

ORIGINAL ARTICLE

**Wheat can acclimate to seawater by pretreatment with kinetin
and spermine through osmotic adjustment and solutes
allocation**

Heshmat S. Aldesuquy*, Zakaria A. Baka and Berdees M. Mickky

Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

*E-Mail: HS-Aldesuquy@hotmail.com

Received March 10, 2013

A key issue in salt adaptation is the osmotic adjustment, therefore, during ear emergence the effect of exogenous application of kinetin and spermine on osmotic pressure (OP) and solutes allocation (total soluble sugars, total soluble nitrogen, proline, organic acids and inorganic ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cl^-) were quantified in flag leaf of wheat plants irrigated by seawater at 25%. Seawater salinity induced significant increase in osmotic pressure. Furthermore, seawater stress induced marked increase in total soluble sugars, total soluble nitrogen, proline, organic acids, as well as Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and P^{+++} in wheat flag leaf. On the other hand, seawater decreased SPR, SAR and PAR in flag leaves of wheat plants. Grain priming with kinetin, spermine or their interaction appeared to mitigate the ill effect of seawater on wheat plants by increasing its own capability to be more tolerant against seawater salinity by inducing additional increase in osmotic pressure and the osmolytes concentrations in flag leaf during ear emergence. Moreover, the effect was more pronounced with the interaction of kinetin and spermine treatment.

Key words: Compatible solutes, kinetin, osmotic adjustment, seawater, spermine, wheat

ORIGINAL ARTICLE

Wheat can acclimate to seawater by pretreatment with kinetin and spermine through osmotic adjustment and solutes allocation

Heshmat S. Aldesuquy*, Zakaria A. Baka and Berdees M. Micky

Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

*E-Mail: HS-Aldesuquy@hotmail.com

Received March 10, 2013

A key issue in salt adaptation is the osmotic adjustment, therefore, during ear emergence the effect of exogenous application of kinetin and spermine on osmotic pressure (OP) and solutes allocation (total soluble sugars, total soluble nitrogen, proline, organic acids and inorganic ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cl^-) were quantified in flag leaf of wheat plants irrigated by seawater at 25%. Seawater salinity induced significant increase in osmotic pressure. Furthermore, seawater stress induced marked increase in total soluble sugars, total soluble nitrogen, proline, organic acids, as well as Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and P^{+++} in wheat flag leaf. On the other hand, seawater decreased SPR, SAR and PAR in flag leaves of wheat plants. Grain priming with kinetin, spermine or their interaction appeared to mitigate the ill effect of seawater on wheat plants by increasing its own capability to be more tolerant against seawater salinity by inducing additional increase in osmotic pressure and the osmolytes concentrations in flag leaf during ear emergence. Moreover, the effect was more pronounced with the interaction of kinetin and spermine treatment.

Key words: Compatible solutes, kinetin, osmotic adjustment, seawater, spermine, wheat

Abbreviations: K- kinetin; LSD- least significant difference; Spm-spermine; SW-seawater; PAR- Potassium adsorption ratio; SAR- Sodium adsorption ratio; SPR- Sodium potassium ratio.

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 as well as

seawater stress (Aldesuquy *et al.*, 2011, 2012). 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).

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), reported that compatible solutes like sugars, amino acids, organic acids and inorganic ions can contribute to this process as well as glycerol (Martnez *et al.*, 2005) and fatty acids. Plants with increased concentrations of these compounds are expected to display increased salt tolerance (Taji *et al.*, 2002). OA allows water uptake, cell enlargement and plant growth during water stress associated with partial stomata opening allowing the CO₂ assimilation at low water potentials that are otherwise inhibitory (Alves and Setter, 2004). Plants respond in many ways to salinity and at a number of levels (Munns and Tester, 2008). One of the best described metabolic responses is the increase in intracellular concentration of a range of soluble, neutral compounds that are collectively termed "compatible solutes" (Widodo *et al.*, 2009). A key issue in salt adaptation is the osmotic adjustment. Salinity decreases soil water potential and thus leads to turgor loss in non-acclimated plants. To maintain water uptake, adjustment of the osmotic potential of the cells is required (Ottow *et al.*, 2005). Typically involved in these responses are sugars, amino acids, organic acids, and inorganic ions (Munns, 2005). Plants with increased concentrations of these compounds are expected

to display increased salt tolerance (Taji *et al.*, 2002).

