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Full Length Research Paper

Efficacy of seawater salinity on osmotic adjustment and solutes allocation in wheat *(Triticum aestivum)* flag leaf during grain filling

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Two wheat (*Triticum aestivum* L.) cultivars (salt sensitive cultivar, Gemmieza-9 and salt resistant cultivar, Sids-1) subjected to different seawater salinity (10 and 25%). Osmotic pressure (OP), osmotic adjustment (OA) and solutes accumulation (TSS, TSN, proline, organic acids, glycerol and inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) were quantified in flag leaf during grain-filling (14 and 21 days post-anthesis). Seawater salinity induced significant increase in osmotic pressure and the magnitude of increase was higher in Sids-1 than in Gemmieza-9. Furthermore, seawater concentrations caused noticeable increase in osmotic adjustment, organic solutes (TSS, TSN, proline, organic acids and glycerol) and inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-). On the other hand, clear reduction in K^+/ Na^+ ratio in the flag leaves of both cultivars was observed. The capacity of osmotic adjustment was greater in younger leaves than in older ones particularly with higher concentration (25%) in both cultivars. Moreover, the production of both organic and inorganic ions tended to be higher in Sids-1 than in Gemmieza-9. Gemmieza-9 appeared to be more sensitive than Sids-1.Osmotic pressure of flag leaf sap appeared to depend mainly on proline, TSN, TSS, organic acids, glycerol and ions content, where there is a positive correlation between osmotic pressure and all of them.

Key words: Wheat, seawater, osmotic adjustment, compatible solutes, glycerol.

INTRODUCTION

Soil and water salinity have been considered a limiting factor to crop production in arid and semiarid regions of the world (Denden et al., 2005). Salinity effects on plants include two distinct types of stress: water stress, caused by the greater difficulty of water absorption, and ionic stress, related to the sodium ion effect on the diverse cellular functions, decreased nutrient absorption, enzyme activities, photosynthesis and metabolism (Zhu, 2001; Hajlaouia et al., 2006).

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Abbreviations: OA, Osmotic adjustment; **d,** days post-anthesis; **LSD,** least significant difference; **R,** resistant; **S,** sensitive; **SW,** seawater; **TSN,** total soluble nitrogen; **TSS,** total soluble sugar

Plants have developed various combating mechanisms to survive with the deleterious effects of salt stress. Among these, osmotic adjustment (OA) is one of the strategies that have been a potential defense toward salt stress (Hajlaouia et al., 2010). This phenomenon is considered to be an important component of salinity tolerance mechanisms in plants (Neocleous and Vasilakakis, 2007) and also necessary to maintain water uptake from a saline soil (Ottow et al., 2005). Hence, OA allows water uptake, cell enlargement and plant growth during water stress associated with partial stomata opening allowing the CO_2 assimilation at low water potentials that are otherwise inhibitory (Alves and Setter, 2004).

According to Blum et al. (1996), OA is usually defined as a decrease in cell sap osmotic potential resulting from a net increase in intracellular solutes rather than from a loss of cell water. The former may operate through the

concentration accretion of inorganic and/or organic solutes (Ben Khaled et al., 2003). As a consequence, the cell's osmotic potential is diminished which in turn attracts water into the cell by tending to maintain turgor pressure (Pérez-Pérez et al., 2009). Furthermore, Munns (2005), compatible solutes like sugars, amino acids, organic acids and inorganic ions can contribute to this process as well as glycerol (Mart nez et al., 2004, 2005) and fatty acids. Plants with increased concentrations of these compounds are expected to display increased salt tolerance (Taji et al., 2002).

The degree of OA also could be affected by the rate of stress intensity and most particularly by organ type and plant age (Alves and Setter, 2004). The occurrence of active OA can be established only if a net increase of solute concentration occurs (Silveira et al., 2009). Moreover, Ouda (2006) reported that many plants are able to partially compensate for low osmotic potential of the soil water by building up higher internal solute contents under saline conditions.

Sugars contributed up to 50% of the total osmotic potential in glycophytes subjected to saline conditions (Ashraf and Harris, 2004). Sugars accumulation in salinity stressed plants prevents structural and functional changes of membranes and destruction of soluble proteins. In accordance with these findings, Hichem et al. (2009) stated that, stressing two maize cultivars with various levels of salinity resulted in marked accumulation of sugars in roots and leaves of such stressed plants. Also, the accumulation of total soluble nitrogen (TSN) is another important adaptive response of plants to the lack of water and salt stress. Nitrogen-containing compounds are well documented to be involved as main osmolytes contributing in the plant osmotic regulation under salt stress. In this respect, Hassan et al. (2004) found that salinity stress increased TSN in fenugreek, mungbean and tomato plants.

Proline is one of the key osmolytes contributing toward osmotic adjustment (Soudry et al., 2005; Iqbal et al., 2008). Salinity increased markedly the proline content in different salt sensitive and tolerant species/cultivars with greater proline accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity (Ashraf and Harris, 2004; Mansour et al., 2005). In connection, salt stress remarkably enhanced proline accumulation in leaves of two rice cultivars differing in salinity tolerance and the rate of increase was more in the tolerant one, which implies the involvement of proline accumulation in osmotic adjustment during salinity (Demiral and Türkan, 2006). Srivastav et al. (2010) noticed that salt stress resulted in significant increase in proline content of mango leaves.

The accumulation of glycerol is another important adaptive response of plants and other organisms to the lack of water and saline stress. Glycerol has been shown to be a compatible solute for cells exposed to lowered water potential (Managbanag and Torzilli, 2002). In this connection, Torzilli (1997) found that, the salt stress increases the cellular concentrations of glycerol in *Aureobasidium pullulans*.

