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Original Research Article

The biotic role of natural biofertilizer application in improvement osmotic defense system of two wheat cultivars grown under osmotic stress

Hamdia, M. Abd El-Samad* and Mohamed, A. A.

1,2Botany and Microbiology Department, Faculty of Science, Minia University, El-Minia, Egypt

***Corresponding Author's E-mail: hamdia10@yahoo.com**

Abstract

The present work was conducting to study the response strategy of salt tolerance of cv. Sakha 94 and cv. Giza 168 and the biotic role of fertilizer *Azospirillum brasilense* inoculation in improvement osmotic defense system of these cultivars. It can be observed that cv. Giza168 was the most tolerant one and cv. Sakha 94 was the sensitive one. This sensitivity in cv. Sakha 94 was concomitant with the decrease in osmotic pressure of the cell sap, soluble sugar and soluble protein, shoot K^+ and Ca^{++} and root K^+, water uptake, fresh, dry matter and length of shoot and root of wheat plant. On the other side the tolerant of cv. Giza in 168 was concomitant with the increase of osmotic pressure, shoot soluble sugar, soluble protein, water content, fresh, dry matter and length of both organs. Sodium increased in shoot and root of both cultivars. *Azospirillum* application was significantly increased in the production of fresh, and dry matter length and water content of both cv. Sakha 94 and cv. Giza 168. Soluble sugar and soluble protein content of both cultivars were markedly accumulated. *Azospirrillum* application induced a marked decrease in the accumulation of Na^+ content in shoot and root of both tested cultivars. *Azospirillum* inoculation was significantly increased K^+, Ca^{++} and Mg^{++} with increasing osmotic stress in shoot and root of cv. Sakha 94. In cv. Giza168, there are a similar trend between shoot and root in the accumulation of K^+ and Ca^{++}, increase with increasing osmotic stress.

Keywords: Osmotic stress, biofertilizers, ameliorated, wheat cultivars.

INTRODUCTION

Soil salinity is a major abiotic stress that limits plant growth and productivity. A high concentration of Na^+ is harmful to plants because of its interference with K^+ nutrition and, consequently, alteration of enzyme activities and cellular metabolism (Ward et al., 2003 and Hamdia and Shaddad, 2014 and 2016). In order to prevent accumulation of toxic amounts of Na^+ in the cytosol, active Na^+ efflux into the apoplast and its compartmentalization inside the vacuole occur (Apse and Blumwald, 2007; Kabala and Russak, 2012). Plant's behavioral response to salinity is complex, and different mechanisms are adopted by plants when they encounter salinity. The soil and water engineering methods increase farm production in the damaged soil by salinity, but achievement of higher purposes by these methods seems to be very difficult (Yokoi et al. 2002; Mahajan and Tuteja, 2005).The high salinity of the soil affected the soil penetration, decreased the soil water potential and finally caused physiological drought (Yusuf et al., 2007).The plants can overcome the changes in environmental conditions either by change their metabolism or by exogenous application of biofertilizers (*Azospirillum)*. Apparently, inoculation with *Azospirillum* improved growth under water stress conditions as was initially demonstrated in the 1980s (El-Komy et al., 2003 and Hamdia and Shaddad, 2010). Coleoptile heigh, and fresh and dry weight of wheat seedlings inoculated with *A. brasilense* Sp 245 were enhanced, despite the water stress (Alvarez et al., 1996). Inoculation with *Azospirillum* alleviated the stress on wheat plants grown under drought and cupper stress conditions (El-Komy et al., 2003 and Hamdia, 2017). Turgor pressure at low water potential was higher in inoculated seedlings in two wheat cultivars under osmotic stress. This could result from better water uptake as a response to inoculation that, in turn, is reflected by faster shoot growth in inoculated seedlings exposed to these stresses. They showed better water status and effects on cell wall elasticity or apoplastic water (Creus et al., 2004).Thus the present work carried out to evaluate the salt tolerance of two wheat cultivars cv. Sakha 94 and cv. Giza 186 and try to ameliorate the deleterious effect of salinity by *Azospirillum* inoculation.