During osmotic adjustment, the cell tends to compartmentalize most of the absorbed ions in vacuoles and at the same time, it synthesizes and accumulates compatible solutes in the cytoplasm in order to maintain the osmotic equilibrium between these two compartments (Hasegawa *et al.*, 2000). In addition to their role in cell water relations, solutes accumulation may also help towards the maintenance of ionic homeostasis and of the C/N ratio, removal of free radicals and stabilization of macromolecules and organelles, such as proteins, protein complexes and membranes a consequence (Bray *et al.*, 2000). As to osmotic regulation exerted by salt-stressed plants, the osmotic pressure of their extract is expected to be higher than that when they are unstressed. In agreement with this concept, Aldesuquy *et al.* (2012) recorded that wheat plants exhibited higher values of osmotic pressure in their leaf water extract as a result of irrigation by seawater when compared with those plants irrigated by normal tap water. Similarly, studies on *Leptochloa fusca* plants irrigated with different doses of seawater indicated that increasing saline irrigation level generally increased the value of osmotic potential (Tawfik *et al.*, 2006).

Seed priming, being an easy, low-cost and low-risk technique, could be a possible solution for the problems associated with salinity in agricultural lands. This approach is being used under both normal and stressful environments (Iqbal and Ashraf, 2007). It was hypothesized that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA (Iqbal *et al.*, 2006). Gadallah (1999) found that kinetin application ameliorated the deleterious effects of salinity on wheat plants. It reduced Na⁺, Ca⁺⁺ and Cl⁻ accumulation and

improved K^+ uptake under salinity stress. Increased K^+/Na^+ ratio helped the plants to avoid Na^+ toxicity and enhanced shoot growth and grain yield. Kinetin also reduced membrane injury by dehydration and heat stress and improved the water status of plants. Also, treating mungbean plants irrigated with 10% seawater with kinetin resulted in an increased dry weight of seawater-stressed plants when compared with their unstressed relatives (Rabie, 2005).

Great attention is paid in the possible involvement of PAs in the defense reactions of plants to various environmental stresses (He *et al.*, 2002; Maiale *et al.*, 2004). Many reports have indicated that stress tolerance of plants is correlated with their capacity to enhance the synthesis of PAs upon encountering the stress (Kasinathan and Wingle, 2004). Which of the three PAs could play a central role in stress response of plants may depend on the plant species and the types of stress (Kasukabe *et al.*, 2004). Spermidine application to salinized nutrient solution resulted in alleviation of the salinity-induced membrane damage as well as plant growth and photosynthesis inhibition, together with an increase in endogenous PAs and proline contents as well as antioxidant enzyme activities in cucumber plants (Duana *et al.*, 2007).

The present study was undertaken to explain the role displayed by exogenous application of kinetin, spermine or their interaction on increasing a potential defense toward seawater stress through osmotic adjustment and solutes allocation in wheat flag leaf at ear emergence.

MATERIALS AND METHODS

Plant material and growth conditions

For soaking experiment, a homogenous lot of *Triticum aestivum* L. var. Sakha 93 grains were

selected. The grains were surface sterilized by soaking in 0.01 M $HgCl_2$ solution for three minutes, then washed thoroughly with distilled water. The sterilized grains were divided into four sets. Grains of the 1st set were soaked in distilled water to serve as control, while those of the 2nd, 3rd or 4th set were soaked in 0.1 mM kinetin, 0.3 mM spermine or 0.1 mM kinetin + 0.3 mM spermine; respectively, each for about 12 hours. After soaking, thoroughly washed grains were drilled in 15th November 2011 in plastic pots (20 cm in diameter) filled with 5.5 kg soil (clay/sand 2/1, v/v), where fifteen grains was 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 (minimum/maximum air temperature and relative humidity were 15/25°C and 35/45% respectively) at mid-day during the experimental period. The plants in all sets 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 of each set were sub-divided into two groups. The 1st group in each set was still irrigated with normal tap water serving as control, whereas the 2nd one was irrigated with 25% seawater. The resulting eight treatments were marked as following: 1. Cont., 2. SW, 3. Cont. K, 4. SW+K, 5. Cont. Spm, 6. SW+ Spm, 7. Cont. K+ Spm, 8. SW+ K+ Spm. Irrigation with seawater was applied after 30 days from sowing with a periodical soil washing (each two weeks) with tap water. 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 $\mu g dm^{-3}$. Its salinity was found to be 38.5 g kg^{-1} ; pH, 8.1 and EC, 47 mmhos

cm⁻¹ (Aldesuquy and Baka, 1998). After thinning and at heading, the plants received 36 kg N ha⁻¹ as urea and 25 kg P ha⁻¹ as super-phosphate. Samples from flag leaf were taken for measurements of osmotic pressure and other osmolytes at ear emergence (65d after sowing).

