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Growing varieties durum wheat (*Triticum durum*) in response to the effect of osmolytes and inoculation by *Azotobacter chroococcum* under salt stress

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This study was conducted to determine the effect of plant growth promoting rhizobacteria *Azotobacter chroococcum* AZ6 isolated from arid soil and osmolytes such as glycine betaine (GB) or proline (P) on the growth of durum wheat varieties under salinity stress. Inoculation by *A. chroococcum* AZ6 in the presence or absence of P (5 mM) or GB (5 mM) reduced substantially the effect of salt stress on plant growth parameters such as root length, plant height, fresh shoot and root weight and dry shoot and root weight. The differences between the two varieties were low but with a fresh and dry weight higher in Waha. The rate of Na^+ accumulation in the roots and the shoots was important up to 100 mM and increased at 200 mM. The K^+ concentration and chlorophyll content decreased but proline and amino acid contents were enhanced with increasing salinity. Treatment by inoculation in the presence or absence of osmolytes improved the chlorophyll (*a* and total) and the K^+ concentrations and reduced intracellular proline accumulation and amino acids contents. Also, as result, the use of *A. chroococcum* AZ6 and osmolytes treatment may provide a means of improving tolerance of durum wheat to salt stress.

Key words: Durum wheat, salinity, osmolytes, *Azotobacter chroococcum*,

INTRODUCTION

Salinity and aridity are major environmental constraints limiting the growth and productivity of crops. In arid and semi-arid areas of the world, rainfall is inadequate for the leaching of salts from the root zone. Accordingly, the soluble salts are accumulated in the soil surface with the Na^+ as a dominant cation. Soil salinity presents a growing threat to agriculture and causes salinization of arable land on the planet. One third of arable land resources in the world are affected by salinity (Munns, 2002). Losses in crop yields in saline areas are important. High

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concentrations of salts cause ion imbalance and hyper-osmotic stress in plants leading to cellular dehydration. The osmotic potential resulting from high concentrations of the Na^+ in the soil prevents the absorption of water which causes a variety of structural, biochemical and physiological seed changes and reduces the rate of germination causing a delay in the development of the plant (Poljakoff-Mayeber et al., 1994). Several experiments have been attempted to reduce the drastic effect of salt stress in the growth and productivity of plants. Most work focuses on the development of salt-resistant varieties. Thus, efforts to reproduce genotypes which are highly salt tolerant are hampered by a lack of understanding the complex nature of tolerance. Plant tolerance seems to be based on a number of mechanisms, many of which are based on physiological processes such as transport mechanisms that reduce or eliminate the Na^+ and the Cl^- of xylem (Tester and Davenport, 2003). Indeed, these varieties have developed various biochemical and physiological mechanisms against this type of stress. Such mechanism, ubiquitous in plants, is the accumulation of some organic metabolites of low molecular weight, especially during germination and early growth (Turan et al., 2007).

Exogenous application of osmolytes such as proline and glycine betaine attracted the attention of many researchers for several years (Wani et al., 2013). Thus, the application of low concentrations of glycine betaine and proline maintains a high concentration of the K^+ and activates the exclusion of the Na^+ and Cl^- ions from the leaves and roots (Cuin and Shabala, 2005).

Another biological approach exists. It is the plant growth promoting rhizobacteria (PGPR). It consists of inoculation of plants by rhizobacteria to promote their growth. PGPR used as biofertilizers and/or antagonists against plant pathogens are a promising alternative to chemical fertilizers and pesticides. Under salt stress conditions, PGPR have shown positive effects on plants, especially on physiological parameters such as germination, tolerance to drought, the weight of stems and roots (Tiwari et al., 2011). These rhizobacteria are able to adapt to adverse conditions and improve plant growth in environments at high osmolarity (Biari et al., 2008) and they develop molecular mechanisms to survive and grow with the increase of salinity (Tripathi et al., 2002).

According to many authors, most salt-tolerant bacteria accumulate or synthesize organic compatible solutes such as proline, glycine betaine and betaine glutamine. The application of compatible compounds and inoculation with rhizobacteria could attenuate the negative effects of salt stress on the growth of wheat.

Through this study, we evaluated the effects of inoculation by *Azotobacter chroococcum* AZ6 isolated from arid soil and exogenous application of glycine betaine (GB) and proline (P) on the morpho-biochemical parameters and on the ionic balance in two varieties of durum wheat under salt stress.

MATERIALS AND METHODS

Bacterial strain isolation

Soil samples were collected from the rhizosphere of wheat in an arid soil located in the region of Sétif situated at 300 km East of Algiers (Algeria). One gram of soil strongly adhering to the roots was extracted from the sample, added to 10 ml of sterile distilled water and shaken for 30 min. To select *Azotobacter*, the isolation was carried out on Ashby medium (Atlas, 2005), incubated at 28°C ± 1/72 h. Typical colonies were subcultured several times on Ashby agar to obtain pure cultures.

Identification of the bacterial strain

Among the isolated strains, AZ6 was identified according to macroscopic appearance (appearance of the colony on solid medium, form, texture and pigmentation), Gram staining, mobility, presence of cysts followed by preliminary biochemical characterization such as catalase test, oxidase and carbohydrate assimilation test (malonate, rhamnose and mannitol) according to Brenner et al.(2005).

16S rRNA gene sequence analysis

PCR fragments obtained by the amplification of a DNA fragment corresponding to a region of the 16S rDNA gene of the isolate were sequenced using the automatic sequencer at DNA Vision Company (http:/www.dnavision.com). The sequence was submitted to the GenBank, and accession number was assigned KT339176. The partial 16S rDNA sequence of the isolate strain was compared with those available in the databases. The phylogenetic tree was constructed on the aligned datasets using the Neighbor-Joining method (Saitou and Nei, 1987). Phylogenetic analyses were conducted in MEGA5 (Tamura et al., 2011).

Plant material

Seeds of two varieties of durum wheat were used: Waha (*Triticum durum* Waha *lcv*) and Bousselam (*Triticum durum* Bousselam *lcv*). They were obtained from Institut Technique des Grandes Cultures (ITGC)-Sétif–Algeria.