MATERIAL AND METHODS

Seeds cultivars of wheat Giza 168 and Skha 94 were surface sterilized by an immersion for 30 minutes in a mixture of 96% ethanol and 95% , H_2O_2 (1:1, V/V). Then they were washed with sterile distilled water several times and germinated in dark on wet sterile filter paper in petri dishes for 3 days at 30°C. The seedlings were transplanted in plastic pots containing 100 g clay soil without salinization treatment (control) and under different salinization concentration 50 mM and 100 mM NaCl concentrations were added to the soil in such a way that the soil solution acquired the assigned NaCl levels at field capacity. The previous treatment group was repeated for *Azospirillum brasilense* strain Z6/12 (isolated from maize rhizosphere) inoculation by El-Komy (1992). Bacterial strain was grown in malate medium supplied with 0.2 g L^{-1} yeast extract for 20 h at 30°C on shaker at 200 rpm cells were h1 cm^3 contained 10^7 colony-forming units (CFU) at the logarithmic phase by centrifugation twice in sterile demineralized water and then used as inoculums at the moment of Cm^3=10^7per used. The noculums was placed on the root surface before immersion. A week after this treatment, the plants were harvested for analysis after 21-days from planting. Dry matter was determined after drying plants in an aerated oven at 70°C to constant mass. Saccharides were determined by the anthrone-sulfuric acids method (Fales, 1951). Soluble protein was measured according to Lowry et al. (1951). Sodium and potassium were determined by flam photometric method (Williams and Twine 1960), and calcium and magnesium by the versene titration method (Schwarzenbach and Biedermann 1948).

Experimental data were subjected to one way analysis of variance and the means were separated by the least significant differences, L.S.D. (Steel and Torrie 1960). Correlation coefficients were calculated using statgraphics 5.0 software**.**

RESULT

Growth parameters

Osmotic stress significantly lowered the production of fresh and dry matter of shoot and root of cv. Sakha 94 as compared with untreated plants (Tab. 1). The percent of reduction at 100 mM NaCl concentration was 73%, 60.7%, 39% and 28.6% of shoot and root respectively. While cv. Giza 168 reflected in tolerance at lower NaCl level (50 mM) especially in shoot than in root. Fresh and dry matter were significantly increased as compared with control plants at that level, above which a significant reduction was recorded (Table 1). The percent of reduction at 100 mM was 59.4%, 27.3%, 2.5% and 21.8% of shoot and root respectively (Tab. 1). The length and water content in shoot and root of the two cultivars were markedly decreased with increasing salinization levels (Tab. 2). The percent of reduction in length at 100 mM NaCl was 39.1%, 60.7% and for water content 63%, 41 % in shoot and root of cv. Sakha 94 respectively. However, water content in cv. Giza 168 showed activation at 50 mM NaCl in shoot and root, while it lowered at 100 mM NaCl compared with control plants (Tab. 2).

Chemical constituents

Osmotic stress induced a significant reduction in soluble sugar of shoot and root of cv. Sakha 94 (Fig. 1). Soluble in sugar run in opposite direction while increase in shoot, it tended to decrease in root of cv. Giza 168 with increasing salinity level (Fig. 1). Soluble protein was markedly decreased in shoot and root of cv. Sakha, while in cv. Giza 168 soluble protein was significantly increased in both organs (Fig. 1).

Mineral contents

Sodium content was significantly increased in shoot and root of both tested cultivars (Tab. 3 and 4). This increasing effect was more obvious in shoot than in root and in cv. Sakha 94 than cv. Giza 186. K^+ content showed a variable response to increasing osmotic stress between two cultivars while it was tended to increase at 50 mM in shoot of cv. Sakha 94 and cv. Giza 168, it was significantly decreased at 100 mM NaCl level. In root K^+ was markedly decreased in cv. Sakha while a huge K^+ accumulation was induced in cv. Giza 168. Calcium content was significantly showed a higher accumulation in both shoots and roots of cv. Giza 168 with increasing salinization levels (Tab. 3 and 4). However, osmotic stress effect variably to the accumulation of Ca^{++} in cv. Sakha 94, induced an increasing effect in root, in shoot induced a decreasing effect (Tab. 3 and 4). Mg^{++} content was markedly and significantly elevated in shoot

Table 1. Effect of different osmotic stress levels on fresh and dry matter in shoot and root of cv. Sakha 94 and cv. Giza 168 grown for 21 days.