The osmotic pressure of flag leaf sap was measured by the cryoscopic method (Walter, 1949) and described by El-Sharkawi and Abdel-Rahman (1974).

Total soluble sugars was extracted and determined by anthrone method of Riazi *et al.* (1985).

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

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

Keto acids were determined according to the method adopted by Friedman and Haugen (1943). Furthermore, the method adapted for estimation of citric acid was essentially described by Snell and Snell (1949).

The procedure adopted for extraction of the different phosphorus compounds were essentially those described by Barker and Mapson (1964) and determined by method described by Humphries (1956).

The extracts of the experimental plants were analyzed for the cations: Na⁺, K⁺ and Ca⁺² Mg⁺² measured by flame emission spectrophotometry according to the method described by Chapman and Pratt (1978) and the anions Cl⁻ chlorides were determined by the AgNO₃ 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:

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

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

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 ≤ 0.05 was performed using least significant difference (LSD) test (Snedecor and Cochran, 1976). The correlation coefficients were estimated according to SPSS programme

RESULTS

Salinity stress induced marked increase (P ≤ 0.05) in osmotic pressure as compared to control value of wheat flag leaf at heading stage (Table 1). Treatments with kinetin, spermine or their interaction caused additional increase in the values of osmotic pressure. Furthermore, kinetin + spermine treatment had the most noticeable effect in increasing osmotic pressure than kinetin or spermine alone.

Salinity induced marked increase (P ≤ 0.05) in total soluble sugars (TSS) in flag leaf extract as compared to control value (Table 2). Application of kinetin and spermine, either alone or in combination, induced additional increase in TSS. In comparison to all treatments, the effect of kinetin + spermine on TSS of wheat flag leaf was the most obvious.

In relation to control value, irrigation of wheat plants with 25% seawater induced massive increase in total soluble nitrogen (TSN) of flag leaf extract as shown in table 2. Grain presoaking in kinetin, spermine or their interaction resulted in additional increases (P ≤ 0.05) in TSN, but the highest values were recorded with kinetin + spermine treatment.

As compared to the control value, salinity stress caused significant increase ($P \leq 0.05$) in proline concentration in the extract of wheat flag leaf at heading stage (Table 2). Grain priming with kinetin, spermine or their interaction caused additional increase ($P \leq 0.05$) in proline content. The highest values were recorded with kinetin + spermine treatment followed by kinetin and finally by spermine.

The pattern of results in table 2 cleared that seawater stress caused marked increase ($P \leq 0.05$) in the content of organic acids (i.e. keto and citric acids) when compared with the control value. Significant additional increase ($P \leq 0.05$) was also recorded when the stressed plants were treated

with kinetin, spermine or their interaction. The effect was more pronounced with kinetin + spermine treatment.

Data in table 3 cleared that salinity stress caused significant increase ($P \leq 0.05$) in Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and P^{+++} contents when compared with control plants. Conversely, the calculated ratios (SPR, SAR and PAR) were noticed to decrease in response to seawater irrigation (Table 4). Grain priming with kinetin, spermine or their interaction caused significant increase ($P \leq 0.05$) in these ionic contents as well as their calculated ratios. Kinetin + spermine treatment had the most pronounced effect.

Table 1. Effect of grain presoaking in kinetin, spermine or their interaction on osmotic pressure (atm) in flag leaf extract of wheat plants (at heading stage) irrigated with seawater.

Treatments	Osmotic pressure (atm)
Cont	1.94
SW	2.05
K	2.28
SW + K	2.80
Spm	2.20
SW + Spm	2.07
K + Spm	2.81
SW + K + Spm	2.92
LSD at $P \leq 0.05$	0.089

Table 2. Effect of grain presoaking in kinetin, spermine or their interaction on some osmolytes (total soluble sugars, total soluble nitrogen, proline, and organic acids) ($\text{mg g}^{-1} \text{d wt}$) in flag leaf extract of wheat plants (at heading stage) irrigated with seawater.