Bacterial inoculation and growth conditions

The strain AZ6 was cultured at 28°C/48 h in Winogradsky broth (Atlas, 2005). The culture was centrifuged (12,000 rpm/10 min) and rinsed twice in Phosphate-buffered saline (PBS) to obtain a density of 10^8 bacteria ml^{-1}. The two varieties of seeds Waha and Bousselam were surface sterilized with sodium hypochlorite solution (2%) for 30 min and rinsed several times with sterile distilled water. Seeds germination was carried out in advance on Whatman paper grade N° 42, in Petri dishes containing 15 ml sterile distilled water at 20°C/48 h in dark. Inoculation was effected on the germinated seeds by an immersion in the bacterial suspension for 30 min. Uninoculated seeds (control) were immersed in sterile distilled water. Plastic pots (Φ=10 cm), of which internal surface was disinfected with 70% of ethanol and the pots were filled with 200 g of sand washed thoroughly with water and autoclaved (120°C/1 h) during three successive days. 20 ml of Hoagland solution at ½ were added in each pot. The pots were then divided into three groups and each group was divided into four subgroups. The three groups represented the concentrations of NaCl used (control, 100 and 200 mM). The four subgroups indicated

the type of treatment: indicator (uninoculed seeds), AZ6, AZ6 + P and AZ6 + GB for each of the concentrations of NaCl used. The GB or P at 5 mM was added to the different NaCl concentrations. The treated seeds were sown (one seed per pot) at a depth of 1 cm from the surface. The experiment was repeated 6 times and was conducted for 45 days in a growth chamber (phytotron) with an average day/night temperature of 26 and 16°C, respectively and a photoperiod of 16 h light of 2100 lux. Soil moisture was adjusted and constantly maintained during the experiment by watering with sterile distilled water. At stage four to five leaves, the plants were harvested and washed with distilled water. The roots and shoots were collected separately. Their sizes and fresh and dry weights (after 72 h at 65°C) were determined. The dosages of chlorophyll, amino acids, proline and the Na^+ and K^+ contents were also performed.

Determination of chlorophyll

Chlorophylls *a* and *b* were determined according to the method of Arnon (Arnon, 1949). 0.5 g of the shoots of each sample were cut into small segments (0.5 cm) and homogenized in 10 ml of acetone at 80% and stored at -10°C overnight. The organic extract was centrifuged at 14000 rpm/5 min and the absorbance of supernatant was measured at 663 and 645 nm to determine the chlorophyll *a* and *b*, respectively.

CH*a* ($mg.l^{-1}$) = 12.41 OD (663) - 2.59 OD (645).
CH*b* ($mg.l^{-1}$) = 22.9 OD (645) - 4.68 OD (663).
CH*t* = CH*a* +CH*b*
CH*a*: chlorophyll *a* concentration.
CH*b*: chlorophyll *b* concentration.
CHt: total chlorophyll concentration.

Content of the Na^+ and the K^+ in shoots and roots

The concentrations of the Na^+ and the K^+ in the shoots and roots were determined by flame spectrophotometer after digestion of the solids (0.1 g of shoots and 0.05 g of roots) in 10 ml of H_2SO_4 98% and 3 ml of H_2O_2 30% during 5 h according to the method of Skoog et al. (2000).

Determination of soluble amino acids and proline

Samples of shoots and roots were stored below -15°C before analysis. The extraction was conducted using the method described by Naidu (1998): 500 mg of shoots were placed in centrifuge tubes containing 5 ml of methanol: chloroform: water (60:25:15). The sealed tubes were heated in a water bath at 60°C for 2 h and centrifuged at 10000 rpm/10 min. The supernatant was then used for assays of soluble amino acids and proline.

Soluble amino acids

1 ml of acetic acid/sodium acetate buffer (pH 4.3) and 1 ml of ninhydrin (5% in ethanol) were added to 1 ml of supernatant. The samples were stirred and heated in a water bath (95°C) for 15 min. The absorbance was determined at 570 nm.

Proline

Proline was determined by a rapid method developed by Singh et al. (1973): 1 ml of the supernatant, 4 ml of ninhydrin solution, 4 ml of glacial acetic acid and 1 ml of distilled water were introduced into 10 ml centrifuge tubes. This mixture was heated in a water bath (90°C) for 45 min and cooled to room temperature. The absorbance was determined at 520 nm.

Statistical analysis

All the data were the mean of six repetitions for growth parameters and three repetitions for other determinations. Two-way analysis of variance (ANOVA) was conducted with the multifactorial Assistat 7.6 Beta software. When an interaction of two-way ANOVA was smaller than 0.05, the Tukey´s HSD test was realized.

RESULTS

Strain certification

Results showed that this AZ6 strain was identified as *Azotobacter chroococcum*. *A. chroococcum* was rod shape, motility occurred through the use of peritrichous flagella, cysts-forming, positive to oxidase and catalase test with insoluble brown or brown-black pigmentation and darken with age, utilized mannitol, malonate and not rhamnose. Partial 16S rDNA sequences confirmed that the AZ6 strain (accession number KT339176) belonged to *A. chroococcum* species with 99% 16S rDNA similarity (Figure 1).

Morphological parameters

Analysis of variance of fresh and dry weight of roots and shoots, root elongation and plant height in both wheat varieties showed that salinity had a significant negative effect on these growth parameters ($p \leq 0.05$) (Table 1). The treatment effect was significant for both wheat varieties. Inoculation by *Azotobacter chroococcum* AZ6 in the presence or absence of P or GB reduced significantly the salt stress effect on these parameters (Figures 2 to 4). The differences between the two varieties were low but with a fresh and dry weight higher in Waha.

Chlorophyll

Salinity reduced contents of chlorophyll *a*, *b* and total ($p < 0.05$) in both varieties but with a lower effect in Waha (Table 2). Treatment by inoculation in the presence or absence of P or GB improved significantly the chlorophyll *a* and total at all levels of salinity. However, these were higher at 100 and 200 mM for plants inoculated in the presence of P or GB and the effect of these factors was not visible on chlorophyll *b* (Figure 5).

Amino acids

Amino acid content was significantly increased under the salt stress (Table 2, Figure 6). This increase was from 30 to 40% at 100 mM and up 73 to 85% at 200 mM in Waha and Bousselam, respectively. Inoculation by *A.*

