doi:10.14720/aas.2018.111.2.17 Original research article / izvirni znanstveni članek

Salt overly sensitive 1 (*SOS1*) gene expression can be regulated via *Azospirillum brasilense* Sp7 in wheat seedlings under saline condition

Hamid Reza GHASSEMI[1], Akbar MOSTAJERAN[2]*, Abolghasem ESMAEILI[3]

Received May 05, 2018; accepted September 23, 2018.
Delo je prispelo 05. maja 2018, sprejeto 23. septembra 2018.

ABSTRACT

Salinity stress reduces plant growth via failure of physiological processes mainly due to the abundance of Na^+ ion. Salt overly sensitive (*SOS*) signaling pathway is considered as an important component of Na^+/K^+ homeostasis system in plants, especially under saline condition. Moreover, it is reported that wheat-*Azospirillum* associated has resulted in an enhanced salinity tolerance. To evaluate involvement of *Azospirillum* species in regulation of *SOS* signaling pathway, inoculated and none-inoculated wheat seedlings with *Azospirillum brasilense* Sp7 were grown for five days. Then uniform seedlings were transferred into saline hydroponic media with and without 200 mM NaCl. The relative expression of *TaSOS1* of root, sheath, and blade as well as Na^+/K^+ ratio was measured after 6, 24 and 48 hours since inoculated and non-inoculated seedling were transferred to NaCl media. Simultaneously Ca, Fe, proline content, root and shoot dry mass and soluble sugars were measured at 72 hour after application of NaCl. Result showed that salinity increased *TaSOS1* gene expression, Na^+, prolin and Na^+/K^+ ratio but Ca and Fe were decreased in root and shoot of wheat seedlings. Although *A. brasilense* Sp7 could improve salinity tolerance in wheat via reduction of Na uptake and upregulation of *TaSOS1* expression, but do not have any effect in sodium distribution within plant parts. Therefore, salinity could increase *TaSOS1* expression in the root, sheath and blade and *A. brasilense* Sp7 also could reduce the adverse effect of salinity via addition of over expression of *TaSOS1*.

Key words: *Azospirillum*; wheat; salinity; *TaSOS1*; Na^+/K^+ ratio

IZVLEČEK

IZRAŽANJE NA SOL PREOBČUTLJIVEGA GENA (*SOS1*) BI PRI PŠENICI V RAZMERAH SLANOSTI LAHKO URAVNAVALI S SEVOM BAKTERIJE *Azospirillum brasilense* Sp7

Slanostni stres zmanjšuje rast rastlin preko odpovedi fizioloških procesov v glavnem zaradi Na^+ iona. Preobčutljiva (*SOS*) solna signalna pot predstavlja najvažnejši del Na^+/K^+ homeostaznega sistema v rastlinah v razmerah slanosti. Še več, poroča se, da je povezava pšenice z bakterijo iz rodu *Azospirillum* rezultirala v povečani odpornosti na slanost. Za ovrednotenje vloge bakterije iz rodu *Azospirillum* pri uravnavanju *SOS* signalne poti so bile gojene z bakterijo *Azospirillum brasilense* Sp7 inokulirane in neinokulirane sejanke pšenice za pet dni. Izenačene sejanke so bile prenešene v hidroponski medij z ali brez 200 mM NaCl. V presledkih 6, 24 in 48 ur po prenosu sejank v slan medij je bila merjena relativna ekspresija gena *TaSOS1* v koreninah, listni nožnici in listni ploskvi in Na^+/K^+ razmerje. Hkrati je bila 72 ur po prenosu v slan medij izmerjena vsebnost Ca, Fe in prolina, suha masa pogankov in vsebnost topnih sladkorjev v istih delih sejank. Rezultati so pokazali, da je slanost povečala ekspresijo gena *TaSOS1*, vsebnost Na^+, prolina in povečala količnik Na^+/K^+, a zmanjšala vsebnost Ca in Fe v koreninah in poganjkih sejank. Čeprav lahko bakterija *A. brasilense* Sp7 izboljša toleranco pšenice na slanost z zmanjšanjem privzema Na in povečano ekspresijo gena *TaSOS1* pa nima nobenega učinka na razporeditev natrija v rastlini. Slanost torej lahko poveča ekspresijo gena *TaSOS1* v koreninah in listih in inokulacija z bakterijo *A. brasilense* Sp7 lahko zmanjša škodljive učinke slanosti preko dodatnega vpliva na povečano ekspresijo *TaSOS1*.

Ključne besede: *Azospirillum*; pšenica; slanost; *TaSOS1*; Na^+/K^+ razmerje

[1] PhD student, Department of Biology, University of Isfahan, Iran.

[2] Plant Science Division, Biology Department, University of Isfahan, Isfahan, Iran. *Corresponding author: mostajeran@sci.ui.ac.ir

[3] Cell and Molecular Biology, Biology Department, University of Isfahan, Isfahan, Iran.

1 INTRODUCTION

Wheat like some other crops cannot tolerate salinity (Tiwari et al., 2010). Salinity has inhibitory effects on wheat growth indexes such as root growth (Neumann, 1995), root/shoot ratio (El-Hendawy et al., 2005), and total dry matter (Pessarakli & Huber, 1991). The saline condition caused an increase in sodium in the root and shoot of plants (Xue et al., 2004), some osmoticum components such as proline, soluble sugars (Hamdia et al., 2004), as well as none-enzymatic (Norastehnia et al., 2014; Amini et. Al., 2017) and enzymatic antioxidant (Baniaghil et al., 2013). In contrast, seedling's dry mass and K^+ content in the roots and shoots of wheat cultivars (Akbarimoghaddam et al., 2011; Lekshmy et al., 2013) and maize (Turan, 2008) decreased by increasing salinity.

It has been reported that plant growth promotion rhizobacteria (PGPRs) could induce more mineral uptake (Askary et al., 2009), phytohormones production (Kang et al. 2014) and consequently better tolerance to abiotic stresses (Vargas et al., 2012). El-Dengawy et al. (2011) reported that inoculation of Carob seedlings (*Ceratonia siliqua* L.) with *Azospirillium lipoferum* (Beijerinck) Tarrand et al. (ATCC® 29707™) under saline condition could improve seedling growth rate, K^+/Na^+ ratio and root characters. In addition, Upadhyay et al. (2012) reported that when wheat co-inoculated with two PGPR strains (*Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 SU47 and *Arthrobacter* sp. SU18) were grown under different salinity conditions (2–6 dS m^{-1}), an increase in dry biomass, total soluble sugars and proline were observed, meanwhile sodium of wheat leaves was reduced in co-inoculated plants under saline conditions.

Creus et al. (1997) also confirmed the reduction of adverse effects of salinity and osmotic stresses on height and dry mass of wheat plants when inoculation with *Azospirillium brasilense* Tarrand, Krieg & Döbereiner, 1978. Vargas et al. (2012) also showed the upregulation of ethylene receptors genes expression in rice plants upregulated due to the inoculation with *A. brasilense* Sp245 as well as an increase in transcripts of some genes involved in nutrition uptake in response to *A. brasilense*. *A. brasilense* could change the pH of soil via proton efflux and increase availably of plant nutrients in wheat cultivars (Amooaghaie et al., 2002). Meanwhile, change in character of rhizosphere due to PGPRs activities and vis versa change in qurom sensing of bacteria due to the change of rhizosphere is a coincide fenomena which affect plant growth and development in stress condition (Pakdaman et al., 2014).