Parameters Treatments	Total soluble sugars ($\text{mg g}^{-1} \text{d wt}$)	Total soluble nitrogen ($\text{mg g}^{-1} \text{d wt}$)	Proline ($\text{mg g}^{-1} \text{d wt}$)	Organic acids ($\text{mg g}^{-1} \text{d wt}$)	
				Keto acids	Citric acid
Cont	36.28	3.70	0.56	0.64	7.59
SW	42.28	4.40	0.66	0.94	9.91
K	43.71	4.28	0.68	0.88	11.38
SW + K	43.29	5.23	0.72	1.33	11.91
Spm	41.29	4.22	0.59	0.81	9.69
SW + Spm	42.34	4.89	0.70	1.23	11.66
K + Spm	44.03	4.97	0.73	0.96	10.31
SW + K + Spm	45.82	5.30	0.74	1.39	12.50
LSD at $P \leq 0.05$	2.97	0.35	0.09	0.17	0.78

Table 3. Effect of grain presoaking in kinetin, spermine or their interaction on ionic content (mmole g⁻¹ d wt) (at heading stage) irrigated with seawater.

Parameters	Ionic content (mmole g ⁻¹ d wt)					
Treatments	Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	P ⁺⁺⁺
Cont	3.54	9.57	8.00	10.00	0.29	0.040
SW	4.77	13.39	9.80	11.20	0.56	0.051
K	4.10	11.48	9.60	10.75	0.49	0.066
SW + K	4.92	17.22	10.40	11.67	0.61	0.072
Spm	4.00	11.28	8.80	10.40	0.43	0.048
SW + Spm	4.84	13.39	10.00	11.20	0.59	0.058
K + Spm	4.15	11.48	10.00	10.80	0.47	0.052
SW +K +Spm	4.97	17.22	10.60	12.00	0.63	0.068
LSD at P ≤ 0.05	0.50	1.65	1.12	0.60	0.04	0.001

Table 4. Effect of grain presoaking in kinetin, spermine or their interaction on ionic ratios in flag leaf extract of wheat plants (at heading stage) irrigated with seawater.

Parameters	Ionic ratio		
Treatments	SPR	SAR	PAR
Cont	0.37	0.85	0.31
SW	0.36	0.68	0.24
K	0.36	0.78	0.28
SW + K	0.29	0.67	0.19
Spm	0.35	0.78	0.27
SW + Spm	0.36	0.67	0.24
K + Spm	0.36	0.78	0.28
SW +K +Spm	0.29	0.68	0.20
LSD at P ≤ 0.05	0.01	0.11	0.05

DISCUSSION

Several reports are available that showed different strategies adopted to counteract the salinity effects (Türkan and Demiral, 2009; Aldesuquy *et al.*, 2012) but the information on mineral nutrient status of plants and salinity tolerance is scarce (Khan, 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. The present work, cleared that irrigation of wheat plants with seawater resulted in marked increase in osmotic pressure, some organic solutes (TSS, TSN, proline and organic acids) and in organic ions (Na⁺, K⁺, Ca⁺, Mg⁺⁺, Cl⁻ and P⁺⁺⁺) in water extract of the flag leaf. In

this connection, Aldesuquy *et al.* (2012) reported that the observed increase in the osmotic potential of salt-stressed plants might be due to the accumulation of inorganic solutes, several organic components such as sucrose, glucose, quaternary ammonium compounds and amino acids including proline. Increased concentrations of compatible solutes in plant cells can contribute to reduced water potential in the cytoplasm by balancing the decreased water potential associated with ions accumulation in the vacuole and the extracellular volume. These compounds can also alleviate the inhibitory effects of high ion concentrations on enzymatic activity without interfering with protein structure and function. Moreover, they may act as antioxidants in scavenging free radicals, and can help in stabilizing membranes (Widodo *et al.*, 2009).