Trea. NaCl mM	Shoot				Root			
Cv. Sakha 94	**f. m.**	**%**	**d. m.**	**%**	**f. m.**	**%**	**d. m.**	**%**
Control	4.18	100	0.107	100	1.13	100	0.077	100
50 mM	1.26	30	0.035	32.7	0.864	76.5	0.0610	71.4
100 mM	1.13	27	0.042	39.3	0.667	61.0	0.047	71.4
Control+*Az.*	2.68	64.1	0.066	61.7	1.78	157.3	0.120	155.8
50 mM+*Az.*	1.35	68.2	0.045	68.2	0.925	81.9	0.065	84.4
100 mM+*Az.*	1.78	89.1	0.059	55.1	0.895	97.2	0.059	76.6
L.S.D. 0.05%	**1.3**		**0.17**		**0.6**		**0.5**	
Cv. Giza 168								
Control	0.838	100	0.033	100	0.564	100	0.055	100
50 mM	1.08	128.9	0.035	106.1	0.578	102.5	0.042	76.4
100 mM	0.340	40.6	0.024	72.7	0.550	97.5	0.04	78.2
Control+Az.	1.78	212.4	0.065	196.9	1.02	180.9	0.043	78.2
50 mM+Az.	1.49	178.5	0.051	154.5	0.795	140.9	0.05	92.7
100 mM+Az.	1.19	142.0	0.04	121.2	0.626	110.9	0.05	90.9
L. S. D. 0.05%	**1.2**		**0.13**		**0.5**		**0.3**	

Table 2. Effect of different osmotic stress levels on length and water content in shoot and root of cv. Sakha 94 and cv. Giza 168 grown for21 days.

Treat. NaCl mM	Length				Water content			
Cv. Sakha 94	**Shoot**	**%**	**Root**	**%**	**Shoot**	**%**	**Root**	**%**
Control	20.5	100	3.5	100	4.07	100	1.05	100
50 mM	11.0	53.7	2.5	71.4	1.23	30.2	0.803	76.5
100 mM	12.5	60.9	2.5	71.4	1.1	27.0	0.62	59.0
Control+Az.	18.5	90.2	5.5	157.1	2.61	64.1	1.66	158.1
50 mM+Az.	16.0	78.0	5.0	142.9	1.31	32.2	0.86	81.9
100 mM+Az.	13.5	65.9	3.0	85.7	1.72	42.3	0.84	80.0
L.S.D. 0.05%	1.5		0.92		1.6		0.85	
Cv. Giza 168								
Control	14.0	100	6.0	100	0.805	100	0.509	100
50 mM	12.0	85.7	4.0	66.7	1.05	130.4	0.536	105.3
100 mM	7.0	50.0	3.0	50.0	0.316	39.3	0.51	100.2
Control+Az.	15	107.1	3.3	55.0	1.72	213.7	0.977	191.9
50 mM+Az.	17	121.4	5.0	83.3	1.43	177.6	0.745	146.4
100 mM+Az.	15.5	110.7	5.0	83.3	1.15	142.9	0.576	113.2
L.S.D. 0.05%	1.8		1.4		1.2		1.2	

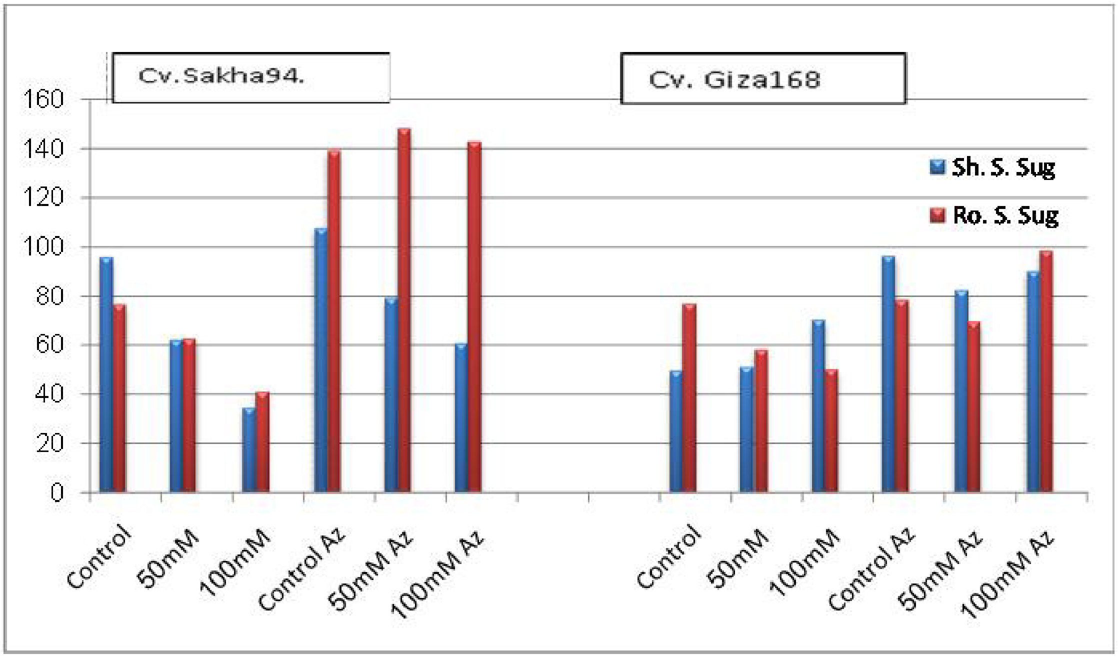
and root of both tested cultivars. It is worthy to mention that at 50 mM NaCl induced a higher values of K^+, Ca^{++} in shoot and Mg^{++} in root of cv. Sakha 94. Whereas in cv. Giza 168, higher values was recorded in shoot and root Ca^{++} and root Mg^{++} (Tab. 3 and 4).