Among the salt overly sensitive (*SOS*) genes, SOS1 is a Na^+/H^+ antiporter located in the cell membrane. Its role has already been demonstrated in cytosolic Na^+ homeostasis by different researchers (Ramezani et al., 2013; Sathee et al., 2015; Shi et al., 2002; Shi & Zhu, 2002; Yadav et al., 2012). It has been reported that *TaSOS1* experssion was up-regulated in the root and the shoot of wheat seedlings under saline condition. For example, Yadav et al. (2012) showed that over expression of *SOS1* gene in transgenic tobacco caused a higher salt tolerance, elevated seed germination, addition of root and shoot length, less dry mass reduction, higher K^+/Na^+ ratio and more sugars relative to wild-type plants. It has been suggested that the SOS signaling pathway has an important role in ion (Na^+ and K^+) homeostasis and salt tolerance under saline condition.

For instance, *TaSOS1* expression in transgenic *Saccharomyces cerevisiae* Meyen *ex* E.C. Hansen (which was already salt sensitive and had high cellular Na^+ content) caused reduction of Na^+ and addition of K^+ of cells (Xu et al., 2008). In addition, Feki et al. (2014) indicated that the *Arabidopsis sos1-1* mutant is hypersensitive to both Na^+ and Li^+ ions, but its hypersensitivity would have disappeared using *TaSOS1* gene. At this condition, better germination and more robust seedling growth, greater water retention capacity, retained low Na^+ and high K^+ in their shoots and roots were observed in nutrient solution containing Na^+ and Li^+ salts. Their work and complementary studies revealed that *TaSOS1* upregulated the ion homeostasis and helped in salinity tolerance (Ramezani et al., 2013).

It has been reported that PGPRs such as *Azospirillum* species can improve wheat growth and productivity under salinity condition (Upadhyay et al., 2011) and co-inoculation of *A. brasilense* and *Rhizobium meliloti* (Dangeard 1926) De Lajudie *et al*. 1994, comb. nov. in different wheat cultivar also showed an enhancement of root colonization and nitrogenize activity (Askary et al., 2008). Help of PGPRs in saline condition in one hand, and increasing 10 % annually saline areas due to the low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultivation in the other hand, create needs for more research in dual effects of salinity and PGPRs.

It is known that *TaSOS1* expression is up-regulated in salt tolerant wheat cultivars (Ramezani et al. 2013) and *A. brasilense* also increased tolerance to salinity. However, it is not known that *Azospirillum* species increase salinity tolerance via up or down regulation of *TaSOS1* gene in wheat seedlings under salinity condition or this phenomena has been done by different process. Therefore, the aim of this study was to evaluate *SOS1* gene expression, Na, K, Ca and Fe uptake, sugar

and proline content and biomass production of root, sheath and blade of wheat cultivars inoculated with *A. brasilense* Sp7 under salinity condition to know more about the effect of *A. brasilense* Sp7 and *TaSOS1* involvment.

2 MATERIALS AND METHODS

2.1 Preparation of inoculant and seeding

Standard strain of *A. brasilense* Sp7 was obtained from NCIMB Company, in Germany and then cultured in an NFb liquid medium supplemented with NH_4Cl (0.25 g l^{-1}) at 30 °C (Baldani & Döbereiner, 1980) in Erlenmeyer flasks for 48 h using a rotary shaker at 200 rpm (logarithmic phase). A high density bacterial culture was obtained by centrifuging at 1000 g for 10 min and was then washed with sterile saline phosphate buffer. Finally, desired concentration (10^7 CFU ml^{-1} of *A. brasilense* Sp7) was prepared from the media.

Seeds of a winter semitolerant wheat cultivar named Sardary (*Triticum aestivum*'Sardari') were obtained from Institute of Agricultural and Research of Isfahan in Iran. Seeds were surface sterilized by dipping in 95 % ethanol for 2 min and then in 1 % sodium hypochlorite (NaOCl) for 1 min followed by six washes in sterile distilled water (Öğüt et al., 2005). Then, sterilized seeds were transferred to autoclaved water agar medium and were kept at 25 ℃ for germination. After 24 hour, uniform germinated seeds were divided into two groups. The first group was inoculated by submerging the germinated seeds in the solution containing 10^7 CFU ml^{-1} of *A. brasilense* Sp7 and the second group treated without bacteria as control. To verify the inoculation success, the root segments stained with tetrazolium chloride dye and also cross section of inoculated root were prepared. After 3 hour, all seedlings (inoculated and none inoculated) were transferred into pots containing sterile perlite and then irrigated with 1/4 strength of Hoagland's nutrient solution (Hoagland & Arnon, 1950). The pots were kept for 5 days in a glasshouse under photoperiod 16/8 h (light/dark) at 25±2 °C. Then, 200 mM of NaCl (as one dose) was added as salinity treatment into only half of the plants in each group via irrigation water to have inoculated and non-inoculated plants under salinity and optimum saline condition. This experiment was conducted in randomized block design with three replicates.

Samples of roots, sheaths and leaves were collected at 6, 24 and 48 h after salinity applied. Some of the collected plant samples were used for Na and K analysis and some other immediately frozen in liquid nitrogen for Real-time quantitative PCR. Simultaneously, some of the plants was allowed to growth up to 72 hours and then their plant parts were collected for Ca, Fe, dry mass, soluble sugar, proline and root and shoot length analysis.

2.2 Real-time quantitative PCR

The total RNA was isolated from frozen roots, sheaths and leaves using Iraizol reagent (RNA biotech, Iran). After RNA extraction, samples were treated with DNase. Then, the first stranded cDNA was synthesized using the M-MLV reverse transcriptase (Fermatas). Real-time PCR was performed in triplicate using SYBER Green Master Mix (RNA Biotech, Iran). Gene-specific primers were designed for an 110 bp fragment of *TaSOS1* (Gen Bank Accession No**.** AY326952). The primer pair was 5'-GGGATGATGAGGAACTTGGG-3' in sense direction and 5'-CTTGTCAGGAACATCGTGGG-3' in anti-sense direction. The primer pair for the housekeeping gene, actin, (Gen Bank Accession No. GI:48927617) was 5'-GTTCCAATCTATGAGGGATACACGC-3' in sense direction and 5'-GAACCTCCACTGAGAACAACATTACC-3' in anti-sense direction with an amplification length of 422 bp (Xu et al., 2008). The PCR conditions were 94 °C for 4 min followed by 40 cycles of 94 °C for 10 s, 62 °C for 40 s, 72 °C for 60 s, followed by 7 min at 72 °C. Serial dilutions of cDNA were used to obtain optimized standard curve amplification efficiency and the best cDNA concentration for real-time PCR was obtained. The relative expression ratio of target and reference genes were calculated based on its real time efficiencies (E) and crossing point difference (ΔCp) of sample versus control as well as reference versus control, respectively (Pfaffl, 2001).

2.3 Dry mass, Na^+ and K^+ determination

Each plant part (root, sheath and blade) was weighted separately and then 100 mg of dried mass of each sample was digested with 10 ml 3 % (w/v) aqueous sulfosalicylic acid for 24 hours. Extracted samples were filtered with Whatman No. 1 filter paper. Then, Na^+ and K^+ concentrations were measured using flame photometric (Perkin-Elmer Coleman 51-ca), using related standard curves for sodium and potassium.

2.4 Determination of soluble sugar content

Soluble sugar content was determined using phenol-sulphuric method (Dubois et al., 1956). To do so, 0.01 g of dried plant sample was extracted in distilled water

and centrifuged at 3000 rpm for 10 min. The extract (0.5 ml) was treated with 0.5 ml phenol (5 %) and 2.5 ml pure sulphuric acid and then after a mild vortex, their absorbance was measured at 490 nm using Shimadzu double beam UV-visible Spectrophotometer.