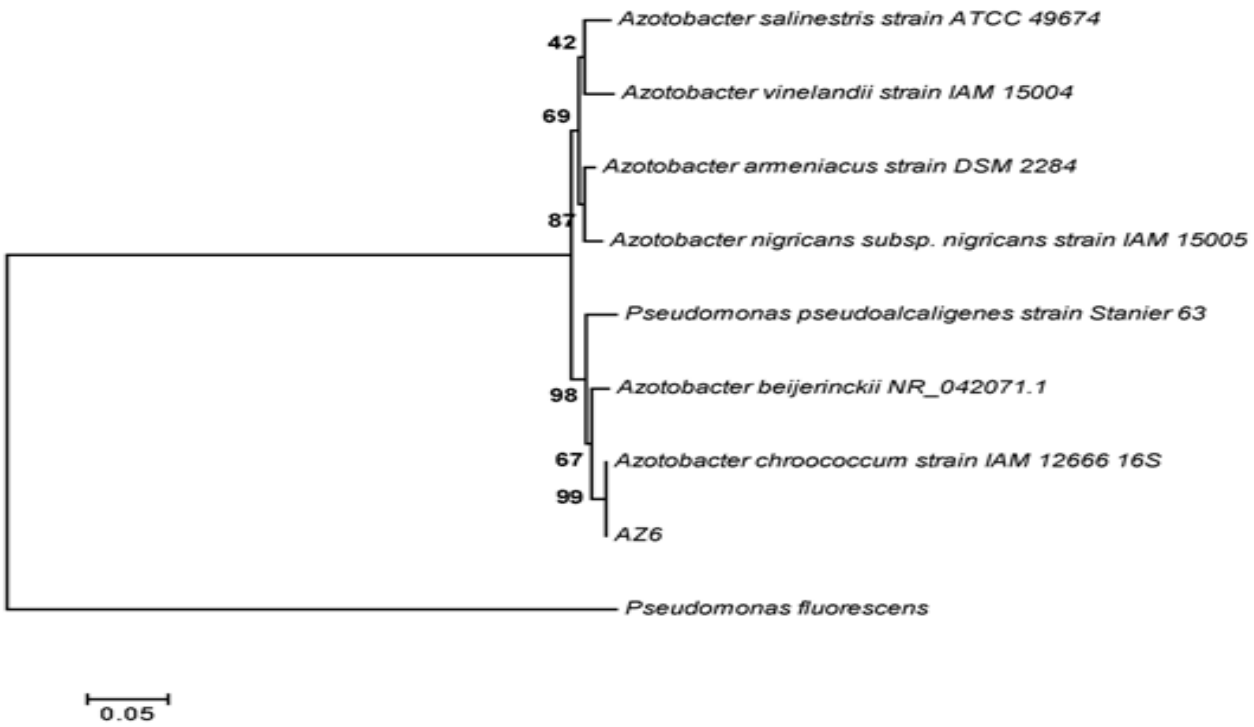

Figure 1. Unrooted phylogenetic tree based on a comparison of the 16S ribosomal DNA sequence of *Azotobacter* (KT339176) and some of their closest phylogenetic relatives (Validly published strains), The tree was created by the neighbor-joining method, The numbers on the tree indicates the percentages of bootstrap sampling derived from 1000 replications, Bar inferred nucleotide substitutions per nucleotides.

Table 1. Analysis of variance summaries (mean squares) of the data for fresh and dry weight, plant height and root length.

Source of variation	df	Shoots fresh Weight (g)	Shoots dry weight (mg)	Roots fresh weight (g)	Roots dry weight (mg)	plant height (cm)	root length (cm)
Treatment (T)	3	14.0200**	83.9662**	18.2932**	14.1300**	1.3319*	14.2141**
Salinity (S)	2	25.8154**	139.1682**	77.7720**	324.0321**	408.100**	329.3861**
S×T	6	2.8527*	4.0371**	3.8491**	31.6417**	8.8541**	1.4071^{ns}
Variety (V)	1	34.5141**	197.4619**	17.3274**	41.1894**	778.94**	26.2284**
S×V	3	2.7577^{ns}	21.7425**	21.2982**	18.2884**	5.5958**	3.5439*
V×T	2	0.0008^{ns}	59.6306**	9.6882**	1.1260^{ns}	15.0568**	3.2453*
S×V×T	6	2.138^{ns}	4.7669**	9.9206**	6.2064 **	1.7873^{ns}	1.8952^{ns}
Error	60	0.253122	21717361	0.02669	2.20650	0.033368	1.923021

*,**:Significative at a level of 5% (p≤0.05) and 1% of probability (p≤0.01), respectively, ns: Non-significative (p≥0.05), df: Degree of freedom.

chroococcum AZ6 reduced these contents. The content of shoot amino acids was lower in the presence of proline or GB at 134 or 167 µg in Waha and 153 or 176 µg in Bousselam.

Proline

Analysis of endogenous proline content in shoot showed that the two varieties of wheat (Waha and Bousselam) strongly accumulated proline under saline conditions. Inoculation by *A. chroococcum* AZ6 decreased the proline content in shoot of the two varieties under the salt stress (Table 2, Figure 7). The exogenous application of P or GB reduced further this content. This effect was visible especially in Waha. In non-saline conditions, the effects of the inoculation treatment and/or application of proline or glycine betaine were not significant.

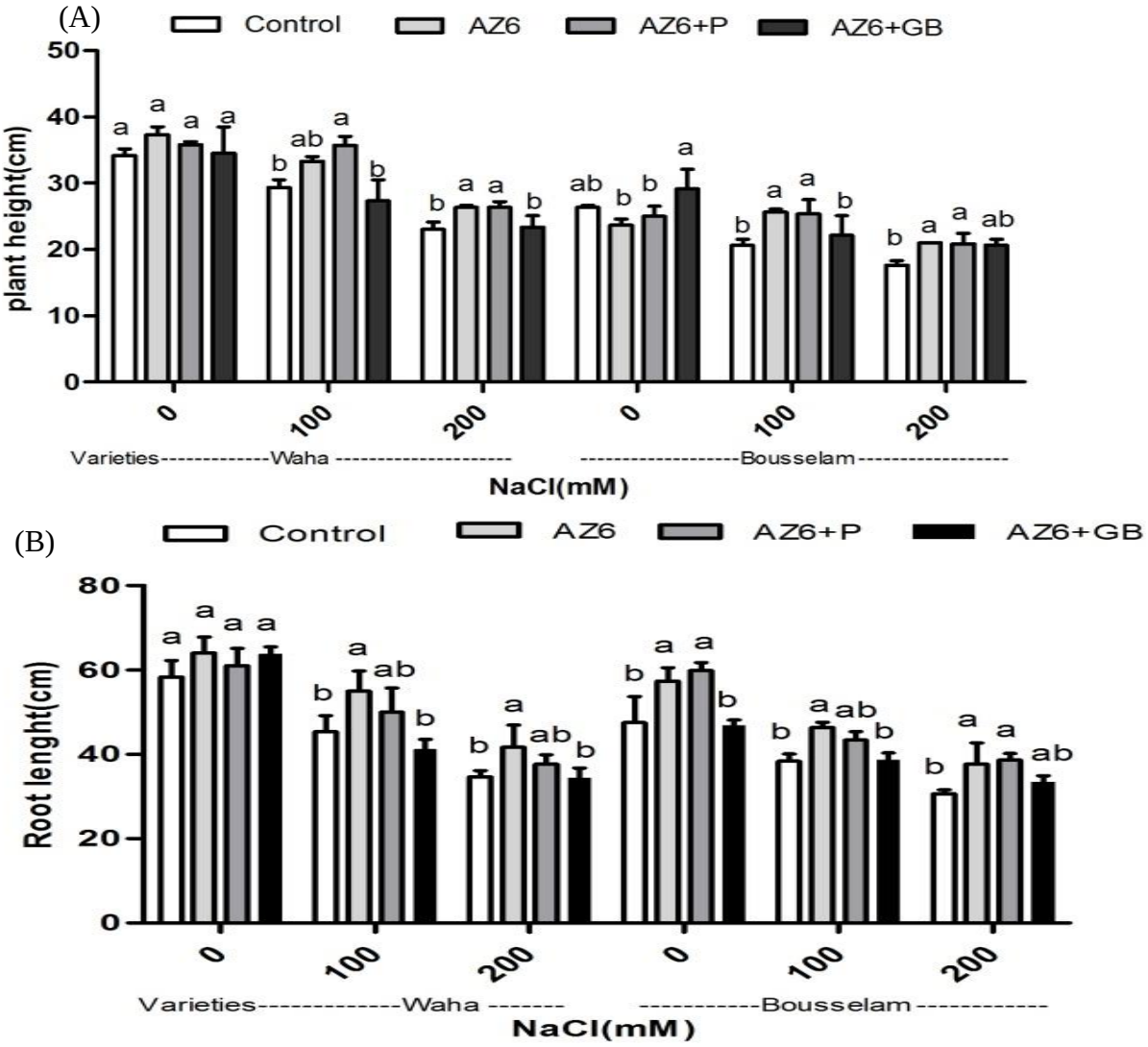

Figure 2. Plant height (A) and root length (B) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from six replicates