Osmotic pressure

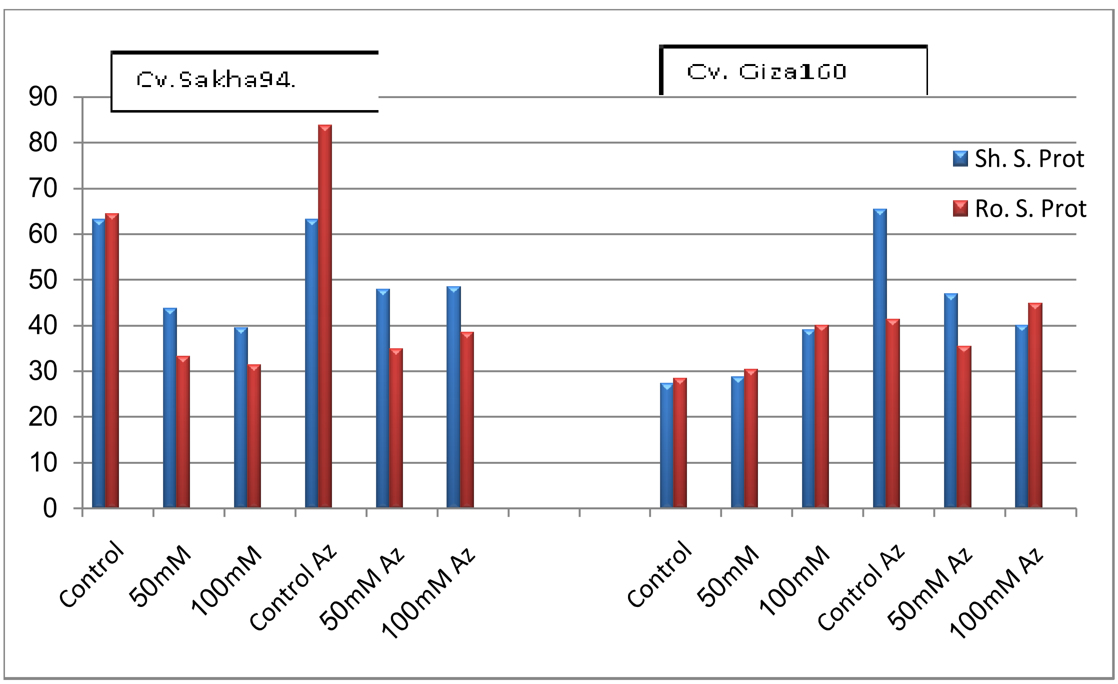
Osmotic pressure significantly elevates with increasing salinization levels in the soil in shoot and root of both tested cultivars (Figure 2). It is worthy to mention that this activation was more effective in shoot than in root organ.

Azospirillum application

Azospirillum inoculation was significantly increased the production of fresh, dry matter, length and water content of both cv. Sakha 94 and cv. Giza 168 (Tab. 1 and 2). Actually it can observed that cv. Giza 168 response more positively to *Azospirillum* treatment than cv. Sakha 94 in production of growth parameters when compared with control plants or with the corresponding osmotic stress level. Soluble sugar and soluble protein of both cultivars were markedly accumulated especially in shoot than in root and at 50 mM NaCl level than 100 mM NaCl compared with uninoculated plants (Fig. 1). *Azospirillum* application induced a marked decrease in the accumulation of Na^+ content in shoot and root of both



a



b

Fig. 1. Effect of different osmotic stress levels on soluble sugar a (mg g^{-1} d. m.) and soluble protein b (mg g^{-1} d. m.) in shoot and root of cv. Sakha 94 and cv. Giza 168 grown for 21 days..