Osmolytes accumulation has also been claimed to facilitate a better translocation of pre-anthesis carbohydrate reserves to the grain during the grain-filling period (Subbarao *et al.*, 2000). To accommodate the ionic balance in the vacuoles, cytoplasm accumulates low-molecular mass compounds, the compatible solutes; because they do not interfere with normal biochemical reactions rather they replace water in these reactions (Zhifang and Loescher, 2003). Measuring the osmotic pressure in the cell sap of salt-stressed plants could provide clear indication for the water-salt status of the plant cells, where the tolerance to salinity is thought to involve raising up the internal osmotic pressure, *via* the accumulation of either organic or inorganic osmotica, to exceed that of the external saline solution. In the present study, osmotic pressure of leaf water extract was found to increase in wheat plants irrigated with seawater.

The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity, despite a significant decrease in net CO₂ assimilation rate (Murakeozy *et al.*, 2003). Irrigation of wheat plants with 25% seawater caused marked increase in TSS in water extract of flag leaf when compared with the control plants. Similarly, Murphy *et al.* (2003) suggested that soluble carbohydrates could act as compatible solutes under high salinity levels. It was suggested that sugars act for osmotic adjustment and/or protect specific macromolecules and contribute to the stabilization of membrane structures, where sugars are thought to interact with polar head groups of phospholipids in membranes so that membrane fusion is prevented (Bartels and Sunkar, 2005). Furthermore, higher levels of TSS in response to salt stress were also reported for many plant species (Oliveira *et al.*, 2009; Liu and Grieve, 2009; Xuan and Catherine,

2009).

As compared to unstressed plants, wheat plants irrigated with seawater had higher TSN content in their leaf extract. In agreement with these results, El-Bassiouny *et al.* (2008) indicated that salt stress, imposed as 50, 100 and 200 mM NaCl, induced accumulated amounts of TSN in wheat plants. Moreover, in presence of high salt concentrations, the content of TSN increased significantly in *Chenopodium quinoa* plants (Koyro *et al.*, 2008). The increase in the TSN content in response to salt stress may result from a sharp increase in total free amino acids and total soluble proteins (Ibrahim, 2004). Moreover, 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). In this connection, salt treatment of grey poplar plants resulted in significant increase in TSN concentration (Ehlting *et al.*, 2007).

Proline accumulation is one of the common characteristics in many plants exposed to salt stress (Ashraf and Harris, 2004). Thus, seawater salinity increased proline content in flag leaf extract of wheat plants. These results may indicate that the increase in proline levels at high salinity concentration might be one of the metabolic responses triggered in the translocation pathway that links the perception of many environmental stresses to the elicitation of physiological responses at the cellular level (Hussein *et al.*, 2006). Consistent with these results, lettuce plants affected by three levels of salinity were characterized by increased proline concentration than their control comparatives (Kohler *et al.*, 2009). Also, Misra and Saxena (2009) cited that lentil plants grown under salinity stress could accumulate proline to a level higher than that in the unstressed plants.

Kavi et al. (2005) reported that proline accumulated in response to several environmental types of stress, such as exposure to salinity, to protect the cell by balancing the osmotic strength of cytosol with that vacuole and external environment. Proline accumulation could be a protective response, not only due to the osmoprotectant role of proline that prevents salinity-induced water deficit, but also for its radical scavenger and protein stabilization properties (Kuznetsov and Shevyakova, 1997). Moreover, Ford and Wilson (1981) proposed that proline plays an indirect role in osmoregulation by increasing the water-binding capacity of plant cell walls to maintain the hydration of protoplasm and to increase membrane permeability.

Organic acids, as micromolecular organic compounds, not only participate in maintaining ionic balance as anions but also cause osmotic adjustment as cell osmolytes. The content of organic acids (mainly citric and keto acids) in flag leaf sap of seawater-stressed plants was higher than that in unstressed plants. The regulation of organic acid metabolism plays a key role when plants encounter unfavorable conditions (Guo et al., 2009). Lecoeur et al. (1992) found that malic acid accumulated in leaves of chickpea plants in response to water stress. In agreement with these findings, when *Puccinellia tenuiflora* seedlings were stressed by exposure to NaCl, the total concentration of organic acids (mainly oxalic, citric, malic and succinic acids) in the shoots was found to increase strongly with increasing salt level (Guo et al., 2009). The organic acids accumulated were suggested to be not only an important organic osmotic regulator, but also an important negative charge contributor, playing important roles in ionic balance and pH adjustment (Guo et al., 2009; Aldesuquy et al., 2011). Hasaneen et al. (1990)

found that α -ketoglutaric and some carboxylic acids of Krebs cycle increased in *Zea mays* seedlings and plants in response to salinity stress. The increase in citric acid content may be enhanced to increase the buffer capacity of the vacuoles (Franco et al., 1992).