The Na^+ and K^+ content in shoots and roots

Salinity also had a significant effect on the Na^+ concentration in shoots and roots in both varieties of wheat ($P \leq 0.01$) (Table 3, Figure 8). This ion increased systematically with the level of salt. However, inoculation with *A. chroococcum* AZ6 in the absence or presence of P or GB reduced the accumulation of Na^+. The accumulation of the Na^+ was more observed in the shoots than in the roots in Bousselam variety. However, the K^+ ions in the roots and the shoots decreased significantly under salt stress in both varieties (Table 3, Figure 9). This decrease was less pronounced for the inoculated samples which were treated with exogenous P or GB. This decrease was also lower in the shoot in Waha for the same samples.

DISCUSSION

The results of experiments on hydroponic medium showed clearly that salt stress led to a reduction in the growth in both wheat varieties resulting in a significant decrease in root length, height of the plant and fresh and dry weight of shoots and roots. These results were similar to those recorded in many plants. In radish (*Raphanus sativus*), for example, the dry weight decreased at high salinity and about 80% of the growth reduction can be attributed to the decrease of the expansion of the leaf area (Marcelis and Hooijdonk, 1999) and therefore, a low light interception. Also, salinity reduced the glycophytes growth by modifying the balance of water and ion in the tissues (Munns, 2002). In leaves, this phenomenon was associated with a decrease in turgor as a result of a

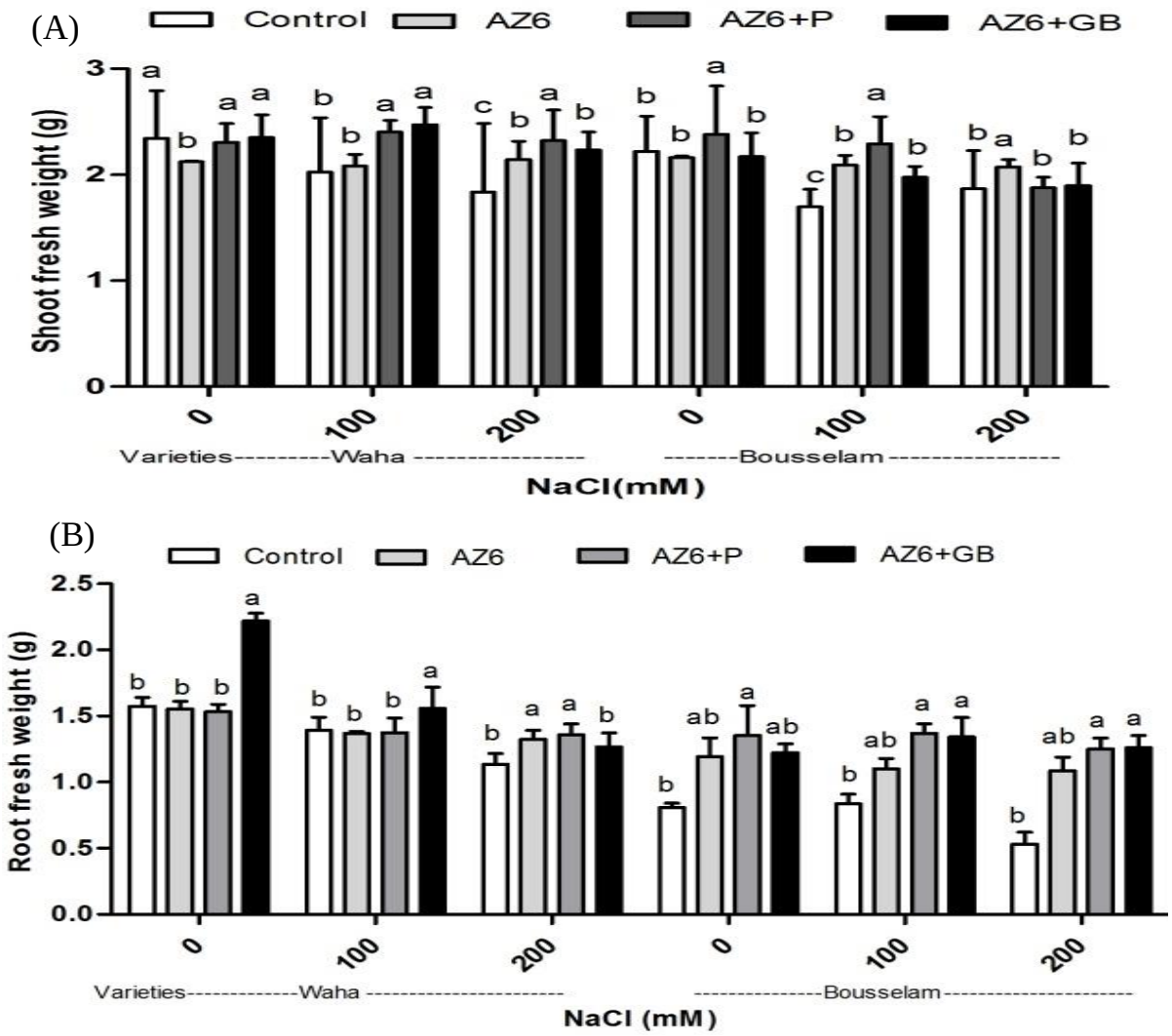

Figure 3. Shoot Fresh (A) and root fresh (B) weight in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from six replicates.

decrease in potential gradient of water between the plant and the environment (Levigneron et al., 1995). In addition, the effects of salinity were attributed to other factors including an increase in the rigidity of the cell wall, probably due to a change in its structure or a reduction in its extensibility and a reduction of new cells production rate (Kinraide and Parker, 1990) and/or a toxicity of ions Na^+ and Cl^-, a nutritional deficiency as well as mineral imbalances (Van Volkenburgh and Boyer, 1985). Salt stress also had an effect on the biochemical characteristics. The decrease in chlorophyll concentration under the influence of salinity has been reported by several authors (Del Zoppo et al., 1999). This decrease was attributed to the salt inducing the weakening protein-lipids complexes and an increased activity of chloro-phyllase. Moreover, salinity reduced the biosynthesis of photosynthetic pigments and caused changes in the integrity and composition of the chloroplasts membranes (Günes et al., 1996).