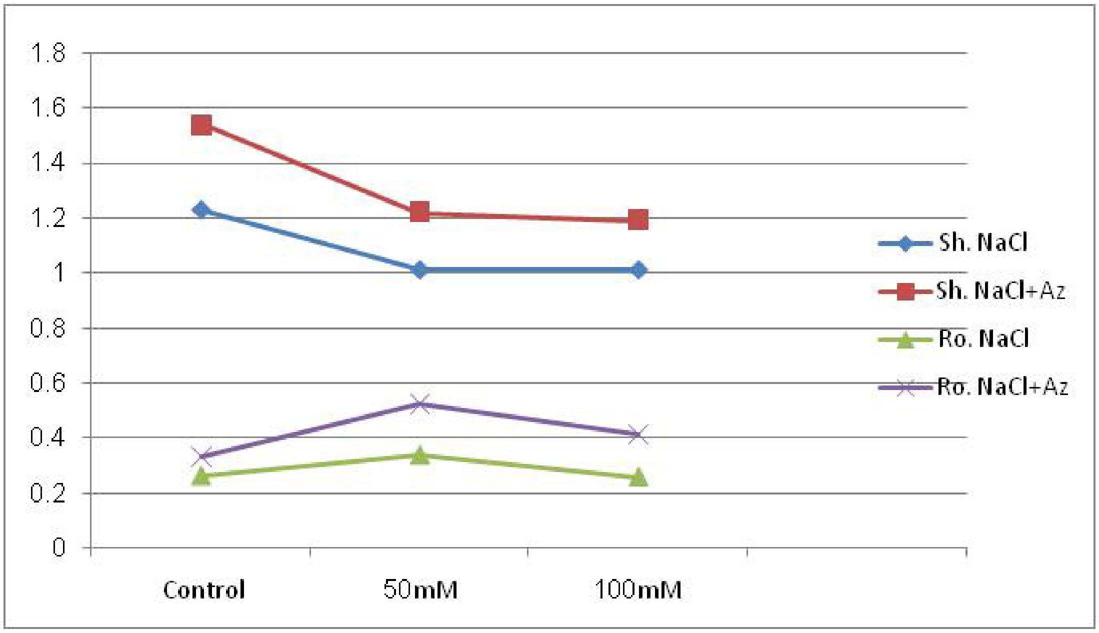
tested cultivars, the reduction was similar in the two cultivars (Tab. 3 and 4). *Azospirillum* inoculation was significantly increased K^+, Ca^{++} and Mg^{++} with increasing osmotic stress as compared with either salinization or corresponding level in shoot and root of cv. Sakha 94. Except of this trend shoot K^+ tended to decrease with raising salinity. In cv. Giza168, there was a similar trend between shoot and root in the accumulation of K^+ and Ca^{++}, increased with increasing osmotic stress (Tab. 3 and 4). It is a surprising situation that *Azospirillum* application was significantly and progressively increased in Mg^{++} content of both organs of the two cultivars. This increase

Table 3. Effect of different osmotic stress levels on mineral contents (mg g^{-1}d. m.) in shoot of cv. Sakha 94 and cv. Giza 168 grown for 21 days.