2.5 Determination of Ca and iron content

Dry mass (100 mg) of each plant sample (roots and shoots separately) was digested in 3 ml of a 1- 4 (v/v) mixture of 37 % (v/v) HCl and 65 % (v/v) HNO3 in Teflon cylinders for 7 h at 140 °C. After adjustment of volume to 10 ml with deionized water, Ca and Fe was determined using an atomic absorption spectrophotometer (AAS, Shimadzu model 6200).

2.6 Determination of proline content

Roots and shoots proline were determined using Bates et al. (1973) method. 100 mg of fresh plant samples was homogenized with 4 ml sulfosalicylic acid (3.0 %) in a mortar. The suspension was centrifuged at room temperature at 3000 rpm for 5 min. The supernatant was mixed well with 4 ml acidic ninhydrin reagent and the reaction mixture was vortexed and the content was placed in a boiling water bath for 60 min. Then, the content was cooled in the ice bath and the mixture was extracted with 4 ml of toluene. The light absorbance of toluene layer was recorded at 520 nm using Shimadzu spectrophotometer (Shimadzu UV-160, Japan) and the concentration of unknown samples was calculated using respected standard curve.

2.7 Statistical analysis

The experimental design was completely randomized design with 3 replicates and MSTAT-C software was used for ANOVA. Duncan multiple range test was used (at 5 % level of significance) to compare the mean values of measured indexes. Excel was used to draw the necessary graphs.

3 RESULTS AND DISCUSSION

3.1 Growth, Ca, Fe, and soluble sugars

The results of length, dry mass and soluble sugars of roots and shoots of inoculated and non-inoculated wheat plants under saline and non-saline conditions are presented in Fig. 1. Root length of 8 days old plants was increased significantly in response to inoculation under saline and non-saline conditions. Simultaneously, shoot length was increased significantly under non-saline condition, while no significant difference was seen between inoculated and non-inoculated plant's shoot length under saline condition.

Root dry mass of inoculated plants was increased significantly under saline (25.8 %) and non-saline conditions (30.1 %) in comparison to control plants (non-inoculated and non-saline condition). However, shoot dry mass of inoculated plants did not improve either under saline or non-saline conditions in a short period of time. In contrast, root and shoot soluble sugars were affected by saline condition and shoot soluble sugars in inoculated (16.7 %) and non-inoculated (13.2 %) plants were increased significantly under saline condition.

Calcium concentration in the roots and shoots of inoculated plants not exposed to salinity was increased by 14.1 and 10.63 %, respectively, when compared to control plants (Fig 2). Salinity caused a significant reduction in Ca content of both roots (38.7 %) and shoots (31.26 %) of seedlings. However, inoculation improved Ca concentration in the root and shoot but its amount was still less than the control plants.

In inoculated seedlings, the amount of Fe in the roots and shoots (Fig 2) was the highest (0.51 and 0.21 mg g^{-1} DM, respectively) meanwhile, a significant reduction was observed in Fe content of the roots (23.68 %) and the shoots (23.68 %) of plants treated NaCl. Inoculation couldn't help the roots to uptake more Fe under salinity condition.

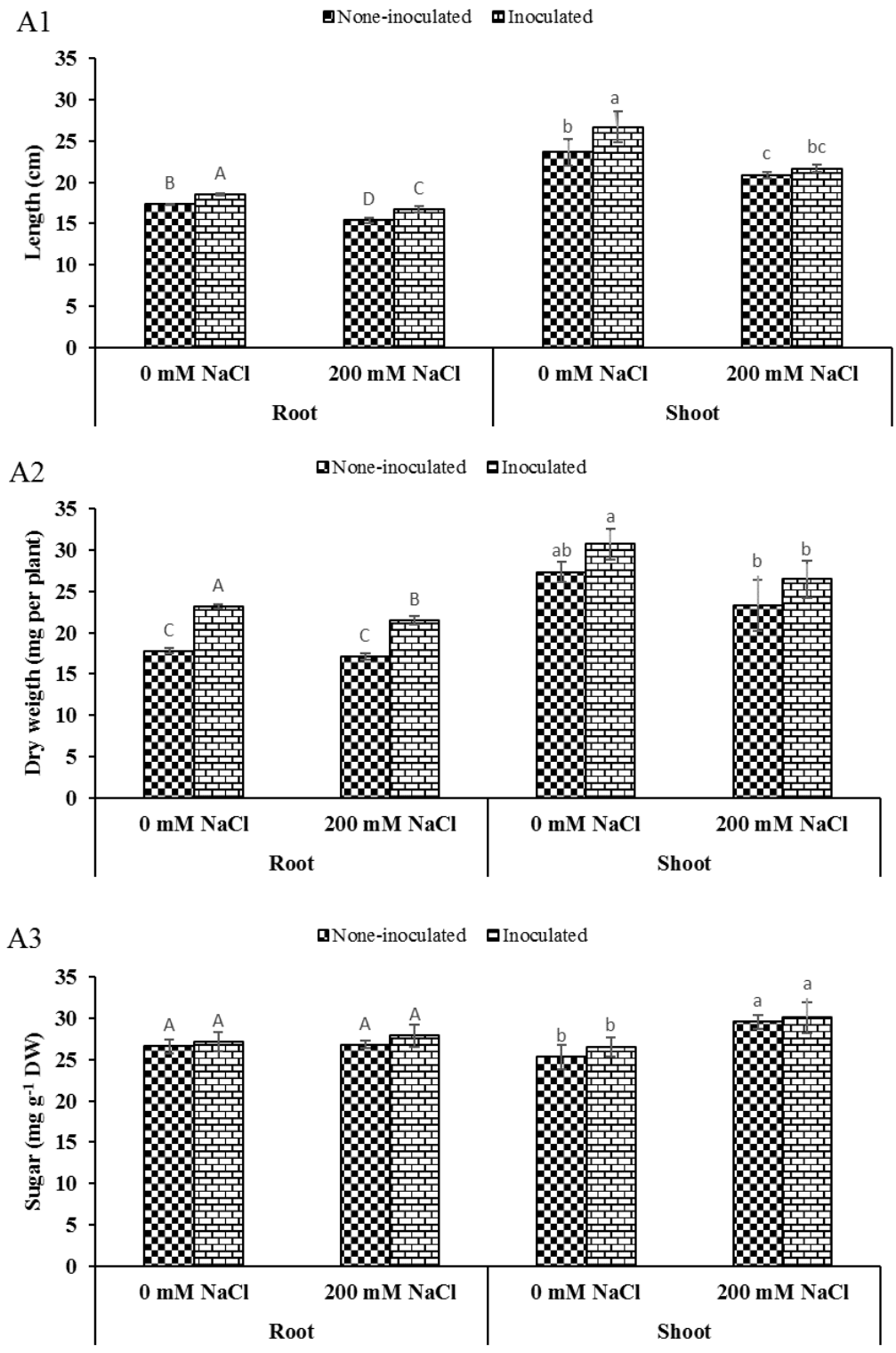

Figure 1: Length (A1); dry mass, DM (A2) and soluble sugars (A3) of root and shoot of wheat plants ('Sardari') in non-inoculated and inoculated with *A. brasilense* Sp7 (10^7 CFU ml^{-1}) grown in saline (200 mmol NaCl) and non-saline conditions. Wheat was grown in 16 h light 8 h dark, photon density 650 µmol m^{-2} s^{-1} and temperature of 25 ºC. Plant samples were collected 72 hours after salt application. Each value represents the mean of three measurements ±SE. Different letters represent significant differences at 5 % level of significance