The compartmentation of ions between the organs (roots/aerial parts), tissue (epidermis/mesophyll) or between cellular compartments (vacuole/cytoplasm) was one of the mechanisms of adaptation to salt stress. Generally, in tolerant plants (as opposed to sensitive plants), the Na^+ ion was well distributed in the vacuole (Cheeseman, 1988). The effect of salt stress also increased the absorption of Na^+ through the roots and the leaves in two wheat varieties (Günes et al., 1996). The same observations have been reported in two barley genotypes in which the salt caused the migration of the Na^+ in the aerial parts with greater accumulation through the leaves compared to roots. The mechanisms of the Na^+ transport in the leaves and those of its root absorption appeared to be regulated separately. However, a gain of

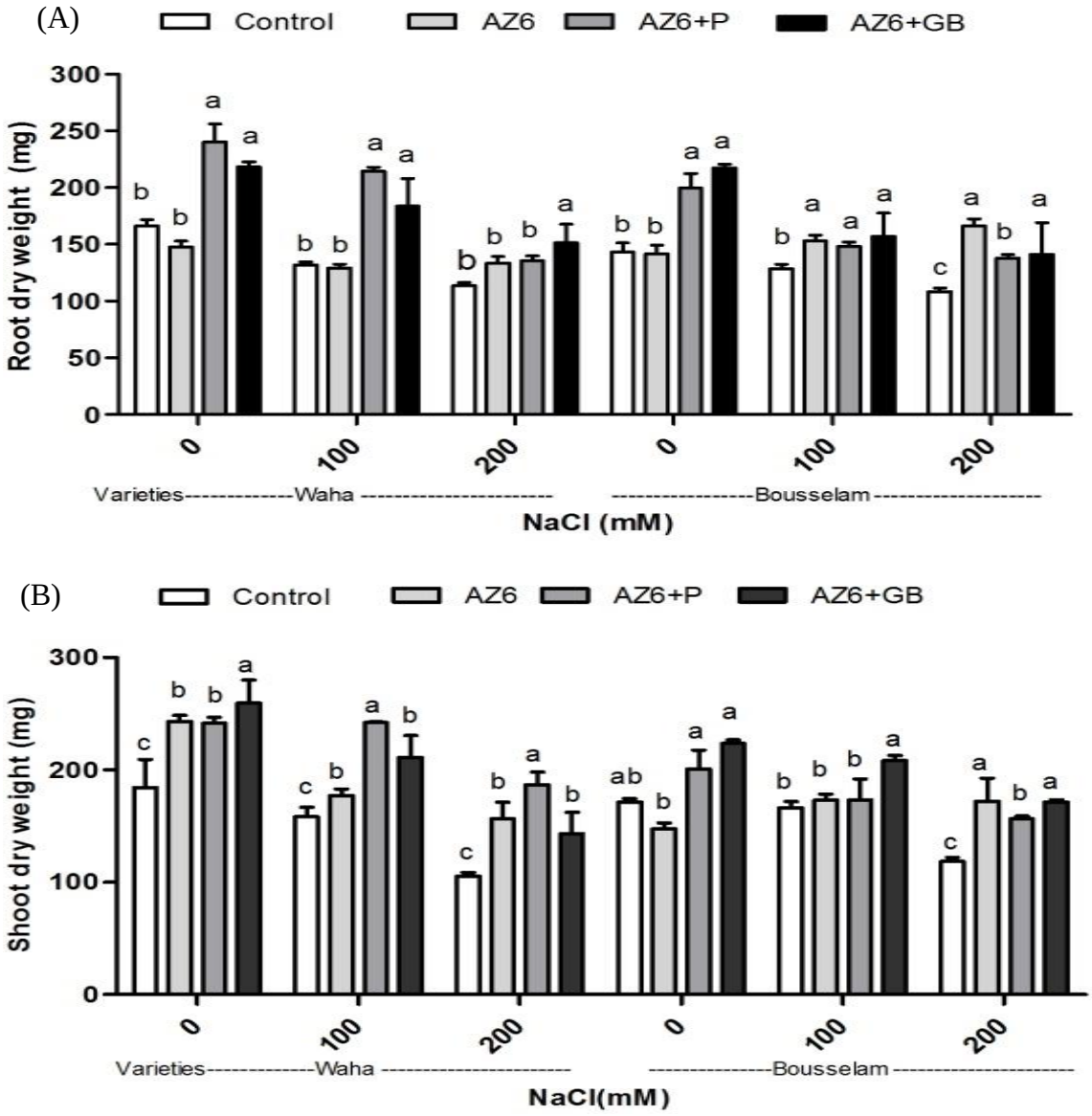

Figure 4. Shoot dry (A) and root dry weight (B) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from six replicates.

Table 2. Analysis of variance of summaries (mean squares) of the data of shoot content chlorophyll *a*, *b* and *a* + *b*, proline and amino acids.

Source of variation	Df	CH*a* (mg/g)	CH*b* (mg/g)	CH*a*+*b* (mg/g)	Proline (µg/g)	Amino acids (µg/g)
Treatment(T)	3	40.5822**	0.8217^{ns}	28.0090**	96.6844**	44.1325**
Salinity (S)	2	415.5958**	7.3617**	221.4564**	2076.5302**	1159.5963**
S×T	6	4.9636**	2.2059^{ns}	1.6871^{ns}	27.6181**	50.3157**
Variety (V)	1	21.6133**	0.4960^{ns}	24.0971**	95.4289**	43.9669**
S×V	3	11.3114**	2.4376^{ns}	5.7110**	11.0311**	0.7574^{ns}
V×T	2	10.0566**	0.6946^{ns}	8.4330**	21.0890**	0.8473^{ns}
S×V×T	6	0.6632^{ns}	0.1517*	0.9138^{ns}	7.5191**	2.2093^{ns}
Error	24	0.40582	0.06554	0.52567	2.31446	4.93056

*,**Significative at a level of 5% (p≤0.05) and 1% of probability (p≤0.01), respectively, ns: Non-significative (p≥0.05), df: Degree of freedom