Accumulation of inorganic solutes, such as cations (Na^+ and K^+) and the anion (Cl^-), can also play a role independently or in combination with other mechanisms in maintaining the osmotic imbalance caused by the salt stress and influence the osmotic potential adjustment of plant cells (Bayuelo-Jimenez et al., 2003; Peng et al., 2004). Since the cytosolic and organellar machinery of glycophytes is equivalently Na^+ and Cl^- sensitive, osmotic adjustment is achieved in these compartments by the accumulation of compatible osmolytes and osmo-protectants "which are small electrically neutral molecules that are nontoxic at molar concentrations and stabilize proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes˝ (Munns, 2002).

The present study was undertaken to examine the effect of seawater salinity on osmotic adjustment and solutes allocation in wheat flag leaf during grain filling.

MATERIALS AND METHODS

Plant material and growth conditions

Pure strains of *Triticum aestivum* L. Gemmieza-9 (salt sensitive cultivar) and Sids-1 (salt resistant cultivar) were kindly supported by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. For soaking experiment, a homogenous lot of *Triticum aestivum* L. (either sensitive or resistant cultivar) grains were selected. The grains were separately surface sterilized by soaking in 0.01 M $HgCl_2$ solution for 3 min, then washed thoroughly with distilled water. The sterilized grains from each cultivar were drilled in plastic pots (25 cm in diameter) filled with 7 kg soil (clay: sand 2/1, v/v), where 15 grains were sown in each pot. The pots were then kept in a greenhouse at Botany Department, Faculty of Science, Mansoura University, Egypt. The plants were subjected to natural day/night conditions (min./max. air temperature and relative humidity were 15/25°C and 35/45%, respectively) at mid-day during the experimental period. The plants were irrigated to field capacity by tap water. After two weeks from sowing, thinning was started so that five uniform seedlings were left in each pot for the subsequent studies. The plants from each cultivar were divided into three sets. The 1st set was still irrigated with normal tap water serving as control, whereas the 2nd or 3rd ones were irrigated with 10 and 25% seawater receptively. Irrigation with seawater was applied after 30 days from sowing with a periodical soil washing (each two weeks) with tap water. After thinning and at heading, the plants received 36 kg N ha^{-1} as urea and 25 kg P ha^{-1} as superphosphate.

The chemical analyses of the employed seawater, collected from the Mediterranean Sea, revealed that it contains Cl^-, 21.6 Kg m^{-3}; Na^+,11.1 Kg m^{-3}; SO_4^{-2}, 2.85 Kg m^{-3}; K^+, 0.49 Kg m^{-3} and P^{+3} 16.6 µg dm^{-3}. Its salinity was found to be 38.5 g kg^{-1}; pH, 8.1 and EC, 47 mmhos cm^{-1} (Aldesuquy and Baka, 1998). Samples were taken for measurements of osmotic pressure and other osmolytes during grain-filling {(14 and 21days post-anthesis) (that is, 99 and106 days after sowing)}.

Measurement of osmotic pressure (OP) and osmotic adjustment (OA)

The osmotic pressure of flag leaf sap was measured by the

cryoscopic method (Walter, 1949) and described by El-Sharkawi and Abdel-Rahman (1974). Osmotic adjustment (OA) was calculated as the difference in OP between salinized and control plants (Martinez-Ballesta et al., 2004)

Determination of total soluble sugars

Total soluble sugars was extracted and determined by anthrone method of Riazi et al. (1985) as modified by Ibrahim (1999).

Determination of the total soluble nitrogen

The total soluble nitrogen was determined by the conventional semi micro-modification of Kjeldahl method (Pine, 1955).

Estimation of proline

The method adopted for estimation of proline was essentially that described by Snell and Snell (1954).

Determination of keto acids

Keto acids were determined according to the method adopted by Friedman and Haugen (1943).

Determination of citric acid

The method adapted for estimation of citric acid was essentially that described by Snell and Snell (1949).

Estimation of glycerol

Glycerol was estimated according to the method described and adopted by Mansour (1972).

Determination of some mineral ions

The extracts of the experimental plants were analyzed for the cations: Na^+, K^+ and Ca^{+2} Mg^{+2} measured by flam emission spectrophotometery according to the method described by Chapman and Pratt (1978) and the anions Cl- chlorides were determined by the $AgNO_3$ titration method as described by Hansen and Munns (1988) .

The sodium adsorption ratio (SAR) and potassium adsorption ratio (PAR) were calculated according to McKell and Goodin (1984) as:

$$SAR = Na+ \{(Ca^{++} + Mg^{++})/2\}1/2$$

$$PAR = K+ \{(Ca^{++} + Mg^{++})/2\}1/2$$

Where, Na^+, K^+, Ca^{++} and Mg^{++} refer to the concentrations of the designated cations.

Statistical analysis

A test for significant differences between means at $P \leq 0.05$ was performed using least significant difference (LSD) test (Snedecor and Cochran, 1976). The correlation coefficients were estimated according to SPSS programme.

RESULTS

Changes in osmotic pressure (OP) and osmotic adjustment (OA)

Seawater stress induced a sharp increase ($P \leq 0.05$) in OP and OA in flag leaf of both cultivars during grain filing as compared to control values (Figure 1). The resistant plants showed higher OP and OA values than the sensitive one under stress conditions. The difference, in terms of OP and OA, between cultivars was more conspicuous especially at higher seawater concentration (25%) where, the young leaves showed to have the maximum OA comparably to older ones particularly (25%). Generally, the Sids-1 cultivar exhibited more osmotic potential and osmotic adjustment.

Changes in total soluble sugar (TSS)

The pattern of results in Figure 2 showed that, there is a noticeable increase in TSS in control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. In relation to wheat cultivar, the resistant one had lower TSS values than the sensitive one. Seawater salinity induced a marked increase ($P \leq 0.05$) in TSS in flag leaf of both cultivars during grain filling as compared to control values. It is clear that the resistant plants accumulated more TSS than the sensitive one under seawater-stress.