It is conceivable that soil salinity alters ionic concentrations in various plant organs (Ramoliya et al., 2004). However, plants differ in their response to salinity. While many salt-tolerant plants may accumulate ions in all tissues (Soares et al., 2002), some plants have effective exclusion mechanisms by which restricts the accumulation of potentially toxic levels of ions in the leaves (Ramoliya et al., 2004).

The obtained results showed that seawater induced noticeable increase in Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and P^{+++} contents when compared with control plants. Conversely, the calculated ratios (SPR, SAR and PAR) were noticed to decrease in response to seawater irrigation. Predominantly, Na^+ and Cl^- contributed to the salt-induced changes in leaf osmotic potential of *Plantago coronopus* plants grown under salt stress (Koyro, 2006). Studies on *Leptochloa fusca* plants grown under different levels of seawater indicated that increasing saline irrigation level generally increased the content of Na^+ and Ca^{++} (Tawfik et al., 2006). More precisely, Ottow (2005) cleared that acclimation to increasing Na^+ concentrations required adjustments of the osmotic pressure of leaves, which were achieved by the accumulation of Na^+ .

Aldesuquy et al. (2012) found that seawater concentrations (10, 25%) 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 two wheat cultivars. 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). 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, salinity increased the level of Na^+ and Cl^- in both root and shoot of rice seedlings (Alamgir *et al.*, 2008). Jin *et al.* (2007) have also cited that irrigating *Aloe vera* plants with 60% seawater caused more accumulated inorganic cations in their stems and roots. Furthermore, Khan and Ahmad (2007) have demonstrated that the exposure of *Pennisetum divisum* plants to irrigation using 10% seawater caused K^+ and Mg^{++} concentration to increase. The increase in Ca^{++} level in flag leaf extract as a result of seawater irrigation may increase the tolerance of wheat plants to salinity stress, since Ca^{++} is a non-toxic inorganic nutrient and has a function of detoxification under saline medium (Izzo *et al.*, 2008).

Kinetin, Spm or their interaction induced additional increases in osmotic pressure, TSS, TSN, proline, organic acids as well as ionic contents and their calculated ratios in the stressed wheat plants.

The potentiality of different growth regulators to enhance the accumulation of various organic and inorganic osmotica in response to stress conditions was similarly recorded in other investigations carried out on wheat plants (Aldesuquy *et al.*, 2011; 2009; El-Sawy, 2009). In addition, application of natural or synthetic cytokinins might partially ameliorate negative effects of water stress mainly by inducing osmotic adjustment to delay the stress-induced senescence (Pospisilova *et al.*, 2000). In *Cicer*, application of cytokinins stimulated the accumulation of various substances which might be responsible for osmotic adjustment (i.e. proline, amino acids and soluble sugars) under water stress (Yadav *et al.*, 1997).

In this investigation, grain priming with kinetin induced marked increase in osmotic pressure of flag leaf in seawater-stressed wheat plants. Also, simulation of osmotic adjustment by kinetin was found in callus cultures of cowpea plants (Agarwal and Gupta, 1995). The enhanced osmoregulatory capacity of kinetin-treated wheat plants may be related to wall loosening (Rayle *et al.*, 1982). Both kinetin and benzyladenin enhanced the accumulation of total soluble sugars and total carbohydrates in barley plants under salt stress conditions. In addition, kinetin was observed to accelerate the enzymatic conversion of reducing sugars (such as glucose) into non-reducing sugars (sucrose), where it was assumed that salt-stressed barley plants may need non-reducing sugars as more effective cytoplasmic osmotic solutes rather than reducing sugars.

Experiments with hydroponically grown tobacco plants indicated that exogenously applied cytokinin led to the accumulation of proline, and this response was also associated with environmental stress conditions, such as salt stress, in many plant

species (Thomas *et al.*, 1995). Similarly, application of cytokinins to *Mesembryanthemum* plants induced the accumulation of proline to promote the osmotic adjustment similarly as NaCl stress (Thomas *et al.*, 1992). The enhanced production of proline under salt stress due to the exogenous application of kinetin was suggested to be of great importance to the stressed plants, where proline produced in the plant leaf is transported to the root of the stressed plants, thereby, helping the plant to regulate the osmotic potential of root cells under salinity (Begum and Karmoker, 1999). Furthermore, Ashraf and Foolad (2007) added that proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions.