Excess of sodium chloride can damage plant growth and development through reducing water and nutrients uptake as well as negative effects on biochemical processes (Akbarimoghaddam et al., 2011). Numerous researchers have reported that growth and morphological indexes such as root length (El-Hendawy et al., 2005), leaf area (Hamdia et al., 2004) as well as soluble sugars, proline (Tavakoli et al., 2016), Ca and Fe content (Askary et al., 2017) are affected significantly by saline condition (Turan, 2008). The result of this study showed that in non-inoculated plant, salinity causes a reduction in root and shoot length. One of the mechanisms that is chosen by plants to cope with environmental stress such as salinity is the accumulation of some organic molecular (such as soluble carbohydrates and proline). It appears that increase in soluble carbohydrates and proline content in the root of wheat seedlings probably cause the better osmotic adjustment and maintained cell turgor for better growth under salinity. However, the soluble sugar of the root and shoot of inoculated and non-inoculated wheat seedlings were increased due to salinity. Meanwhile, proline was increased just in the root of non-inoculated seedlings. This result is similar to the results obtained by Maghsoudi and Arvin (2010). They reported a significant reduction in dry matter of susceptible wheat varieties under saline condition, when compared to the salt tolerate wheat cultivars. Moreover, the results of this study showed that inoculation of wheat plants with *A. brasilense* Sp7 under saline and non-saline conditions had no significant effect on the amount of root and shoot soluble sugars in short period of time. But, inoculation increased significantly dry mass and length of the roots. Similar results were reported by different researchers. Zarea et al. (2012) showed that *Azospirillum* had no effect on soluble sugars content of wheat plants. In similar work Hamdia et al. (2004) reported that root dry mass and root length of maize were increased significantly in response to inoculation with *A. brasilense*. However, growth reduction under saline condition may be either due to lowering the external water potential or ion toxicity on metabolic processes. Other reports indicated that *Azospirillum* Spp. may produce various plant growth regulators that increase plant growth indexes (dry mass, root and shoot length), nitrogen fixation, and absorption of water and minerals (El-Dengawy et al., 2011, Askary et al., 2017). In this study, Ca and Fe content of root and shoot seedlings significantly reduced under salt stress while inoculation with *A. brasilense* shown some improvement in Ca uptake. Upadhyay et al. (2011) reported that some of the native strains of bacteria which separated from the wheat rhizosphere of the soils were able to establish salt tolerance by bacterial secretion such as exopolysaccharides that affect the mineral availability.

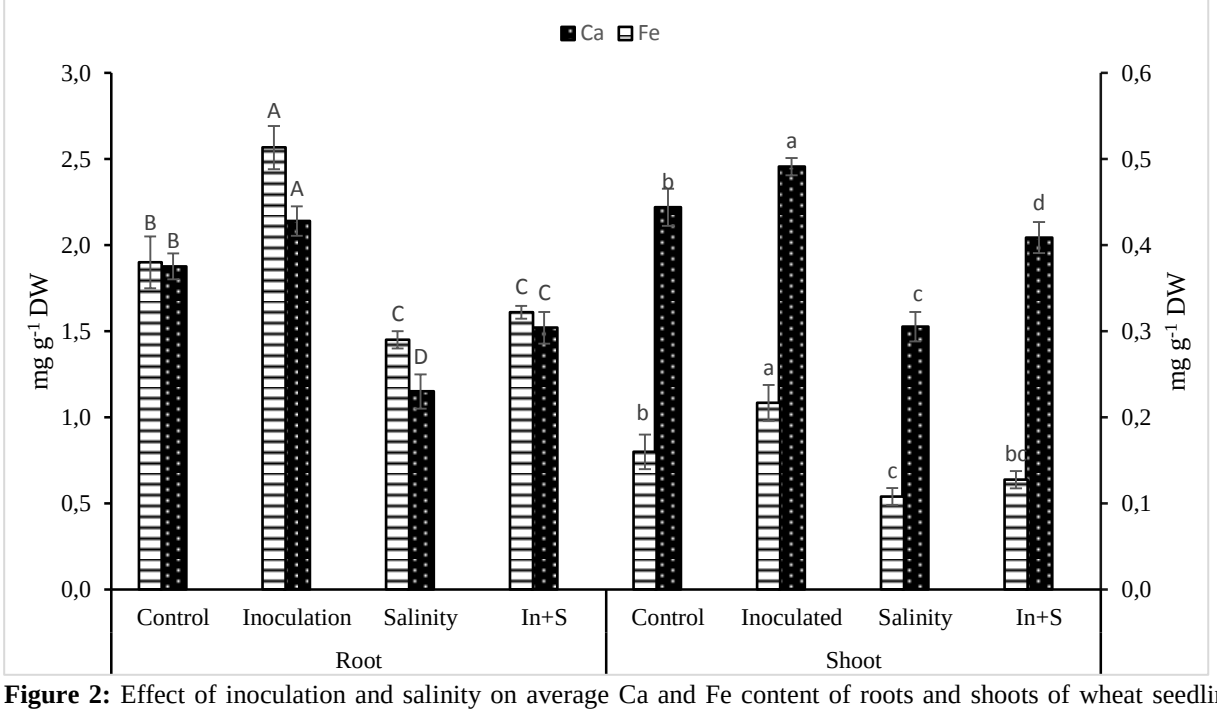

Figure 2: Effect of inoculation and salinity on average Ca and Fe content of roots and shoots of wheat seedlings. Differences in small (shoot) and cap (root) letters on the bar graph indicated difference in their mean (n = 3) values based on Duncan's multiple range tests

3.2 Sodium, potassium and Na^+/K^+ ratio

In control condition, sodium content of roots, sheaths and leaf blades showed an increasing trend over time, and so, the sodium content was the highest at 48 hour (at the end of experiment) after salinity applied (Table 1), it means sodium was accumulated during the experiment in all plant parts. The sodium content of roots, sheaths and leaf blades were also increased in same pattern in non-inoculated plants under saline condition as compared to control plants. Under inoculation and non-saline condition, the sodium content of root, sheath and leaf blade were significantly less than control plants. Although salinity raised sodium accumulation in root, sheath and leaf blade of inoculated plants over time, their sodium content were still significantly less than non-inoculated plants that were grown under saline condition. Moreover, the maximum sodium content in root, sheath and leaf blade of non-inoculated plants which exposed to 200 mmol NaCl was 7.94, 4.44 and 2.70 mg g^{-1} DM, respectively at 48 hour after salinity applied. According to the result (Table 1), we observed that wheat inoculation by *A. brasilense* could help to prevent the sodium entrance to the roots of plants (17.22 % at non-saline and 9.5 % at saline conditions) meanwhile didn't have considerable effect on sodium allocation within plant parts.

Although sodium content was increased in inoculated plants under saline condition, it was significantly less than sodium content of non-inoculated plants which grown under saline condition. In addition, we observed that *A. brasilense* could help to prevent sodium uptake by root of inoculated wheat plant. This might be due to producing and secretion of bacterial exopolysaccharides to the root environment and reducing the availability of Na^+ for plant uptake. This result is consistent with the results presented by Upadhyay et al. (2011). They indicated that some of the native strains of bacterial which separated from the wheat rhizosphere in the soils of Varanasi and India were able to establish salt tolerance by bacterial secretion such as exopolysaccharides.

