BT	0.132b±0.002	4.48b±0.06	4.48c±0.31	18.33fg±0.72	16.99b±0.34	12.35a±0.89
100 mM NaCl + BW	0.101d±0,004	3.4c±0.18	6.51b±0.31	44.67ab±0.71	10.1d±0.37	6.83de±0.36
200 mM NaCl + BW	0.122bc±0.003	3.78c±0.08	4.3c±0.29	29.67d±1.19	22.17a±1.29	13.31a±0.37

The proline content in wheat plants was significantly increased under 200 mM salinity and seed priming by BW, increased proline content in wheat which were under 200 mM salinity. Salinity (100 and 200 mM) enhanced sugar content in control group. The soluble sugars content was significantly increased in plants which were priming with BW when compared with control plants.

Also, the BW inoculation increased sugars content in wheat plants which were under 200 mM salinity as shown in Table 3.

In this study, it was observed that salinity reduced copper and potassium and increased sodium and calcium content, but did not affect the

Table 3.
Effect of PGPRs pre-treatment on phenolic, flavonoids, anthocyanins, sugars and proline content of wheat plants under control and salinity stress. Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ($p \leq 0.05$).

Treatment	Phenols Content (mg/g FW)	Total Flavonoids Content (mg/g FW)	Total Anthocyanin Content (µmol/g FW)	Soluble Sugars Content (mg/g FW)	Proline Content (µmol/g FW)
Control	1.45c±0.023	2.04de±0.09	0.66d±0.03	71.9d±2.03	11.09e±0.45
E. cloacae (BT)	1.18d±0.038	2.07cd±0.12	0.69d±0.02	75.36cd±1.58	12.3de±0.19
B. cereus (BW)	1.37c±0.012	2.10bc±0.16	0.9b±0.01	78.64bc±1.18	11.89e±0.13
100 mM NaCl	1.65ab±0.062	1.92e±0.12	0.67d±0.02	81b±1.25	12.84cde±0.16
200 mM NaCl	1.52bc±0.009	1.83cd±0.06	0.74cd±0.03	79.93bc±0.84	21.67b±1.44
100 mM NaCl + BT	1.43c±0.036	2.20cd±0.25	0.82c±0.01	76.33bcd±0.72	14.86cd±0.12
200 mM NaCl + BT	1.7a±0.010	2.18cd±0.13	0.82c±0.01	74.64cd±0.98	23.29b±0.50
100 mM NaCl + BW	1.41c±0.047	2.18ab±0.12	0.95b±0.02	77.67bc±0.98	15.17c±0.14
200 mM NaCl + BW	1.65ab±0.065	2.22a±0.11	1.08a±0.01	87.99a±1.63	39a±1.25

Table 4.
Effect of PGPRs pre-treatment on Na, K, Ca, Mg, Fe and Cu content of wheat plants under control and salinity stress. Each value represents mean ± SEM. Means were compared using Duncan's multiple range test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

Treatment	Na^{+} Content (mg/g FW)	K^{+} Content (mg/g FW)	Ca^{+2} Content (mg/g FW)	Mg^{+2} Content (mg/g FW)	Fe^{+2}Content (mg/g FW)	Cu^{+2} Content (µg/g FW)
Control	4.37e±0.16	40.05ab±0.38	8.65b±0.09	4.22bc±0.102	5.93ab±0.18	24a±1.22
E. cloacae (BT)	4.31e±0.10	40.98a±0.28	8.7b±0.09	4.97a±0.001	5.86ab±0.11	21a±1.06
B. cereus (BW)	5.14e±0.07	40.32ab±0.21	8.91ab±0.18	5.04a±0.044	6.55a±0.09	23a±0.65
100 mM NaCl	9.84c±0.09	39.41ab±0.24	8.76b±0.09	4.11c±0.046	5.79ab±0.18	16b±0.86
200 mM NaCl	15.01a±0.10	38.75b±0.14	9.25ab±0.14	4.1c±0.057	5.75b±0.14	15b±0.13
100 mM NaCl + BT	8.19d±0.30	40.5a±0.28	9.05ab±0.08	4.9a±0.058	5.71b±0.11	16b±1.05
200 mM NaCl + BT	11.66b±0.06	39.75ab±0.43	9.44ab±0.22	4.51b±0.029	5.17b±0.14	15b±0.02
100 mM NaCl + BW	9.61c±0.18	39.75ab±0.14	9.42ab±0.010	4.55b±0.107	5.8ab±0.11	15b±0.27
200 mM NaCl + BW	11.85b±0.31	38.75b±0.14	9.7a±0.22	4.23bc±0.029	5.77b±0.03	15b±0.45