Changes in total soluble nitrogen (TSN)

In relation to control values, seawater-stress induced a noticeable increase ($P \leq 0.05$) in TSN from the younger leaves to the older leaves of both cultivars during anthesis stage, but the resistant plants accumulated more TSN than the sensitive one (Figure 3).

Changes in proline

As compared to the control values, salinity stress caused apparent increase ($P \leq 0.05$) in proline concentration in flag leaf of both cultivars during grain filling (Figure 4). Comparing both cultivars, under saline conditions, Sids-1 had more proline level than Gemmieza-9.

Changes in organic acids

Changes in keto-acids

Perusal of the data shown in Figure 5 showed that, there is a marked decrease in keto-acids in control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. In relation to control

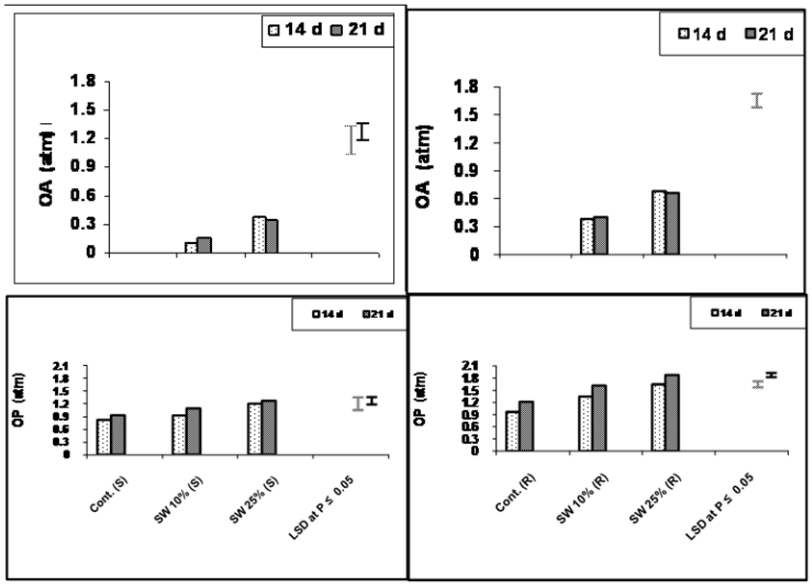

Figure 1. Effect of different concentrations of seawater on OP and OA (atm) in flag leaf extract of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

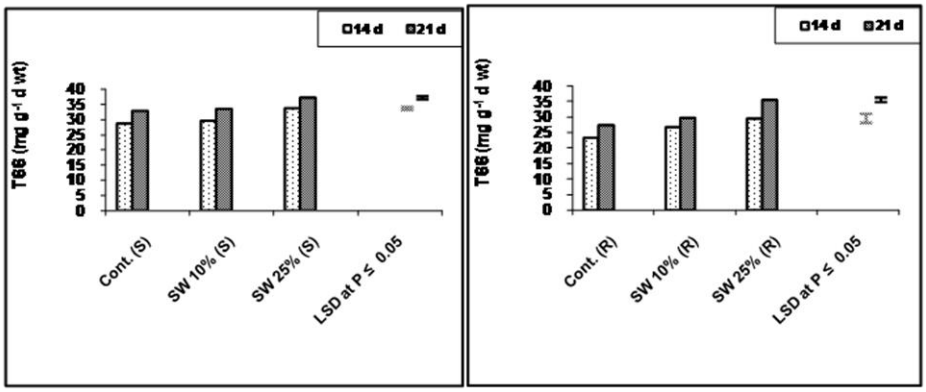

Figure 2. Effect of different concentrations of seawater on TSS (mg g^{-1}d wt) in flag leaf extract of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

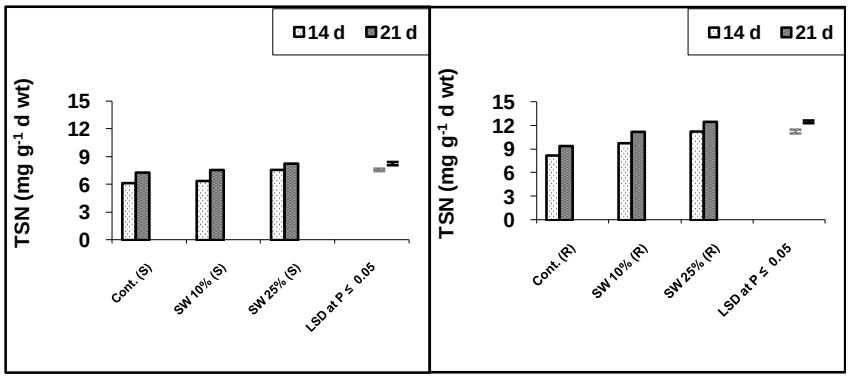

Figure 3. Effect of different concentrations of seawater on TSN (mg g^{-1}d wt) in flag leaf extract of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

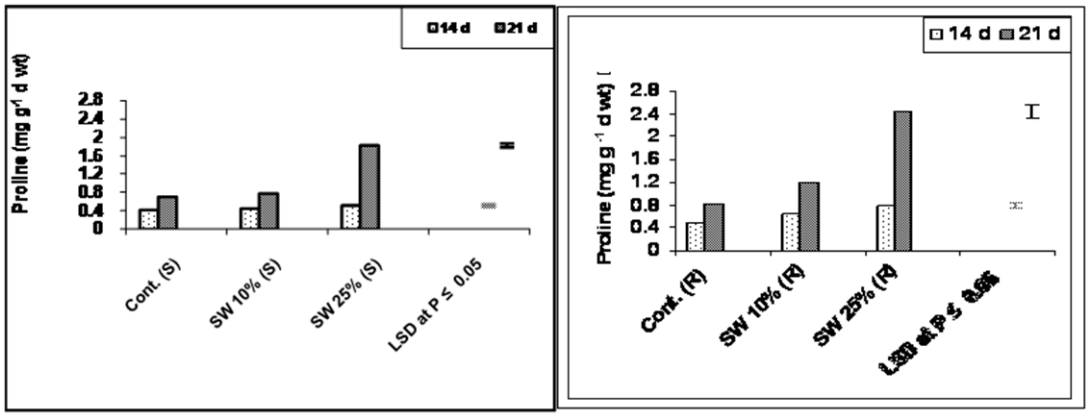

Figure 4. Effect of different concentrations of seawater proline (mg g^{-1}d wt) in flag leaf extract of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