Previous studies revealed that sodium accumulation in germinated seeds was increased gradually under saline condition (Akbarimoghaddam et al., 2011; Hamdia et al., 2004). In addition, El-Dengawy et al. (2011) showed that Carob seedlings (*Ceratonia siliqua* L.) inoculated with *A. lipoferum* under saline condition improved the addition of sodium-to-potassium ratio and gave better root characteristics. Al these results show that establishing a cooperation system between wheat and *Azospirillum* help to facilitate better growth through direct or indirect mechanisms (Hamdia et al., 2004; Nadeem et al., 2006).

Table 1: Mean values of Na^+ content (mg g^{-1} D M) in inoculated and non-inoculated wheat plants with *Azospirillum brasilense* SP7 (10^7 CFU ml^{-1}) grown under saline (200 mmol NaCl) and non-saline conditions for 6, 24 and 48 h. The growth condition was light with photon density 650 µmol m^{-2} s^{-1} and temperature of 25 ºC. Different letters in each plant parts separately represent a significant difference at 5 % level of significance.

	6 h			24 h			48 h		
	Root	Sheath	Blade	Root	Sheath	Blade	Root	Sheath	Blade
None inoculated									
Control	3.94^{E}	1.38^{H}	1.11^{G}	4.22^{DE}	1.89^{E}	1.24^{F}	4.37^{D}	1.81^{EF}	1.38^{E}
Salinity	4.56^{D}	2.28^{D}	1.28^{EF}	6.94^{B}	3.94^{B}	1.91^{C}	7.94^{A}	4.44^{A}	2.70^{A}
Inoculated									
Control	3.45^{G}	1.09^{I}	0.86^{H}	3.69^{FG}	1.50^{GH}	1.03^{G}	3.54^{G}	1.63^{FG}	1.29^{EF}
Salinity	4.47^{D}	2.25^{D}	1.06^{G}	5.99^{C}	3.33^{C}	1.60^{D}	7.12^{B}	3.36^{C}	2.55^{B}

Table 2: Mean (n = 3) values of K^+ content (mg g^{-1} D.M) in inoculated and non-inoculated wheat plants with *Azospirillum brasilense* (10^7 CFU ml^{-1}) grown under saline (200 mmol NaCl) and non-saline conditions for 6, 24 and 48 h. The growth conditions were light with photon density 650 µmol m^{-2} s^{-1} and temperature of 25 ºC. Different letters in each plant parts represent significant differences at 5 % level of significance.

	6 h			24 h			48 h		
	Root	Sheath	Blade	Root	Sheath	Blade	Root	Sheath	Blade
None inoculated									
Control	1.20^{DE}	2.47^{G}	2.06^{H}	1.23^{D}	3.53^{BC}	2.98^{E}	1.28^{DE}	3.69^{B}	3.75^{C}
Salinity	0.999^{G}	2.02^{H}	1.97^{H}	1.05^{FG}	3.04^{DF}	2.71^{FG}	1.03^{FG}	2.94^{EF}	3.85^{C}
Inoculated									
Control	1.51^{C}	2.79^{F}	2.57^{G}	1.52^{C}	3.74^{B}	3.46^{D}	2.16^{A}	4.15^{A}	5.03^{A}
Salinity	1.12^{EF}	2.16^{H}	2.08^{H}	1.29^{D}	3.22^{D}	2.76^{F}	1.24^{D}	3.44^{C}	4.11^{B}

Table 3: Mean values of Na^+/K^+ ratio in inoculated and non-inoculated wheat plants with *Azospirillum brasilense* (10^7 CFU ml^{-1}) grown under saline (200 mmol NaCl) and non-saline conditions for 6, 24 and 48 h. The growth conditions were light with photon density 650 µmol m^{-2} s^{-1} and temperature of 25 ºC. Different letters in each plant parts represent significant differences at 5 % level of significance.

	6 h			24 h			48 h		
	Root	Sheath	Blade	Root	Sheath	Blade	Root	Sheath	Blade
None inoculated									
Control	3.28^{FG}	0.56^{E}	0.54^{DE}	3.51^{F}	0.54^{E}	0.42^{F}	3.41^{F}	0.49^{E}	0.37^{G}
Salinity	4.56^{D}	1.13^{C}	0.65^{B}	6.61^{B}	1.30^{B}	0.71^{A}	7.70^{A}	1.51^{A}	0.70^{A}
Inoculated									
Control	2.30^{GH}	0.39^{F}	0.34^{GH}	2.44^{GH}	0.40^{F}	0.30^{HI}	1.64^{I}	0.39^{F}	0.26^{I}
Salinity	3.98^{E}	1.04^{D}	0.51^{E}	4.67^{D}	1.03^{D}	0.58^{CD}	5.74^{C}	0.98^{D}	0.62^{BC}

Potassium content of plant's root in treated and non-treated wheat seedlings with *A. brasilense Sp7* and *Azospirillum* plus salinity was higher compared to salt stressed plants alone (Table 2). Also, potassium content of plant's sheath and leaf blade in all treated and non-treated wheat seedlings showed an increasing trend over time (Table 2). Under saline condition, potassium accumulation decreased in root and sheath as compared to control plants while potassium content of leaf blade was preserved. The reduction of potassium content was more severe in root at 48 hour after salinity applied (46.6 %). Potassium accumulation of root, sheath and leaf blade in control plants were 1.94, 3.69 and 3.75 mg g^{-1} DM, respectively. While potassium content decreased to 1.03, 3.04 and 3.85 mg g^{-1} DM when salinity applied. However, with inoculation, potassium content increased to 2.16, 4.15 and 5.03 mg g^{-1} DM in root, sheath and leaf blade, respectively. In dual effect (salinity and inoculation) the potassium content was higher than control condition but less than in inoculated plants. The average potassium content of whole plant was 3.13 (control), 2.64 (salinity), 3.78 (inoculation) and 3.47 mg g^{-1} DM for dual effects of inoculation and salinity.

In root and sheath of non-treated plants, Na^+/K^+ ratio didn't show variation over time while this ratio in leaf blade was significantly decreased over time (Table 3). Under saline condition, the Na^+/K^+ ratio of non-inoculated plant's root, sheath and leaf blade was higher than control plants and also showed an increasing trend over time. Moreover, the maximum Na^+/K^+ ratio in non-inoculated plants was seen in root at 48 hour after salinity applied (278 %). Under non-saline condition, Na^+/K^+ ratio of root, sheath and leaf blade in inoculated plants were less than non-inoculated plants. Although the Na^+/K^+ ratio of inoculated plants under saline condition was more than control plants, but it was less than non-inoculated plants treated with salinity. Moreover, the maximum reduction (29.73 %) of Na^+/K^+ ratio was observed in leaf blade of inoculated plants not exposed to NaCl as compared to control plants.

Under saline and non-inoculated condition, potassium content of root and sheath showed a significant reduction compare to control plants. Meanwhile, potassium content of inoculated plants was more than that of non-inoculated plants under saline condition. In addition, under non-saline condition, potassium content of all plant parts was increased due to inoculation. The

results of this study show that the wheat plant inoculation with *A. brasilense* Sp7 has a significant effect on K^+ accumulation under saline and non-saline conditions. Under saline condition, the competition between uptake of sodium and potassium by non-inoculated plants favored sodium ions (Wakeel, 2013), but the wheat seedlings inoculated with *Azospirillum* increases potassium uptake. Therefore, sodium entry into the cell and potassium leakage out of the cell is decreased; this leads to a reduction of Na^+/K^+ ratio (Fraile-Escanciano et al., 2010; Hamdia et al., 2004; Upadhyay et al., 2011). Ardakani et al. (2011), showed that the potassium content of inoculated wheat plants by *A. brasilense* was increased and causes better mineral nutrient uptake. The results of this study are also similar to Askary et al. (2009), who showed that *A. brasilense* Sp7 improves potassium, phosphorus and nitrogen uptake by different wheat cultivars. In addition, the results are the same as Omar et al. (2009), who showed that Na^+/K^+ ratio of wheat plant after inoculation with *A. brasilense* was decreased due to increasing K^+ and limiting Na^+ uptake. In inoculated and non-inoculated plants, Na^+/K^+ ratio of leaf blade significantly was lower than the root. This might be due to less sodium accumulation in leaf blade that have already confirmed by Davenport et al. (2005).