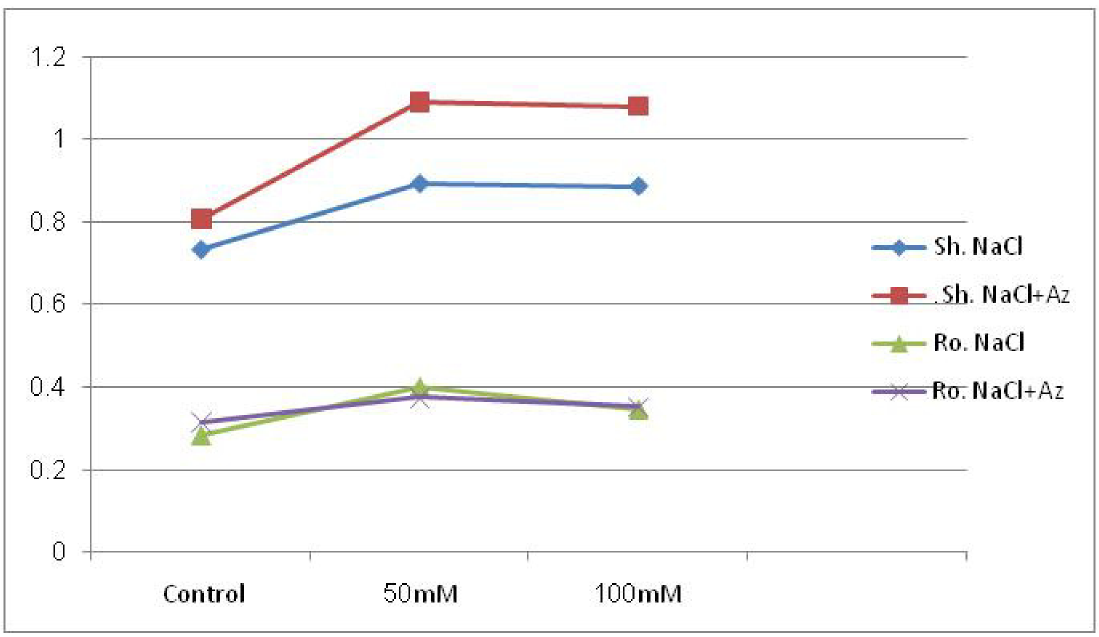
Treat. NaCl mM				Shoot				
Cv. Sakha 94	**Na^+**	**%**	**K^+**	**%**	**Ca^{++}**	**%**	**Mg^{++}**	**%**
Control	1.8	100	4.2	100	16.3	100	1.8	100
50 mM.	3.0	166.7	4.6	109.5	12.0	73.6	1.8	100
100 mM.	3.3	183.3	1.6	38.1	5.5	33.7	2.8	155.6
Control +*Az.*	1.0	55.6	2.1	50	12.5	76.7	9.0	500
50 mM+*Az.*	2.1	116.7	2.6	61.9	21	128.8	9.0	500
100 mM+*Az.*	2.4	133.3	1.3	30.9	22.5	138.0	5.4	300
L. S. D. 0.05%	1.1		1.2		1.1		1.4	
Cv. Giza 168								
Control	2.6	100	2.7	100	15	100	3.0	100
50 mM	2.9	115	3.1	114.8	21	140	5.1	170
100 mM	3.9	150	2.1	77.8	21	140	3.8	126.7
Control + Az.	1.5	57.7	3.4	125.9	12.5	83.3	6.3	210
50 mM+Az.	2.1	80.8	3.8	140.7	13.5	90	10.8	360
100 mM +Az.	2.1	80.8	2.9	107.4	13.5	90	10.8	360
L. S. D. 0.05%	1.0		1.3		1.0		1.2	

Table 4. Effect of different osmotic stress levels on mineral contents (mg g^{-1}d.m.) in root of cv. Sakha 94 and cv. Giza 168 grown for 21 days.

Treat. NaCl mM				Root				
Cv. Sakha 94	**Na^+**	**%**	**K^+**	**%**	**Ca^{++}**	**%**	**Mg^{++}**	**%**
Control	1.5	100	3.3	100	15.0	100	1.8	100
50 mM	2.1	140	1.0	30.3	19.5	130	4.5	250
100 mM	2.1	140	0.78	23.6	18.0	120	2.7	150
Control+*Az.*	1.2	80	4.3	130.3	15.0	100	3.4	188.9
50 mM+*Az.*	1.1	73.3	4.5	136.4	18.0	120	5.4	300
100 mM +*Az.*	1.9	126.7	1.0	30.3	15.0	100	10.8	600
L. S. D. 0.05%	1.3		1.3		1.2		1.0	
Cv. Giza 168								
Control	2.1	100	1.0	100	7.8	100	2.9	100
50 mM	2.2	104.8	2.1	210	21	269.2	3.6	124.1
100 mM	2.4	114.3	2.1	210	16.5	211.5	3.7	127.6
Control+*Az.*	1.1	52.4	1.2	120	14.4	184.6	5.1	175.9
50 mM+*Az.*	1.18	56.2	2.1	210	28.2	361.5	5.8	200
100 mM+*Az.*	1.2	57.1	2.9	290	18.0	230.8	3.6	124.1
L. S. D. 0.05%	0.9		1.0		1.4		0.8	



(a)



(b)

Fig. 2. Effect of different osmotic stress levels on osmotic pressure (mOsmo/ H_2O) of shoot and root of cv. Sakha 94 a and cv. Giza 168 b grown for the 21 days.

was reached 2 to 5-folds in shoot and root of Cv. Sakha 94 respectively and 2-folds in shoot cv. Giza168 compared with control plants. *Azospirillum* application induced in most cases an increase in osmotic pressure of cv. Sakha94 and cv. Giza 168 at both osmotic stress levels (Figure 2).