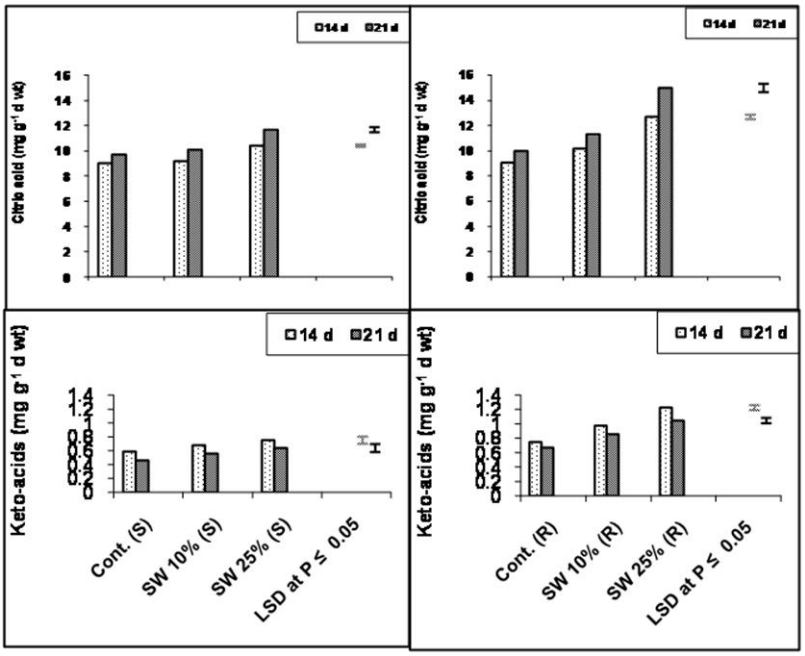

Figure 5. Effect of different concentrations of seawater on keto acids and citric acid (mg g^{-1} d wt) in the extract of the flag leaf of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

values, keto-acids accumulation in response to seawater-stress was more pronounced (P ≤ 0.05) in flag leaf of both cultivars during grain filling where the resistant plants accumulated more keto-acids than the sensitive ones (Figure 5).

Changes in citric acid

There is a tendency among control and seawater-stressed plants to a progressive increase in citric acid from 14 to 21 days post-anthesis in both wheat cultivars. Seawater at all examined concentrations caused a marked increase (P ≤ 0.05) in citric acid in flag leaf of both cultivars at 14 and 21 days post-anthesis in comparing with control plants (Figure 5). Moreover, the resistant plants accumulated more citric acid than the sensitive one (Figure 5).

Changes in glycerol

The pattern of results in Figure 6 showed that, there is a noticeable increase in glycerol in control and seawater-

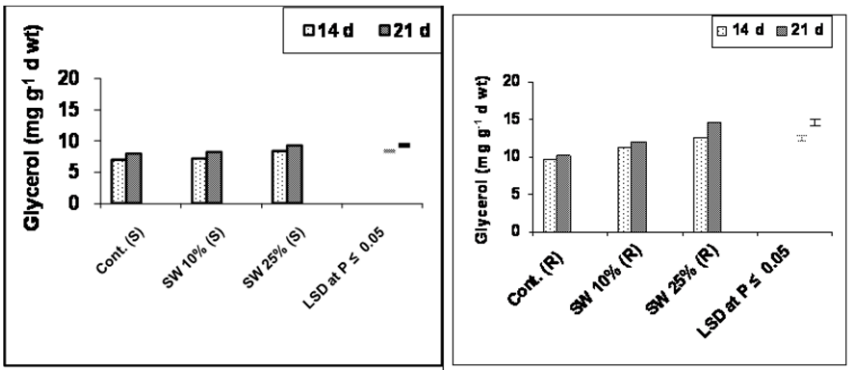

Figure 6. Effect of different concentrations of seawater on glycerol (mg g^{-1} d wt) in the extract of the flag leaf of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. In relation to control, seawater irrigation stimulated a progressive increase (P ≤ 0.05) in glycerol content in flag leaf of both cultivars during grain filling. The resistant cultivar accumulated more glycerol than the sensitive one under salt-stress. Almost, the increase was non-significantly at 10% in case of Gemmieza-9.

Changes in ionic content

Perusal of the data shown in Figures 7 and 8 cleared that, there is a tendency among control and seawater-stressed plants to a progressive increase in ionic contents from 14 to 21 days post-anthesis in both wheat cultivars. In relation to control values, the two examined concentrations of seawater induced significant increase (P ≤ 0.05) in ions content (Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-) as well as Na^+ adsorption ratio and K^+ adsorption ratio and induced a noticeable decrease in K^+/ Na^+ ratio in flag leaf of both cultivars during anthesis stages (Figures 7 and 8).

In the majority of cases, the applied 10% dose of seawater revealed a non-significant increase on all the previous parameters (OP, TSS, TSN, keto-acids, citric acid, K^+, Ca^{2+}, Mg^{2+}, Na^+ adsorption ratio and K^+ adsorption ratio) (in case of Gemmieza-9 in both stage except for Na^+ and Cl^- ions, the increment in them was significantly in Gemmieza-9 and non-significantly in Sids-1. Hence, Gemmieza-9 depicted more susceptibility to salt toxicity in comparison to Sids-1 under saline conditions.