3.3 Proline

Inoculated seedlings had lower proline concentration in the root and the shoot when compared to seedlings which were exposed to saline conditions. The lowest amount of proline (0.4 µmol g^{-1} FM) was measured at the root of inoculated plants not exposed to salinity ($P < 0.05$, Fig. 2). Salinity didn't have a significant effect on shoot proline, meanwhile, the maximum content of proline was observed in the root of salt-affected that non-inoculated with *Azospirillum* ($P < 0.05$, Fig. 3). Proline production is one of the mechanisms that enable the plant to tolerate adverse effect of environmental stresses. Proline is thought to contribute to osmotic adjustment, detoxification of ROS, and protection of membrane integrity (Heuer, 2010).

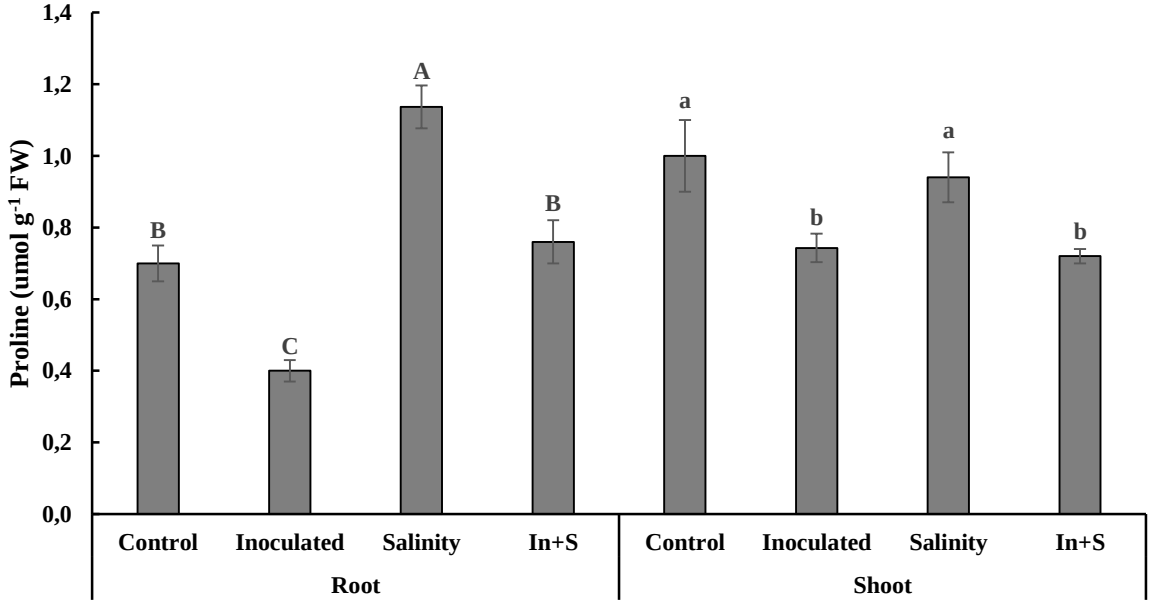

Figure 3: Effect of inoculation and salinity on average (n = 3) proline content of roots and shoots of wheat seedlings. Differences in small and cap letters on the bar graph indicated difference in their mean values based on Duncan's multiple range tests.

3.4 Relative *TaSOS1* (*Triticum aestivum* salt overly sensitive 1) gene expression

In control plants, the relative expression ratio of root *TaSOS1* didn't show any variation in its transcript level over time (Table 4) while a considerable reduction in *TaSOS1* expression was observed in sheath and leaf blade. When wheat seedlings exposed to 200 mmol NaCl and inoculated with *A. brasilense* Sp7 (10^7 CFU ml^{-1}), *TaSOS1* gene expression at mRNA level varied differently in different plant parts.

Root: Due to salt stress, *TaSOS1* over-expression in non-inoculated plant's root as compared to the control

plants (non-inoculated and non-saline condition) was observed. Although the maximum (224 %) over-expression happened at 24 h after salinity applied, its value declined almost by 50 % afterward (at 48 h). In non-saline condition, the root *TaSOS1* relative expression of inoculated plants showed an up-regulation trend during the experiment. While, inoculated roots under salinity condition showed a considerable up-regulation (260 %) at 24 h and then down-regulated at 48 h after salinity applied but still the *TaSOS1* expression was higher than its control plant. In addition, *TaSOS1* over expression was higher in inoculated plants, than non-inoculated under non-saline condition.

Sheath: In non-inoculated plants, the relative expression ratio of *TaSOS1* was increased immediately when NaCl was added, then its value was reduced at 24 h, and finally reached to its maximum (120 %) at 48 hour. In non-saline condition, inoculation caused a significant increase in sos*1* gene expression as compared to control plants and reached to its maximum at 48 h (188.4 %). In saline and inoculated condition, the relative expression of *TaSOS1* was increased in sheath by 119 % when compared to its corresponded control plants. However, *TaSOS1* up-regulation due to dual treatments (salinity and inoculation) was significantly higher than inoculation or saline condition.

Leaf blade: There was a significant increase (almost 14 %) in *TaSOS1* expression after salinity applied as compared to non-treated plants. In inoculated and non-saline condition, the relative expression of *TaSOS1* showed an increasing trend and reached to 1.66 at 48 h. However, in saline condition, the expression of *TaSOS1* in inoculated plants was higher than control plants at 24 (70 %) and 48 h (27 %) after salinity applied. The *TaSOS1* mRNA level was higher in inoculated plants treated with NaCl as compared to control plants and also seedlings exposed to salinity.

Table 4: *TaSOS1* gene relative expression inoculated and non-inoculated wheat plants with *Azospirillum brasilense* (10^7 CFU ml^{-1}) grown under saline (200 mmol NaCl) and non-saline conditions for 6, 24 and 48 h. The plants were grown under the light density of 650 µmol m^{-2} s^{-1} and temperature of 25 ºC. Each value represents the mean of three individual measurements ±SE. Different letters in each plant parts represent significant differences at 5 % level of significance.

	6 h			24 h			48 h		
	Root	Sheath	Leaf	Root	Sheath	Leaf	Root	Sheath	Leaf
None inoculated									
Control	0.660^{G}	0.696^{E}_{F}	1.010^{D}	0.557_{G}	0.737^{E}	0.818^{F}	0.594^{G}	0.576^{F}	0.908^{E}_{F}
Salinity	1.163^{D}_{E}	0.911^{D}	1.011^{D}	1.805_{B}	0.741^{E}	0.936^{D}_{E}	1.262^{CD}_{E}	1.262^{B}	1.026^{D}
Inoculated									
Control	0.885^{F}	0.931^{D}	0.884^{E}_{F}	1.135_{E}	1.015^{C}_{D}	1.181^{C}	1.190^{DE}	1.660^{A}	1.661^{A}
Salinity	1.365^{C}	0.893^{D}	0.869^{E}_{F}	2.01^{A}	0.923^{D}	1.390^{B}	1.282^{CD}	1.153^{B}_{C}	1.155^{C}

The relative expression ratio of *TaSOS1* in different plant parts shown that the highest up-regulation of *TaSOS1* was observed in root at 24 h whereas the highest in sheath and leaf blade was happend at 48 h after salinity applied (with a delay) as compared to the root.