magnesium and iron content. PGPRs (BT and BW) pretreatment of seeds decreased sodium absorption under salt stress. Moreover, in this study, seed inoculation by BT, increased magnesium and potassium content in control condition as well as under salt stress and BW inoculation increased their magnesium content in control condition and under 100 mM salinity. Also, inoculation of seeds by BW increased iron and calcium content in control condition and 200 mM salinity respectively (Table 4).

Discussion

Rhizobacteria have been well reported to promote plant growth as well as overcome various abiotic stresses including salinity. Improvement of water absorption and production of phytohormones such as auxin and cytokinin by bacteria can be due to the increment of growth in plants which were treated with growth-promoting bacteria (El-Ghany et al., 2015). It has been reported that microbial secretions stimulate the synthesis of hormones such as auxin, gibberellin and cytokinin in plants that play a role in improving growth (La Torre-Ruiz et al., 2016). It has also been observed that in salinity stress conditions, plant growth-promoting bacteria help to increase the amount of water available to the plant, and there is a higher hydration in inoculated plants, which results in better water use efficiency and improved photosynthesis and biomass (Shukla et al., 2012).

The effect of salinity on the synthesis of chlorophyll depended on the specific concentration of sodium chloride. Our result showed that salinity reduced photosynthetic pigments which were according to other research (Ashraf et al., 2007; Shukla et al., 2012). In the present study, the pre-inoculation of seeds in stressed wheat plants significantly enhanced chlorophylls content as compared to plants grew under salt stress without seed priming. Similar findings have also been observed in other researchers (El-Ghany et al., 2015). In previous research it has been reported that PGPRs with availability of iron improved the pathway of chlorophyll synthesis.

Also, these bacteria reduce membrane permeability and peroxidation of lipids and maintain the integrity and function of membranes (El-Ghany et al., 2015; Shukla et al., 2012). Therefore, one of the reasons for increasing the content of chlorophyll after application of growth promoting bacteria priming in conditions of salt stress could be due to the effective role of bacteria in providing iron with the protection of the membrane and the structure of chloroplasts by eliminating active oxygen species.

Both biotic and abiotic stresses are involved in over production of ROS causing peroxidation of lipid membranes due to increase in malondialdehyde (MDA) content (Singh and Jha, 2010; Mohammadkhani and Abbaspour, 2017). In the present study, salinity increases the content of MDA and other aldehyde which is the indicator of lipid peroxidative damage of biomembranes (Masood et al., 2006). Seed priming of plants with PGPR-leads to decrease of lipid peroxidation level, suggesting that PGPR protect the cell membrane from the harmful effect of oxidative stress caused by salinity. Shukla et al. (2012) reported the PGPR-mediated reduction of MDA content in inoculated plants compared to un-inoculated plants under salt stress. The effect of seed priming with growth-promoting bacteria on the reduction of malondialdehyde levels, emphasizes the protective role of these microorganisms in the membrane against stress-induced damage. Abiotic stresses cause an increase in cellular level of ROS, leading to lipid peroxidation of membranes.