DISCUSSION

The present results indicated that the salt tolerant mechanisms not only varied according to different cultivars but also varied between different organs of the same plant. Additionally, this effect seems to be more expressed in shoot than in root of both tested cultivars. It can be recorded that cv. Giza 168 was the most tolerant cultivar and cv. Sakha 94 was the sensitive one during this stage of plant development. The sensitivity in cv. Sakha94 was paralleled with the reduction in osmotic pressure of the cell sap which was inhibited in the accumulation of soluble sugar and soluble protein in shoot and root of this cultivar. This reduction was reflected on the lowering of water uptake that lead to a reduction in fresh and dry matter, length in shoot and root of cv. Sakha 94 plant. Also, the reduction of K^+ and Ca^{++} content in shoot and K^+ in root may play a role in this strategy. On the other side the tolerant of cv. Giza 168 was coincided with the increase of osmotic pressure especially at lower salinity levels. This tolerant strategy was related with the increase of shoot soluble sugar and soluble protein in both organs which increased the ability of cv. Giza 168 to uptake more water content (Zahra et al., 2010; Zhao et al. 2010 and Rabie and Almadini, 2014). The enhancement uptake of water increase photosynthetic activity which confirm in the production of fresh and dry matter in this cultivar. Enhancement of organic and inorganic solutes can be used as a suitable trait to discriminate genotypes for salt tolerance and osmotic stresses. Mohammed (2007) reported that it is important to know how the sink source relationships are affected in plant growth under salt stress conditions, because the efficient use of assimilate may be a limiting factor to plant growth under salinity. The relative ability of the plant or plant organ to stimulate the accumulation of cytosol substances in its tissues (osmotic adjustment) will partially determine its tolerance to stress conditions (Kukreja et al., 2005). The marked increase in soluble sugars as well as soluble protein and tissue water contents in shoots might indicated the superiority of shoots and over roots to alleviate the imposed salt stress, either via osmotic adjustment as in cv. Giza 168 (D'Onofrio and Lindbeg, 2009; Hamdia, 2013) or by conferring desiccation resistance to plant cells as in Cv. Sakha 94 (Hamdia, 2016 and Hamdia and Shaddad 2014 and 2016). Hamdia et al. (2017) study the response of wheat plants to different osmotic stress levels varied among the different organs root, shoot and spike and the situation of these organs with application of two Cu^{++} levels 5 mM and 25 mM as $CuSO_4$. The sensitivity of root organ was related with reduction in fresh, dry matter and length. This resulted from reduction of soluble sugar reflected a reduction in water uptake and K^+ content of the cell sap. In the moderate organ spike, the reduction in fresh, dry matter and length were concomitant with the accumulation of soluble sugar and a huge accumulation of soluble protein. In the higher organ shoot, this related with more water uptake which in turn induced an accumulation of soluble protein and cofactor K^+ content. It can be recorded that shoot was higher Na^+ accumulation than root and spike. It is worthy to note that the differential distribution of Na^+ between two cultivars and between the shoot and root of the same cultivar may explain the tolerance strategy mechanism of two tested cultivars. The increase translocation of Na^+ from root to shoot is similar between the two cultivars leads to the inhibition in fresh and dry matter in shoot more than in root. While the accumulation of Na^+ in higher values in salt sensitive cv. Sakha 94 more than salt tolerant cv. Giza 168 support the previous view. Our results distinguished another point that the increase of Na^+in shoot cv. Sakha 94 was related with reduction uptake of $K^+$and Ca^{++} while in root this reduction was related with the decrease in K^+ only lead to inhibition of growth parameter in shoot more than in root. In cv. Giza 168 the increase in Na^+ was parallel with increase in K^+, Ca^{++} and Mg^{++} in shoot and root which induced an increase in OP and finally enhanced growth parameter of this cultivar.