The result of this study showed that addition of sodium in the root rhizosphere and inoculation of wheat seedlings with *A. brasilense* Sp7 cause an increase in *TaSOS1* expression. This result is similar to that obtained by Ramezani et al. (2013), who showed that under saline condition *TaSOS1* and *TaSOS4* gene expression would increase in such cases. Moreover, Xu et al. (2008) showed that after 3 h of salt stress implementation, *TaSOS1* expression of root was increased immediately and then decreased. But, these changes occurred in the leaf with lower intensity and with a delay (after 9 h of salt stress).

Wheat seedling treated with *A. brasilense* and salinity showed that *TaSOS1* expression was increased in compared to non-inoculated and saline condition. Therefore, it can be concluded that *A. brasilense* can increase *TaSOS1* expression under saline and non-saline condition and can help sodium and potassium ions to be adjusted in plant. Numerous studies have already showed that gene expression changes in host plant after inoculation with plant growth-promoting rhizobacteria (PGPR). For instance, Vargas et al. (2012), showed that

rice ethylene receptor gene expression was increased after inoculation with *A. brasilense* Sp245. In addition, it was reported that some genes involved in nutrient uptake were increased when wheat plant inoculation with *A. brasilense* (Camilios-Neto et al., 2014).

It seems that the tolerance of wheat plant to high concentration of salt is related to their ability to prevent Na uptake, avoid accumulation of toxic levels of sodium, regulation of osmotic pressure and maintaining adequate amount of potassium especially in the leaf blade. To achieve these goals, some genes related to such indexes should be express differently, e.g. *TaSOS1* which is a regulator for Na^+ uptake and upregulated at salinity condition. Establishment of an associated relationship between wheat plants and PGPR such as *A. brasilense* may help to regulate expression of such genes (e.g. *SOS1*) and consequently regulate the balance of cytosolic sodium and potassium. This is coincidence with, reduction of Na^+ availability for plant uptake via secretion of bacterial exopolysaccharides to the root environment.

4 CONCLUSION

This study showed that *TaSOS1* gene expression, growth, and biochemical indexes increased due to inoculation and saline conditions. Furthermore, the highest up-regulation of *TaSOS1* was observed in sheath and leaf blade with a delay as compared to the root. Also, *A. brasilense* had an important role in preventing the sodium entry into the plant. Further research might explore plasma membrane *TaSOS1* antiporter proteins by proteomic techniques in inoculated wheat seedlings under saline condition.

5 ACKNOWLEDGEMENTS

Thanks to the University of Isfahan for financial support, and also thanks to the Department of Biology for cooperation and providing space and facilities**.** We also thank Dr. Manoochehr Tavassoli for some gene expression explanation.

6 REFERENCES

Akbarimoghaddam, H., Galavi, M., Ghanbari, A., & Panjehkeh, N. (2011). Salinity effects on seed germination and seedling growth of bread wheat cultivars, *Trakia journal of Sciences, 9*(1), 43-50.

Amini, F., Askary, M., Haghir, M., & Ghassemi, H. R. (2017). Changes in antioxidant system and oxidative stress under water stress in four cucumber cultivars. *Indian Journal of Plant Physiology*, *22*(1), 114-119. doi:10.1007/s40502-017-0285-0

Amooaghaie, R., Mostajeran, A., & Emtiazi, G. (2002). The effect of compatible and incompatible *Azospirillum brasilense* strains on proton efflux of intact wheat roots, *Plant and soil*, *243*(2),155-160.

Ardakani, M.R., Mazaheri, D., Mafakheri, S., & Moghaddam, A. (2011). Absorption efficiency of N, P, K through triple inoculation of wheat (*Triticum Aestivum* L.) by *Azospirillum brasilense*, *Streptomyces* sp., *Glomus intraradices* and manure application, *Physiology and molecular biology of plants : an international journal of functional plant biology*, *17*(2), 181-192.

Askary, M., Mostajeran, A., & Emtiazi, G. (2008). Colonization and nitrogenase activity of *Triticum aestivum* (cv. Baccross and Mahdavi) to the dual inoculation with *Azospirillum brasilense* and *Rhizobium meliloti* plus 2,4-D. *Pakistan Journal of Biological Sciences*, *11*(12), 1541-1550.

Askary, M., Mostajeran, A., Amooaghaei, R., & Mostajeran, M. (2009). Influence of the co-inoculation *Azospirillum brasilense* and *Rhizobium meliloti* plus 2, 4-D on grain yield and N, P, K content of *Triticum aestivum* (cv. Baccros and Mahdavi), *American-Eurasian Journal Agriculture Environment Science, 5*, 296-307.

Askary, M., Talebi, S. M., Amini, F., & Bangan, A. D. B. (2017). Effects of iron nanoparticles on *Mentha piperita* L. under salinity stress. *Biologija*, *63*(1), 65-75.

Baniaghil, N., Arzanesh, M., Ghorbanli, M., & Shahbazi, M. (2013). The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress, *J Appl Environment Biology Science, 3*, 17-27.

Bates, L., Waldren, R., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*(1), 205-207. doi:10.1007/BF00018060

Camilios-Neto, D., Bonato, P., Wassem, R., Tadra-Sfeir, M.Z., Brusamarello-Santos, L.C.C., Valdameri, G., Donatti, L., Faoro, H., Weiss, V.A., Chubatsu, L.S., Pedrosa, F.O., & Souza, E.M. (2014). Dual RNA-seq transcriptional analysis of wheat roots colonized by *Azospirillum brasilense* reveals up-regulation of nutrient acquisition and cell cycle genes, *BMC Genomics, 15*(1), 378.

Creus, C.M., Sueldo, R.J., & Barassi, C.A. (1997). Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses, *Plant physiology and biochemistry-paris, 35*, 939-944.

Davenport, R., James, R. A., Zakrisson-Plogander, A., Tester, M., & Munns, R. (2005). Control of sodium transport in durum wheat. *Plant Physiology, 137*(3), 807-818. doi:10.1104/pp.104.057307

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances, *Analytical Chemistry, 28*(3), 350-356.

El-Dengawy, E., Hussein, A.A., & Alamri, S.A. (2011). Improving growth and salinity tolerance of carob seedlings (*Ceratonia siliqua* L.) by *Azospirillum* inoculation, *American-Eurasian Journal of Agricultural & Environmental Sciences, 11*(3), 371-384.

El-Hendawy, S.E., Hu, Y., Yakout, G.M., Awad, A.M., Hafiz, S.E., & Schmidhalter, U. (2005). Evaluating salt tolerance of wheat genotypes using multiple parameters, *European journal of agronomy*, 22 (3), 243-253.

Feki, K., Brini, F., Ben Amar, S., Saibi, W., & Masmoudi, K. (2014). Comparative functional analysis of two wheat Na^+/H^+ antiporter *SOS1* promoters in *Arabidopsis thaliana* under various stress conditions, *Journal of Applied Genetics, 56*(1), 15-26.