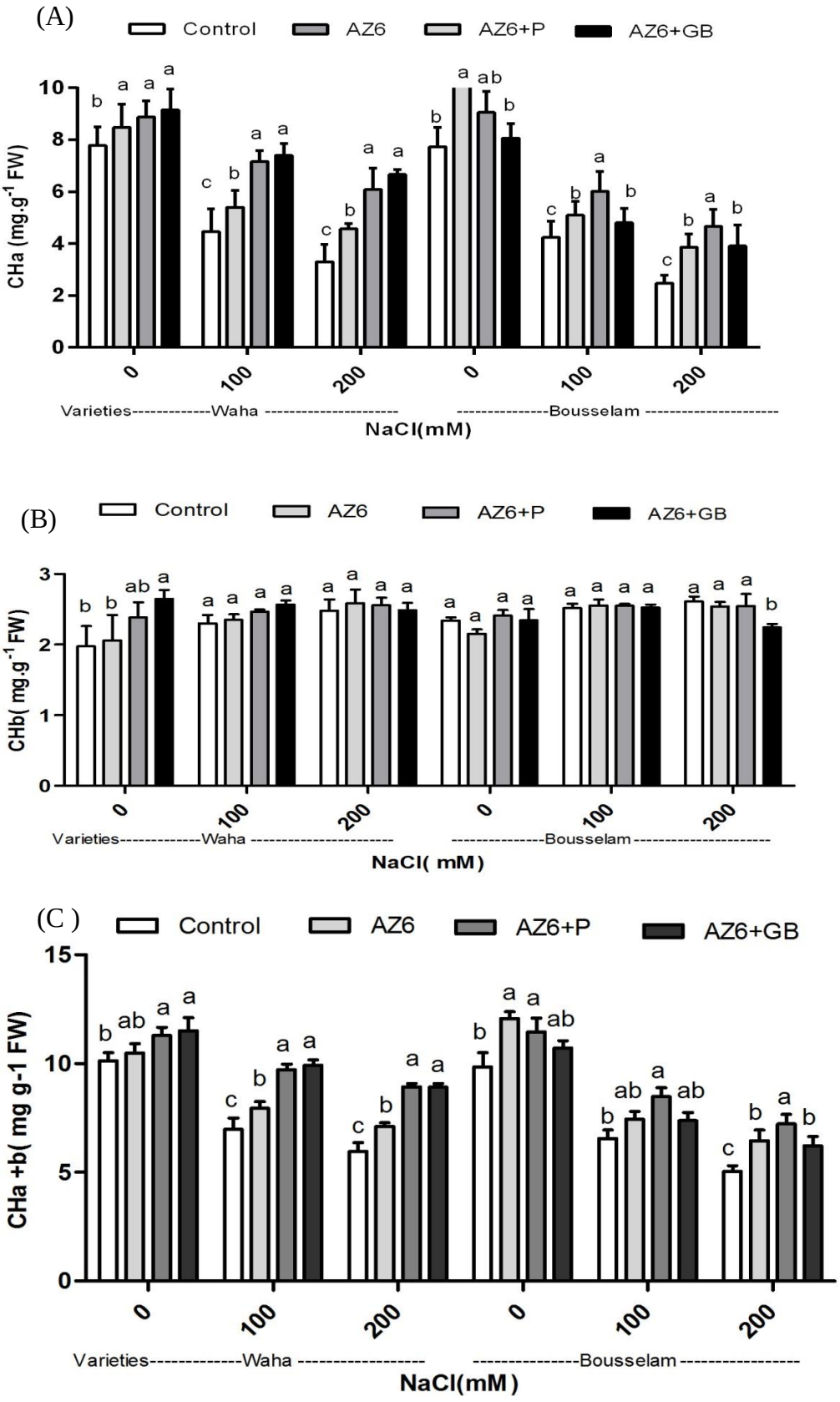

Figure 5. Chlorophyll *a* (A), *b* (B) and *a*+*b* (C) content(mg.g^{-1} FW) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from three replicates.

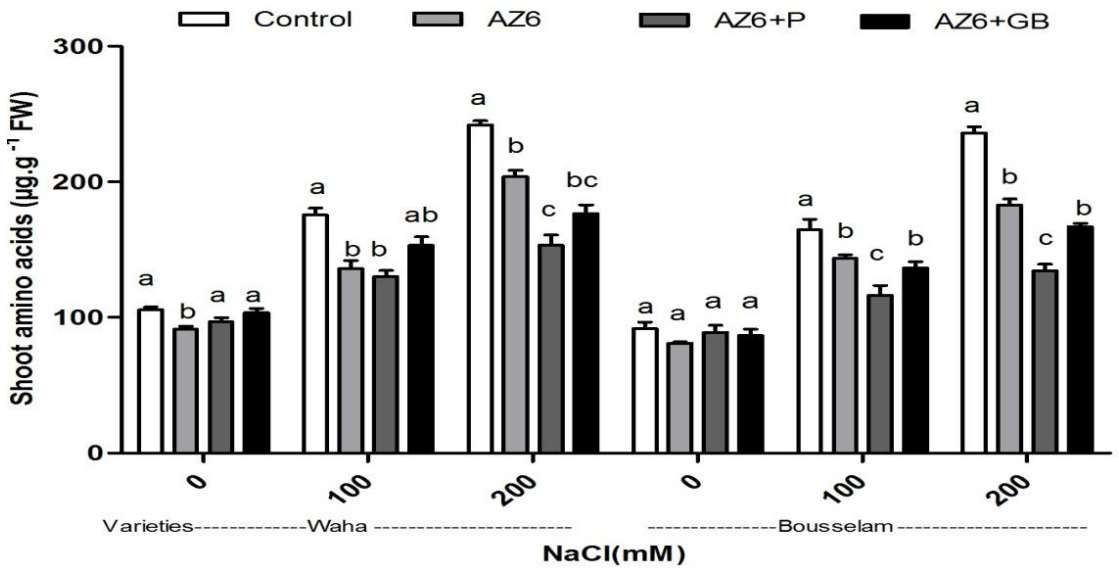

Figure 6. Shoot amino acids content (µg.g^{-1} FW) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from three replicates.

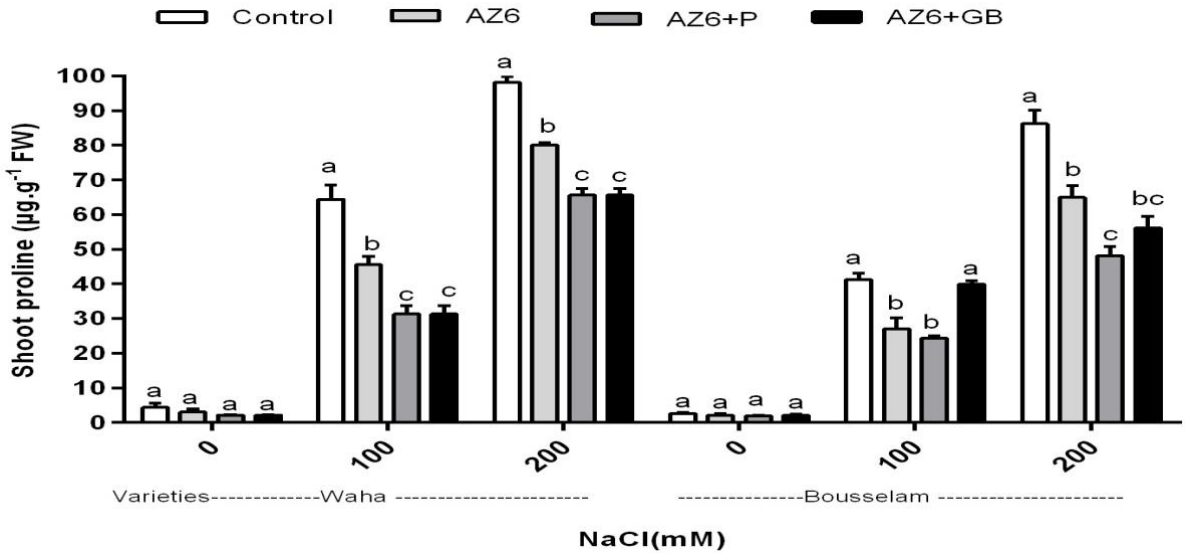

Figure 7. Shoot proline content(µg.g^{-1} FW) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from three replicates.

tolerance was observed in plants with improved ability to recirculate sodium, which protected the aerial parts from the saline invasion (Munns, 2002).