DISCUSSION

Several reports are available that showed different strategies adopted to counteract the salinity effects (Ashraf, 2009; Türkan and Demiral, 2009) but the information on mineral nutrient status of plants and salinity tolerance is scarce (Khan et al., 2009b, 2010; Khorshidi et al., 2009). Plants have developed various combating mechanisms to survive with the deleterious effects of salt stress. Among these, osmotic adjustment is one of the strategies that have been a potential defense toward seawater-salinity.

In the present study, seawater at the two used concentrations induced noticeable increase in osmotic pressure, some organic solutes (i.e. TSS, TSN, proline, organic acids and glycerol) and inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) content as well as Na^+ adsorption ratio and K^+ adsorption ratio while induced a noticeable decrease in K^+/ Na^+ ratio in water extract of the flag leaf of both cultivars. Sids-1 accumulated more osmolytes than Gemmieza-9 (Figures 1-8). This incresease in osmotic potential might be due to the increase in organic acids, TSS, TSN and inorganic osmolytes (i.e. Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-). Furthermore, osmotic pressure appeared to be positively correlated with organic osmolytes [TSS (r = 0.99, 0.94); (r = 1.00, 0.93), TSN (r = 0.99, 0.97); (r = 0.99, 1.00), proline (r = 0.99, 0.90); (r = 0.99, 0.91), keto-acids (r = 0.94, 0.99); (r = 0.99, 0.99), citric acid (r = 0.98, 0.95); (r = 0.96, 0.92) and glycerol (r = 0.99, 0.95); (r= 0.99, 0.97)] as well as inorganic ions [(Na+ (r =1.00, 0.96); (r = 0.98, 0.87), K+ (r = 1.00, 0.91); (r = 0.96, 0.92), Ca2+ (r = 0.99, 0.93); (r = 0.94, 0.95), Mg^{+2} (r = 0.99, 0.93); (r = 0.99, 0.96), Cl- (r = 0.98, 1.00); (r = 0.98, 1.00)] for both Gemmieza-9 and Sids-1 during grain filling respectively (Table 1. In accordance to these results, Kusaka et al. (2005) reported that the observed increase in the osmotic potential of salt-stressed plants might be due to the accumulation of inorganic solutes(e.g., K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO^{3-}, SO_4^{2-} and HPO^{4-}), several organic components such as sucrose, glucose, quaternary ammonium compounds and amino acids.

Osmotic adjustment is a mechanism used for maintaining turgor and reducing the deleterious effects of salt stress on vegetative and reproductive tissue. Thus, salinity stress induced a marked increase in osmotic potential and osmotic adjustment of wheat flag leaf of both cultivars (Figure 1).

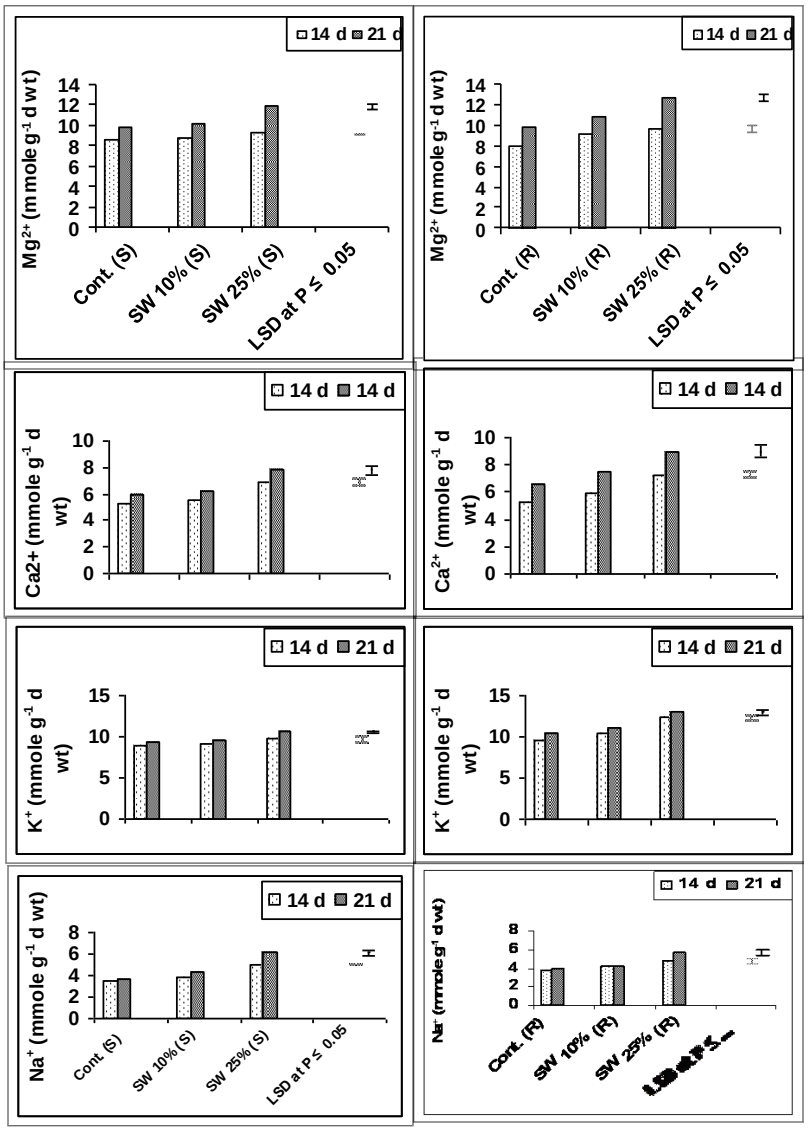

Figure 7. Effect of different concentrations of seawater on Na^+, K^+, Ca^{2+} and Mg^{2+} (mmol g^{-1} d wt) in flag leaf extract of wheat cultivars during grain-filling (14 and 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

These results were in agreement with those obtained by Ottow et al. (2005) who cleared that, by doubling NaCl concentration in the nutrient solution of *Populus euphratica* plants, these plants were able to adjust the osmotic pressure of leaves to levels just exceeding those of the nutrient solution, which is important to maintain water uptake and to prevent dehydration.