It is important to note that the low levels (50 mM) osmotic stress induced a higher values of K^+, Ca^{++} in shoot and Mg^{++} in root of cv. Sakha 94. Whearas in cv. Giza 168, higher vaues was recorded in shoot and root Ca^{++} and root Mg^{++}.This is a good criteria reflected on the salt tolerance of cv. Giza 168 (Tammam et al. 2008 and Hamdia and Shaddad, 2016) and Wilson et al. (2000) suggested that K^+ might be recirculates from the leaves to the roots along with organic anions. After eventual metabolism of the organic acids in the roots, K^+ is available for renewed xylem transport to the shoot. It is well detected that the increase of sodium in shoot and root of both cultivars represented a sign for salt sensitivity and tolerance. This activation was more detected in cv. Sakha 94 than cv. Giza 168 and in shoot than in root. This correlated with reduction of fresh and dry response matter was in shoot more than in root. This suggested that salt-sensitive cultivars accumulate more Na^+ than salt tolerant for rice Lutts et al. (1996) and Hamdia and Azooz (2002) for maize and Tammam et al. (2008) for wheat Hamdia et al. (2004) and Hamdia and Barakat (2013), working with wheat cultivars and broad bean, respectively found that K^+/Na^+ ratio was high in salt tolerant than sensitive cultivars and recommended it as a suitable selection criterion for salt tolerance. Al- Alfocea et al. (1993) and Hamdia et al. (2004) reported that K^+ nutrition is not affected by excessive Na^+ in salt tolerant tomato and wheat plants respectively. This situation was interpreted by Garacia et al. (1997) who reported that in rice there was no correlation between K^+ and Na^+ transport and concluded that the genes affecting Na^+ uptake had not apparently related with those involved in K^+ uptake. However, this situation contrasts with that in triticeae (Hamdia, 2013& 2016). Antagonistic relations between Na^+ and K^+ or negative effects of salinity on K^+ uptake in different plants were recorded by other authors (Hamdia and Shaddad, 2014; 2016). The mechanisms of ion distribution increased the osmotic pressure of the shoot which facilitates the steepness of osmoregulation towards the aerial parts which in turn increases the water flow from the root to the shoots which in turn maintained the water status (the conservation and utilization). Also, when treated both cultivars with *Azospirillum* inoculation under osmotic stress respond passively to this treatment. This effect was more prominent in salt tolerance cv. Giza 168 than in salt sensitive Sakha 94. This was detected in the accumulation of soluble sugar and soluble protein, shoot and root K^+ and root Ca^{++}, which reflected on the increase of growth parameters production (fresh, dry matter, length and water content). Finally it can be said that the tolerance of cv. Giza 168 enabled the cultivar to overcome the saline injury and respond more positively than salt sensitive cv. Sakha 94 to *Azospirillum* inoculation. Supporting this view Hamdia and El-Komy, (1997) and Hamdia *et al*. (2004) showed that the *Azospirillum* inoculation in maize at NaCl concentrations up to –1.2 MPa significantly increased chlorophyll, K^+, Ca^{++}, soluble saccharides and protein contents compared with control maize growing without NaCl. Similarly, under high NaCl concentration inoculation of wheat with *A. lipoferum* reduced some of the deleterious effects of NaCl (Bacilio et al., 2004). Finally, *Azospirillum*-inoculated lettuce seeds had better germination and vegetative growth than no inoculated controls after being exposed to NaCl (Barassi *et al*., 2006; Bashan et al., 2004: Bashan 2010, Kang *et al*. 2014, Abd-Alla *et al*., 2014 and Hassen *et al*., 2016). Hamdia (2017) stated that *Azospirillum brasilense* inoculation increased the accumulation of soluble sugar and soluble protein which reflected an increase in the production of fresh, dry matter and water content with increasing values of osmotic pressure of the tested plants under Cu^{++} treatment. Finally, wheat plants differentially to Cu^{++} treatment according to its organ and *Azospirillum brasilense* treatment improved wheat plant efficiency to tolerate the effect of Cu^{++} stress.

CONCLUSION

The present work was conducting to study the response strategy of salt tolerance of two wheat cultivars cv. Sakha 94 and cv. Giza 168 and the biotic role of fertilizer *Azospirillum* inoculation in improvement osmotic defense system of these cultivars. It can be observed that cv. Giza 168 was the most tolerant one and cv. Sakha 94 was the sensitive one. This strategy reflected on growth and metabolities, mineral and osmotic pressure of the tested two cultivars and on their response to natural fertilizers *Azospirillum brasilense* applications.

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