In the same context, the content of the K^+ decreased in the roots and leaves in both varieties of wheat under salinity effect. The same findings were observed in tomato plants grown in the presence of NaCl at 50 and 100 mM (Heuer, 2003). This salt inhibitory effect on the absorption of the K^+ existed also in wheat and in the olive (Ottow et al., 2005). These observations were attributed to a competitive interaction between the Na^+ and the K^+ at the absorption sites of the plant. Reducing the concentration of the K^+ resulted in a reduction of growth by decrease of the osmotic adjustment and the maintenance of the turgor (Bernstein et al., 1995).

The concentrations of amino acids and proline of the shoots increased substantially and reached a remarkable content when the two varieties of wheat were subjected

Table 3. Analysis of variance of summaries (mean squares) of the data for concentrations of Na^+ and K^+ in shoots and roots.

Source of Variation	df	Na^+ shoots	K^+ shoots	Na^+ roots	K^+ Roots
Treatement(T)	3	10.3670*	5.6212*	29.1332**	6.2192*
Salinity(S)	2	17.4079**	280.5441**	320.7020**	1084.6339**
S×T	6	184.9180**	1.8350^{ns}	8.8642**	11.6436**
Variety (V)	1	25.4238**	0.0965^{ns}	58.0627**	5.6434*
S×V	3	0.1888^{ns}	0.0839^{ns}	0.2287^{ns}	3.0335*
V×T	2	3.1246*	6.4001**	14.8168**	3.0335*
S×V×T	6	4.5620**	2.6609*	4.7274^{ns}	2.6055*
Error	24	1.38218	2.76145	0.78264	1.15570

*,**Significative at a level of 5% (p≤0.05) and 1% of probability (p≤0.01), respectively, ns: Non-significative (p≥0.05), df: Degree of freedom.

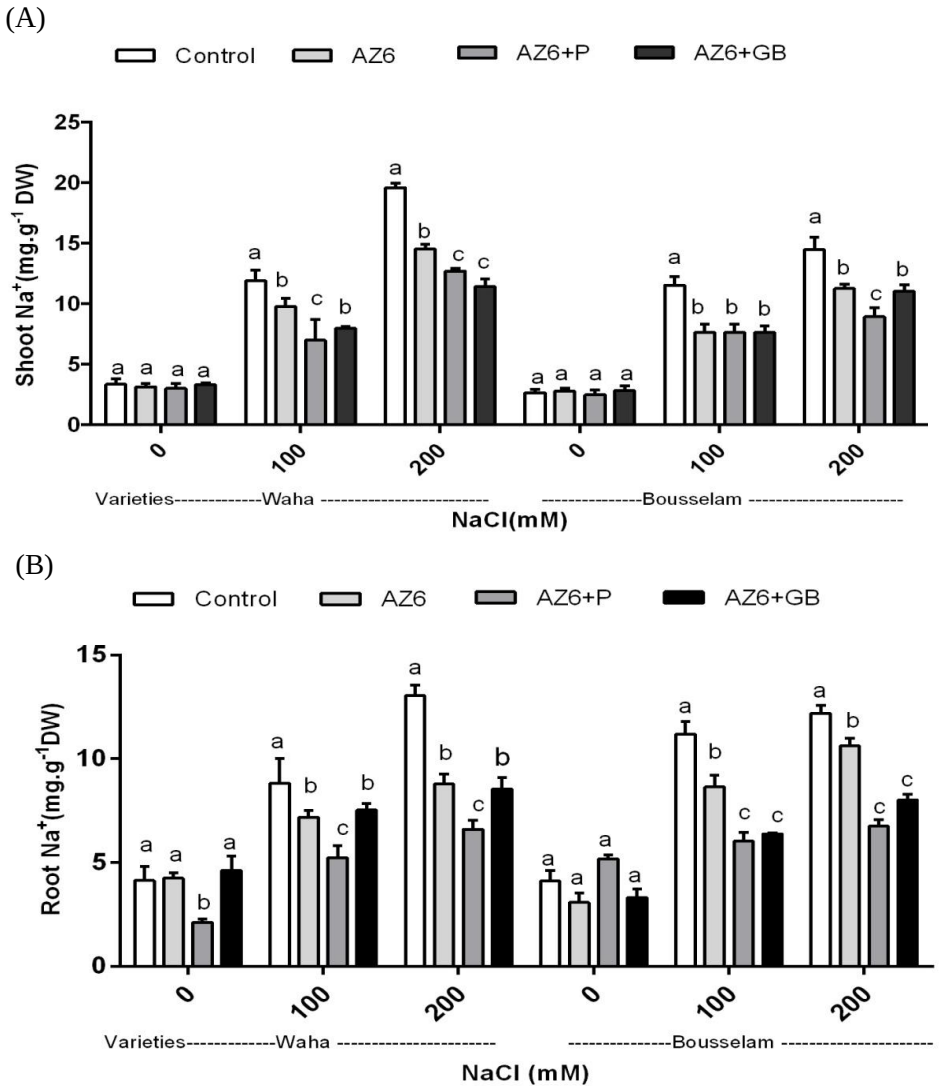

Figure 8. Shoot an root Na^+ content($mg.g^{-1}$ DW) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error (p≤0.05) from three replicates.