In comparing with control plants, soil drench with seawater at the two examined concentrations caused marked increase in TSS in flag leaf of both cultivars during grain filling with marked accumulation in Sids-1 comparing to Gemmieza-9 (Figure 2). In support, Hassanein et al. (2009) reported that various doses of NaCl, induced significant increases in soluble sugars in shoots of maize plants. Currently, sugar accumulation in plants in response to seawater stress, is also quite well documented and confirming previous results reporting that soluble sugars are the main contributors to osmotic adjustment in stressed tissues (Xuana and Catherine, 2009; Gorai et al., 2010). In this respect, soluble carbohydrates (glucose, fructose, sucrose, fructans) accumulated under salt stress play a leading role in osmoprotection, osmotic adjustment, carbon storage and radical scavenging (Parida et al., 2002) and can be used as an indicator of salt tolerance (Juan et al., 2005; Almodares et al., 2008). Furthermore, Bartels and Sunkar (2005) suggested that in cotton leaves sugars act for osmotic adjustment and/or protect specific macromolecules and contribute to the stabilization of membrane structures, where sugars are thought to

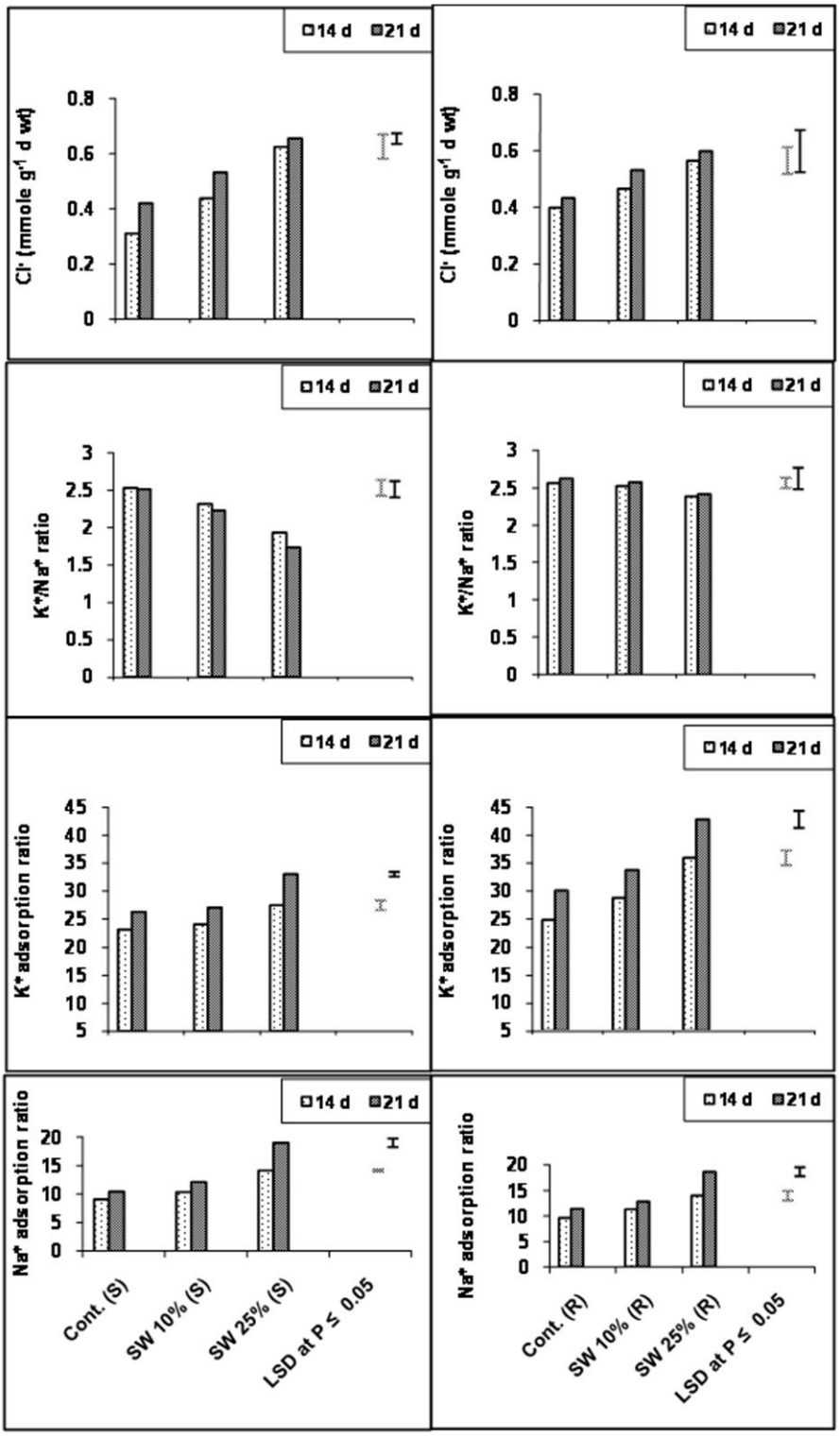

Figure 8. Effect of different concentrations of seawater on Cl- (mmol g-1 dwt), K+/Na+ ratio, Na+ adsorption ratio and K+ adsorption ratio in flag leaf extract of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at P ≤ 0.05.

interact with polar head groups of phospholipids in membranes so that membrane fusion is prevented.

The nitrogen contents of plants growing under salt stress show great diversity (Mahmood et al., 2008).

Table 1. Correlation coefficient (r) between osmotic pressure, some osmolytes and ions of the two seawater-stressed wheat cultivars during grain-filling (14 & 21 days post-anthesis).