Plants possess several defense systems to scavenge ROS to protect themselves from the oxidative stress (Cheeseman, 2007). To protect against oxidative stress, plant cells produce both antioxidant enzymes such as peroxidase and catalase, and non-enzymatic antioxidants (Miller et al., 2010). Mittler (2002) stated that antioxidant enzyme activities are usually affected by salinity and used as indicators of oxidative stress in plants. In the present investigation, the responses of wheat plant to salinity were reflected by increased of GPX activities and PGPRs pretreatment of seeds increased catalase and peroxidase activities of cultivated wheat under salt stress which trend has also been observed in other researchers (Eraslan et al., 2008; Molassiotis et al., 2006). These activated antioxidant systems are useful for plant performance and have an essential role in alleviating oxidative stress damage in plants, by removing excess ROS and inhibiting lipid peroxidation. Previous reports have also demonstrated that PGPR-inoculated plants attenuate higher antioxidant enzyme activities resulting in enhanced salt tolerance (Baltruschat et al., 2008; Bharti et al., 2016). Singh and Jha (2017) reported that peroxidase activity increased in plants inoculated with PGPR in control and saline condition.

In current research, the protein content increased in plants which were priming with

PGPRs growing with 100 mM or without sodium chloride. Some of these proteins may have direct correlation with salinity tolerance. In previous studies in maize and rice plants it has been observed that many proteins directly or indirectly related to plant growth promotion which was differentially found expressed by the interaction of *Pseudomonas fluorescens* (Kandasamy et al., 2009).

Proline and soluble sugars are very important biochemical indicators of salinity tolerance in plants (Ashraf and Harris 2004). When plants face salt stress, proline accumulates in the cytosol and helps in cytoplasmic osmotic adjustment (Shukla et al., 2012). Proline also assistances the plant cell by stabilizing subcellular structures such as proteins and membranes and scavenging free radicals under salt stress (Ashraf and Foolad, 2007). Similar to proline, the soluble sugars in higher plants under salinity increased. In this study, seed inoculation with BW induced enhanced proline and sugars accumulation with or without salt stress compared to un-inoculated plants. High proline and sugars contents were also observed under salt stress in the presence of PGPRs in paddy and maize plants (Sandhya et al., 2010; Jha and Subramanian, 2013).

Results showed that in wheat plants which were under 200 mM salinity phenolic content significantly increased. Similar results were reported in plants that phenolic compounds increased under stress conditions (Ksouri et al., 2007). For example, it was stated that, phenolic compounds enhanced in wheat plants under salinity. The phenolic are implicated in plant defense through scavenging reactive oxygen species (Keles et al., 2004). Moreover, in this study, higher production of phenolics, flavonoids and anthocyanins in seed inoculated-plants might be involved in increasing plant resistance. Accumulation of phenolics compounds were recorded in rice roots inoculated with *P. fluorescens* (Kandasamy et al., 2009). Salinity of soil and water is caused by the presence of excessive amounts of salts.

Salt stress reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity (Parida and Das, 2005). Reducing many ions content and increasing sodium content are the most significant effects of salinity, which are mentioned in many reports (Juan et al., 2005; Kafi et al., 2003). In the present study, we observed that PGPRs-seed priming decreased sodium absorption under salt stress. Similarly, several PGPR are reported to reduce sodium absorption in various plants (Bano and Fatima, 2009; Kohler et al., 2009). Our data showed that, bacterial inoculation of seeds increased magnesium and potassium content of wheat plant in both control and stress condition. Potassium is an osmotically active solute that contributes to water absorption at the cell and whole plant level. It plays a key role in plant water stress tolerance (Caravaca et al., 2004). This shows that PGPR strains could increase nutrient uptake which was reported previously (Nadeem et al., 2006). It has also been reported that PGPRs produce exopolysaccharides which bind with cations such as Na^+ and reduced their absorption through plant (El-Ghany et al., 2015). In addition, it has been shown that inoculation with bacteria has led to an increase in the expression of SOS genes. SOS1 is one of the important Na^+/H^+ antiporters of plasma membrane that improves salinity tolerance and is very important for long-term regulation of sodium transfer from root to shoot. SOS4 genes also increase the production of ethylene and auxin, which lead to improved root growth and ultimately lead to an increase in mineral absorption (Bharti et al., 2016).

Comparison of data shows that in this study, pretreatment of seeds by PGPRs have improved the plant's condition under stress through different ways and are an effective tool for protecting wheat plants from salinity damaged and this strategy could be applied for sustainable agriculture.

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