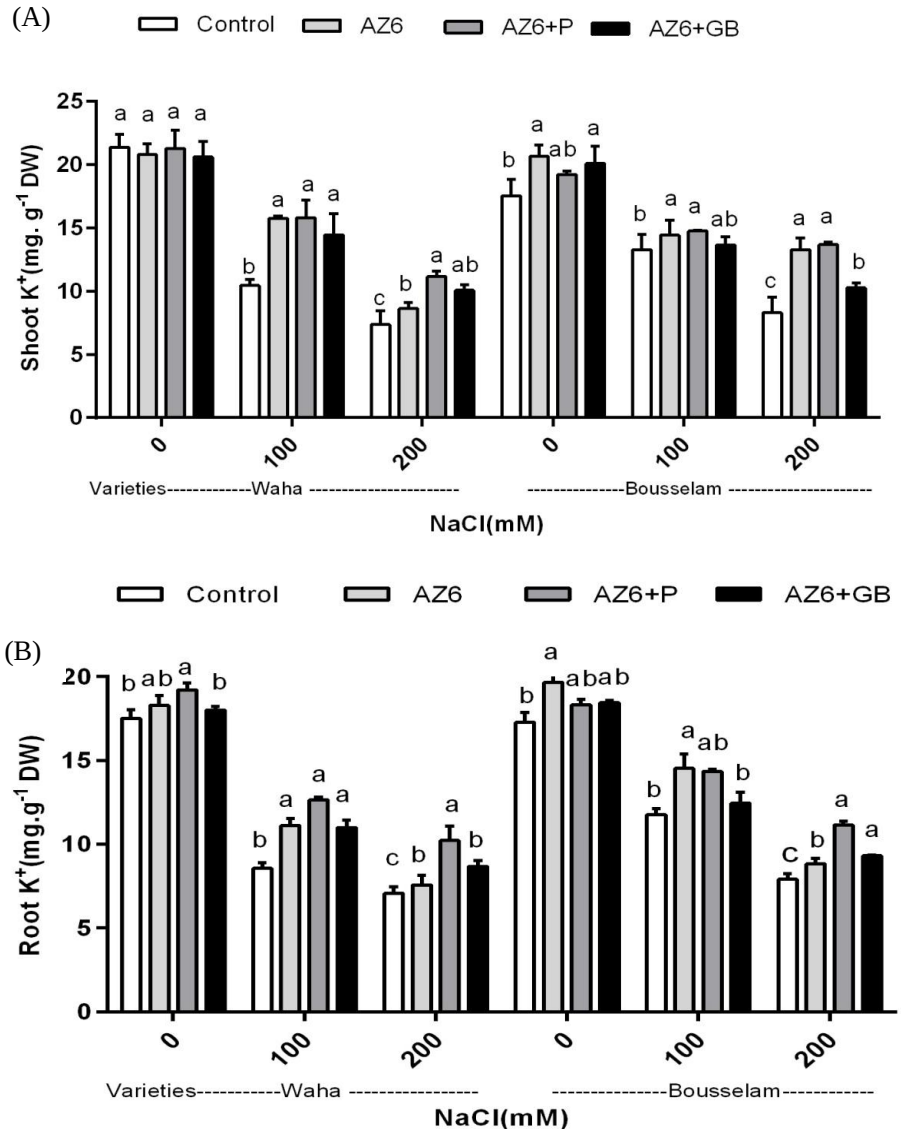

Figure 9. Shoot (A) and root (B) K^+ content($mg.g^{-1}$ DW) in two wheat durum varieties at different treatments before and after exposure to 100 and 200 mM NaCl stress, results are shown as Mean±standard error ($p \leq 0.05$) from three replicates

to salt stress. According to Abd-El-Baki et al. (2000) increased salinity caused an accumulation of amino acids in foliar and root maize. The shoots were more affected by this accumulation. The most important amino acids involved were alanine, arginine, glycine, serine, leucine, valine, proline and non-protein amino acids such as citrulline and ornithine (Mansour, 2000). Proline was probably the most widely accumulated amino acid by plants but also by other organisms (McCue and Hanson, 1990).

In many plant species (including cereals), the presence of proline in high concentrations was one of the manifestations of water and salt stress (Ali Dib et al., 1992). On one hand, the correlation between proline content and concentration of Na^+ ion confirmed the remarkable osmoregulatory role of proline (Chowdhury et al., 1993).

On the other hand, it was also reported that sensitive wheat varieties significantly accumulated proline concentrations higher than tolerant varieties (Lutts et al., 1999). Proline played several physiological roles in plants subjected to salt stress (osmoregulation, a source of energy, of carbon and nitrogen and a senescence signal) (Aspinall and Palleg, 1981). Its accumulation in the plant tissues under stress conditions was reported as the result of a decrease in its degradation due to an inhibition of proline dehydrogenase, an increase of its biosynthesis by expression of a gene encoding the pyrolline-5-carboxylate

synthetase (Lutts et al., 1999), and a decrease in the synthesis or protein hydrolysis (Viégas and Silveira, 1999). However, the significance of the accumulation of proline in osmotic adjustment is still unclear and varies by species (Rhodes and Hanson, 1993).

The salt inhibitory effect was due to differential genetic potential. In this study, the salinity effect on the morpho-biochemical parameters of both wheat varieties revealed a tolerance of Waha more important than Bousselam under different treatments. This tolerance was explained by the acquisition of resistance genes or a natural adaptation to salt stress which is related to the ability of the plant to restrict the accumulation of the Na^+ ion while promoting that of the K^+ ion and those of osmolytes, such as proline (Botella et al., 2005).

Under salt stress, the addition of glycine betaine (5 mM) or proline (5 mM) stimulated root length, the morphological parameters of the growth, the chlorophyll *a* and total and the K^+ content of roots and shoots. However, the amino acids, the endogenous proline and the Na^+ ions concentrations were reduced. The salt stress induced a higher level of proline content, whereas GB and proline application reduced the proline accumulation when compared with salt treatment without GB and proline, suggesting that proline accumulation is just a symptom of salt stress rather than a cause of tolerance (Ashraf, 1989). These results are in agreement with those of Shaddad (1990) and Gagnon and Dansereau (1990) on *Raphanus sativus* (turnip), *Gossypium hirsutum* and *Vicia faba* (broad beans), respectively. These observations were explained by the fact that glycine betaine, zwitterionic ampholyte can interact directly with the ions accumulated to protect membranes from the deleterious effects of toxic ions (Papageorgiou and Murata, 1995). The addition of proline or glycine betaine promoted a smaller ion transport due to a decreased respiration. These osmolytes also increased cell turgor through osmotic adjustment and contributed to the increase in stomatal conductance in leaves (Heuer, 2003).

Inoculation of durum wheat seeds by *A. chroococcum* AZ6 significantly increased the growth parameters plant height, root length and fresh and dry weight of root and shoot under salt stress. The chlorophyll and the potassium contents were improved while the concentrations of amino acids, proline and sodium were reduced. These results were reported by many authors (Nadeem et al., 2006; Chaudhary et al., 2013). According to Glick et al. (1998), the use of PGPR (for example, *Pseudomonas*) as inoculant of wheat seeds in saline soils improved the plant height, root length, the grain yield, chlorophyll content and the ratio K^+/Na^+. The role of *Azotobacter* in the production of growth-promoting substances and plant improving resistance to abiotic and biotic stresses must also be considered (Chaudhary et al., 2013; Sahoo et al., 2014).

Moreover, it was established that the PGPR strains producing exopolysaccharides (EPS) that bind with the Na^+ ions were capable of reducing the concentration and absorption of this ion in root and thus attenuated the salt stress in plants (Ashraf et al., 2004).

According to many authors, the addition of osmolytes and essentially proline can have a direct and beneficial effect on both the survival and growth of *A. chroococcum* AZ6 and tolerance of the plant to salt stress. Indeed, the middle salinity was a stressful environment for the rhizobacteria. The exposure of the bacteria to conditions of high osmolarity decreased the activity of water in their cytoplasm (Epstein, 1986) and led to harmful effects on cell proteins and other macromolecules. In addition, the salinity reduced the number of root colonizing bacteria. The contribution of osmolytes (proline or glycine betaine) in the environment played an important role in the adaptation of these rhizobacteria to salt stress and as osmoprotectant or as carbon and nitrogen sources (Alloing et al., 2006).

The addition of osmolytes, glycine betaine and essentially proline, attenuated the salinity effects by improving the morpho-biochemical parameters. The use of *A. chroococcum* AZ6 as an inoculant and providing osmoprotective molecules will be, therefore, promising biofertizant in arid and saline soils.

Conflict of interests

The authors have not declared any conflict of interest.

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