Fraile -Escanciano, A., Kamisugi, Y., Cuming, A. C., Rodríguez-Navarro, A., & Benito, B. (2010). The SOS1 transporter of *Physcomitrella patens* mediates sodium efflux in planta. *New Phytologist, 188*(3), 750-761. doi:10.1111/j.1469-8137.2010.03405.x

Hamdia, M.A.E.-S., Shaddad, M., & Doaa, M.M. (2004). Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions, *Plant Growth Regulation, 44*(2), 165-174.

Heuer, B. (2010). Role of proline in plant response to drought and salinity, *Handbook of plant and crop stress. CRC Press, Boca Raton*, 213-238.

Hoagland, D.R., & Arnon, D.I. (1950). The water-culture method for growing plants without soil, *Circular. California Agricultural Experiment Station*, 347 (2nd edit).

Kang, S.-M., Khan, A.L., Waqas, M., You, Y.-H., Kim, J.-H., Kim, J.-G., Hamayun, M., & Lee, I.-J. (2014). Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*, *Journal of Plant Interactions, 9*(1), 673-682.

Lekshmy, S., Sairam, R., & Kushwaha, S. (2013). Effect of long-term salinity stress on growth and nutrient uptake in contrasting wheat genotypes. *Indian Journal of Plant Physiology, 18*(4), 344-353. doi:10.1007/s40502-014-0059-x

Maghsoudi, K., & Arvin, M.J. (2010). Salicylic acid and osmotic stress effects on seed germination and seedling growth of wheat (*Triticum aestivum* L.) cultivars, *World Applied Sciences Journal, 2*(1), 7-11.

Mostajeran, A., & Gholaminejad, A. Effect of salinity on sodium & potassium uptake and proline, carbohydrates contents of turmeric plant parts, *Journal of Current Chemical & Pharmaceutical Sciences, 4*(1) (1), 10-21.

Nadeem, S.M., Zahir, Z.A., Naveed, M., Arshad, M., & Shahzad, S. (2006). Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress, *Soil Environment, 25* (2), 78-84.

Neumann, P.M. (1995). The role of cell wall adjustments in plant resistance to water deficits, *Crop Science, 35* (5), 1258-1266.

Norastehnia, A., Niazazari, M., Sarmad, J., & Rassa, M. (2014). Effects of chloride salinity on non-enzymatic antioxidant activity, proline and malondialdehyde content in three flue-cured cultivars of Tobacco, *Journal of Plant Development, 21*.

Öğüt, M., Akdağ, C., Düzdemir, O., & Sakin, M.A. (2005). Single and double inoculation with *Azospirillum*/*Trichoderma*: the effects on dry bean and wheat, *Biology and Fertility of Soils, 41*(4), 262-272.

Omar, M.N.A., Osman, M.E.H., Kasim, W.A., & Abd El-Daim, I.A. (2009). *Improvement of salt tolerance mechanisms of barley cultivated under salt stress using Azospirillum brasilense*, chapter

title, in: Ashraf, M., Ozturk, M., Athar, H.R. (Eds.), *Salinity and Water Stress: Improving Crop Efficiency*. Springer Netherlands, Dordrecht, pp. 133-147. doi:10.1007/978-1-4020-9065-3_15

Pakdaman, N., Mostajeran, A., & Hojati, Z. (2014). Phosphate concentration alters the effective bacterial quorum in the symbiosis of Medicago truncatula-Sinorhizobium meliloti, *Symbiosis, 62*(3), 151-155.

Pessarakli, M., & Huber, J. (1991). Biomass production and protein synthesis by alfalfa under salt stress, *Journal of Plant Nutrition, 14*(3), 283-293.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT–PCR, *Nucleic Acids Research, 29*(9), e45-e45.

Ramezani, A., Niazi, A., Abolimoghadam, A.A., Babgohari, M.Z., Deihimi, T., Ebrahimi, M., Akhtardanesh, H., & Ebrahimie, E. (2013). Quantitative expression analysis of *TaSOS1* and *TaSOS4* genes in cultivated and wild wheat plants under salt stress, *Molecular Biotechnology, 53*(2), 189-197.

Sathee, L., Sairam, R. K., Chinnusamy, V., & Jha, S. K. (2015). Differential transcript abundance of salt overly sensitive (*SOS*) pathway genes is a determinant of salinity stress tolerance of wheat. *Acta physiologiae plantarum, 37*(8), 169-179. doi:10.1007/s11738-015-1910-z

Shi, H., Quintero, F.J., Pardo, J.M., & Zhu, J.-K. (2002). The putative plasma membrane Na^+/H^+ antiporter *SOS1* controls long-distance Na^+ transport in plants, *The Plant Cell, 14*(2), 465-477.

Shi, H., & Zhu, J.-K. (2002). Regulation of expression of the vacuolar Na^+/H^+ antiporter gene *AtNHX1* by salt stress and abscisic acid, *Plant molecular biology, 50*(3), 543-550.

Tavakoli, M., Poustini, K., & Alizadeh, H. (2016). Proline accumulation and related genes in wheat leaves under salinity stress. *Journal of Agricultural Science and Technology, 18*(3), 707-716.

Tiwari, J.K., Munshi, A.D., Kumar, R., Pandey, R.N., Arora, A., Bhat, J.S., & Sureja, A.K. (2010). Effect of salt stress on cucumber: Na^+/K^+ ratio, osmolyte concentration, phenols and chlorophyll content, *Acta physiologiae plantarum, 32*(1), 103-114.

Turan, N.G. (2008). The effects of natural zeolite on salinity level of poultry litter compost, *Bioresource Technology, 99*(7), 2097-2101.

Upadhyay, S., Singh, J., & Singh, D. (2011). Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. *Pedosphere, 21*(2), 214-222. doi:10.1016/S1002-0160(11)60120-3

Upadhyay, S.K., Singh, J.S., Saxena, A.K., & Singh, D.P. (2012). Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions, *Plant Biology, 14*(4), 605-611.

Vargas, L., de Carvalho, T.L.G., Ferreira, P.C.G., Baldani, V.L.D., Baldani, J.I., & Hemerly, A.S. (2012). Early responses of rice (*Oryza sativa* L.) seedlings to inoculation with beneficial diazotrophic bacteria are dependent on plant and bacterial genotypes, *Plant and Soil, 356*(1), 127-137.

Wakeel, A. (2013). Potassium–sodium interactions in soil and plant under saline-sodic conditions, *Journal of Plant Nutrition and Soil Science, 176*(3), 344-354.

Xu, H., Jiang, X., Zhan, K., Cheng, X., Chen, X., Pardo, J.M., & Cui, D. (2008). Functional characterization of a wheat plasma membrane Na^+/H^+ antiporter in yeast, *Archives of Biochemistry and Biophysics, 473*(1), 8-15.

Xue, Z.-Y., Zhi, D.-Y., Xue, G.-P., Zhang, H., Zhao, Y.-X., & Xia, G.-M. (2004). Enhanced salt tolerance of transgenic wheat *(Tritivum aestivum* L.) expressing a vacuolar Na^+/H^+ antiporter gene with improved grain yields in saline soils in the field and a reduced level of leaf Na^+, *Plant Science, 167*(4), 849-859.

Yadav, N.S., Shukla, P.S., Jha, A., Agarwal, P.K., & Jha, B. (2012). The *SbSOS1* gene from the extreme halophyte *Salicornia brachiata* enhances Na^+loading in xylem and confers salt tolerance in transgenic tobacco, *BMC Plant Biology, 12*, 188-188.

Zarea, M., Hajinia, S., Karimi, N., Goltapeh, E.M., Rejali, F., & Varma, A. (2012). Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl, *Soil Biology and Biochemistry, 45*, 139-146