Sensitive			Resistant		
Variable	r		Variable	r	
	14 d	21 d		14 d	21 d
Na^+	1.00	0.96	Na^+	0.98	0.87
K^+	1.00	0.91	K^+	0.96	0.92
Ca^{2+}	0.99	0.93	Ca^{2+}	0.94	0.95
Mg^{2+}	0.99	0.93	Mg^{2+}	0.99	0.96
Cl^-	0.98	1.00	Cl^-	0.98	1.00
K^+ / Na^+ ratio	-0.99	-0.99	K^+ / Na^+ ratio	-0.92	-0.91
Na^+ adsorption ratio	1.00	0.95	Na^+ adsorption ratio	0.98	0.89
K^+ adsorption ratio	0.99	0.92	K^+ adsorption ratio	0.97	0.93
TSS	0.99	0.94	TSS	1.00	0.93
TSN	0.99	0.97	TSN	0.99	1.00
Proline	0.99	0.90	Proline	0.99	0.91
Keto-acids	0.94	0.99	Keto-acids	0.99	0.99
Citric acid	0.98	0.95	Citric acid	0.96	0.92
Glycerol	0.99	0.95	Glycerol	0.99	0.97

Wheat plants irrigated with seawater had higher TSN content in their flag leaf extract as compared to unstressed plants. Sids-1 was able to keep out its nitrogen content under seawater-stress at higher levels than Gemmieza-9, in order to increase their ability to withstand salinity stress (Figure 3). These results were in accordance with those of (Ehlting et al., 2007; Koyro et al., 2008) on Grey poplar and *Chenopodium quinoa* respectively.

The accumulation in TSN may result from a sharp increase in total free amino acids and total soluble proteins (Ibrahim, 2004). Likewise, this change in nitrogen content may be related to the inhibition of translocation from root to shoot, inhibition of protein synthesis or the increase in protease activity (Khalil and Mandurah, 1990). Moreover, this increase in the soluble nitrogen compounds is of important in plant osmoregulation in response to salinity stress.

The pattern of proline accumulation in the flag leaves of the two wheat cultivars showed gradual increase with increasing salinity level however Sids-1 had higher proline accumulation than Gemmieza-9 (Figure 4). Sids-1 is better performing than Gemmieza-9 under salinity stress. Similar observations were recorded by many workers (Pagter et al., 2009; Khan et al., 2009a; Salah et al., 2011) in different crops. Proline accumulation is one of the common characteristics in many plants exposed to salt stress (Ashraf and Harris, 2004). Therefore, proline could be used as is a good parameter for the evaluation of tolerance or sensitivity of plants to stress (Misra and Gupta, 2004; Shi and Wang, 2005). Therefore, proline accumulation may contribute to osmotic adjustment at the cellular level (Mahajan and Tuteja, 2005; Demiral and Türkan, 2006). In this respect, proline accumulation could be a protective response not only due to the its osmoprotectant role that prevents salinity-induced water deficit but also for its hydroxyl scavenger, stabilization of membrane and protein structure, a sink of carbon and nitrogen for stress recovery, and buffering cellular redox potential under stress (Matysik et al., 2002; Khedr et al., 2003; Lee et al., 2008). Also, as an alternative to direct ROS scavenging feature, proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways (Szabados and Savouré, 2009).

Organic acids content (mainly citric and keto-acids) were increased in flag leaf sap of seawater-stressed plants during grain filling with higher accumulation in Sids-1 (Figure 5). These results were in a good conformity with those obtained by many authors Shi and Sheng (2005) and Guo et al. (2009). In this respect, organic acids not only an important organic osmotic regulator but also an important negative charge contributor, playing important roles in ionic balance and pH adjustment. Additionally, they might reflect an adaptation trait of wheat plant in response to seawater irrigation. It is well known that organic acids play a major role in the salt stress adaptation of plants (Gilbert et al., 1998). The increase in citric acid content might be explained on the fact that, the role of citric acid decarboxylation to increase CO_2 concentration in mesophyll during the day, while night-time citric acid accumulation increases the buffer capacity of the vacuoles (Franco et al., 1992).

In comparing with control plants, seawater irrigation caused a marked increase in glycerol content of flag leaf of both cultivars during grain filling with marked accumulation in Sids-1 than Gemmieza-9 (Figure 6).

These results were also in line with those obtained by Ben-Amotz and Avron (1973) and Frank and Wegrnann (1974), who reported that cells tended to accumulate high concentrations of glycerol with increasing the external salinity level in order to increasing the internal cell osmotic pressure. Such increment would improve the performance of the cell at high salinity by preventing leakage of the compatible solute out of the cell and diffusion of potentially harmful ions into the cell Elenkov et al. (1996). Glycerol has been shown to be a compatible solute involves in osmotic adjustment in cells exposed to lowered water potential (Mart nez et al., 2004, 2005). Moreover, Torzilli (1997) suggests that increases in the cellular concentrations of glycerol contribute to the acquisition of stress tolerance. Also, glycerol was required to balance the osmotic potential of Na^+ and Cl^- (Flowers and Colmer, 2008). The effect of hypertonic concentration of NaCl has been attributed to the formation of glycerol as osmotic agent to facilitate the retention of cellular water (Berry and Brown, 1987) and maintain enzyme activity (Borowitzka and Brown, 1974).

Excess or deficiency of any mineral nutrient is crucial for the reason that the plant growth depends on supply of inorganic nutrients (Marschner, 1995). Although organic compounds are the major compounds of osmoregulation in plant cells during water deficit stress, inorganic ions would also contribute to the osmotic adjustment (Guo et al., 2009). In addition, synthesis and accumulation of organic solutes consume more energy than uptake of inorganic ions (Xu and Yu, 1990). Generally, the obtained results in Figures 7 and 8 showed that seawater at all examined concentrations induced marked increase in Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- contents as well as Na^+ adsorption ratio and K^+ adsorption ratio. A clear reduction in K^+/Na^+ ratio was observed in wheat flag leaf of both cultivars during grain filling. These results were in agreement with those obtained by Jin et al. (2007) who suggested that seawater salinity increased the level of inorganic cations in both root and shoot of *Aloe vera* plants. Na^+ and Cl^- contents increased due to salinity in the two wheat genotypes however genotypes Gemmieza-9 maintained the higher leaf Na^+ and Cl^- concentrations than Sids-1 (Figures 7 and 8). These results were in agreement with those obtained by Patakas et al. (2002), who observed an increase in the Na^+ and Cl^- concentrations in grapevine leaves under water stress. Moreover, Silva et al. (2010) cleared that a significant increase in both Na^+ and Cl^- concentrations in *Jatropha curcas* plants treated with 50 mM NaCl.

Na^+ in C_4 species such as wheat is related to its involvement in photosynthesis mainly in the maintenance of mesophyll chloroplast structure, mainly in relation to granal stacking (Brownell and Bielig, 1996). In addition, under saline conditions Na^+ influx across the plasma lemma to the vacuole might play a major role in permitting turgor maintenance, and this accumulation of Na^+ inside the vacuoles reduce its toxic levels in cytosol and increase the vacuolar osmotic potential with the concomitant generation of a more negative water potential that favors water uptake by the cell and better tissue water retention under high salinity levels (Tawfik et al., 2006). Additionally, the greatest accumulation of Na^+ by plants at high salt concentration may be attributed to the damage of the protoplasm of plant cells and as a result, the selective salt absorption is replaced by passive absorption which causes abnormal accumulation of salts in plant organs (Kader and Lindberg, 2005). To date, very little is known about the toxic effects of Cl^- ions in the plant cell (Natasha and Stephen, 2010). However, in the leaves of citrus plants, Cl^- ions are more toxic than Na^+ ions but responsible mechanisms are yet unknown (Storey and Walker, 1999). It is also reported that sensitivity of some crops to salinity is due to the inability to keep Na^+ and Cl^- out of transpiration streams (Gorham et al., 1990). Since Na^+ and Cl^- are toxic elements whose higher concentration disturbs the different metabolic activities, Sids-1 which successes in retaining Na^+ and Cl^- in the root was more tolerant than Gemmieza-9 which translocated maximum Na^+ and Cl^- in leaves.

Data in Figure 7 showed that in response to salinity stress, K^+ concentration increased in flag leaves of both cultivars with a higher rate in Sids-1. This is consistent with findings reported on *Medicago truncatula* and *Medicago laciniata* populations (Yousfi et al., 2010). K^+ is known to function in osmotic adjustment in the guard cell controlling the stomatal movements and thus CO_2 assimilation in photosynthesis (Degl'Innocenti et al., 2009; Cha-um et al., 2010). Similarly, changes in the potassium content may contribute substantially to osmoregulation (Shabala and Cuin, 2007) and may occur in concert with changes in sugars and amino acids (Pérez-Pérez et al., 2009).

Seawater stress caused a decrease in K^+/ Na^+ ratio in wheat flag leaves of both wheat cultivars with a higher magnitude of response in Gemmieza-9 (Figure 8). This reduction in K^+/Na^+ ratio in wheat leaves with increasing salinity level might be due to competition of Na^+ with K^+ where this competition could be at uptake level and/or transport level. Since maintenance of a high cytosolic K^+/Na^+ ratio is a key feature of plant salt tolerance, as indicated by Cuin et al. (2008) and Kronzucker et al. (2008). Additionally, intracellular K^+ and Na^+ homeostasis bears importance for the activities of many cytosolic enzymes, maintaining membrane potential and a suitable osmoticum for cell volume regulation (Shabala and Cuin, 2007; Munns and Tester, 2008). These results are supported by Grewal (2010) who observed that increasing levels of subsoil NaCl salinity had significant depressing effect on leaves K^+/ Na^+ ratio of wheat, barley, canola and chickpea.

Perusal data showed that Ca^{2+} content was increased to some extent in flag leaf extract of both wheat cultivars during grain filling with more increment in Sids-1 (Figure 7). These results were in a harmony with those obtained

by Hessini et al. (2009) who observed that, Ca^{2+} presented a significant augment at the mild stress level in *Spartina alterniflora*. The increase in Ca^{2+} level as a result of seawater irrigation may enhance the tolerance of wheat plants to salinity stress since Ca^{2+} is a non-toxic inorganic nutrient and has a function of detoxification under saline medium (Izzo et al., 2008). In this regard, Ca^{2+} enhanced salt tolerance in barley (Huang and Redman, 1995) and sorghum (Bernstein et al., 1993).

Moreover, plants respond directly and specifically to Na^+ within seconds by increasing cytosolic Ca^{2+} (Munns and Tester, 2008). In fact, Ca^{2+} is known to play an important role in processes that preserve the structural and functional integrity of plant membranes, regulate ion transport and control activities of cell wall enzymes (Rengel, 1992). Moreover, many researches showed that calcium signals decoding elements are involved in ABA-induced stomatal closure and plant adaptation to salt and other abiotic stresses and some new studies show that Ca^{2+} is dissolved in water in the apoplast and transported primarily from root to shoot through the transpiration stream (Song et al., 2008).

In conclusion, genotype Sids-1 appears to be more tolerant to seawater salinity than Gemmieza-9 since it able to maintain less amounts of Na^+ and Cl^- and higher amounts of organic solutes (TSS, TSN, proline, organic acids, glycerol and inorganic ions (K^+, Ca^{2+}, Mg^{2+} and K^+/Na^+ ratio) in their flag leaves. In addition, this study provides scarce evidence supporting the hypothesis that OA plays a preponderant role in the resistance to seawater stress in both wheat cultivars. Also, the degree of OA also affected by the rate of stress intensity. Results of this research suggest also that OA could be a part of the salt tolerance mechanisms developed by wheat and could be exploited in breeding programs for improved salt stress tolerance.

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