It is clear from the present results that polyamines play an important role in protecting wheat plants from seawater stress by alleviating the osmotic injury, as judged by increase in RWC of wheat leaves (Liu *et al.*, 2005). Therefore, exogenous application of PAs might be a useful method to improve growth and productivity of wheat plants and retard aging process under seawater stress conditions. The ability of exogenous PAs to induce high levels of osmolytes was revealed in the increased value of osmotic pressure. Grain priming with Spm increased the content of TSS in flag leaf of seawater-stressed and unstressed wheat plants. This increase in TSS might be explained on the fact that PAs increases the leaf area (Liu *et al.*, 2004), production of photosynthetic pigments as well as its biogenesis (Besford *et al.*, 1993) and consequently stimulated the photosynthetic activity.

The pronounced increase in TSS by PAs treatments in seawater-treated plants may

probably be due to an increase in α -amylase activity in wheat flag leaves (El-Sawy, 2009). In this connection, exogenously applied Spm, Spd and Put during germination of barley grains induced an increase in α -amylase activity of such seedlings as compared with control untreated seedlings (Tipirdamaz *et al.*, 1995). Furthermore, the contents of starch, maltose and sucrose were higher in response to treatments with PAs in relation to control levels (Lee *et al.*, 1994). Kakkar and Naggari (1996) reported that treatment of *Camella sinensis* plants with different concentrations of Spm, Spd and Put increased the contents of starch and sucrose.

The application of Spm to wheat plants induced additional increase in TSN content in flag leaf at heading stage. These data are comparable to those obtained by Chen and Ching (1996) using rice plants and Ibraheem (1999) using French bean plants. This increase in TSN may probably be due to the increase of biosynthesis of nitrate reductase and nitrite reductase with inhibition of their degradation in different plant species (Reed and Hageman, 1980; Pandey and Srivastava, 1995). Furthermore, PAs are considered as one of the reserves of carbon and nitrogen in plant tissues (Kakkar *et al.*, 2000). The higher level of proline content in wheat plants treated with Spm may be due to expression of gene encoding key enzymes of proline synthesis and low activity of the oxidizing enzymes which are controlled by osmotic and salinity stress (Amini and Ehsanpour, 2005). In support to these results, Jimenez-Bremont *et al.* (2006) found that a mixture of Put, Spd and Spm increased proline concentration in bean plants during salinity stress. In this connection, proline was assumed to be able to activate multiple responses that are component of the adaptation process

(Maggio *et al.*, 2002). It was also reported that proline acts as free radical scavenger and/or enzyme protectant as well as compatible solute (Hoque *et al.*, 2007).

Generally, the application of Spm led to additional increases in ionic contents (Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and P^{+++}) and the calculated ratios (SPR, SAR and PAR). The obtained results were in good agreement with those obtained by Wang *et al.* (2007) who found that cucumber seedlings treated with exogenous PAs and combined with salinity exhibited higher level of K^+ accumulation compared with the seedlings treated only with salt stress. Similar results were also recorded by El-Sawy (2009) working on wheat plants.

The increases in ion contents as a result of PAs application might be explained on the fact that exogenous Spm treatment alleviated significantly osmotic stress injury to wheat seedlings and also activated ATPase in mitochondrial membranes, increased ATP content which used in the uptake of minerals by carriers in the plasma membranes and also to pump H^+ across the inner mitochondrial membrane (Liu *et al.*, 2005). Moreover, PAs increased water uptake by root and consequently increases the uptake and translocation of K^+ , Na^+ and Ca^{++} contents which were driven by transpiration (Alcázar *et al.*, 2006).

This study provides scarce evidence supporting the hypothesis that OA plays a preponderant role in the resistance to seawater stress. 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. Furthermore, pretreatment with kinetin and spermine increased wheat acclimatization to seawater through osmotic adjustment and solutes allocation.

REFERENCES

- Agarwal, R. K. and Gupta, S. C. (1995) Plant growth substances as osmoregulants under salt stress in callus cultures of cowpea. *Indian J Plant Physiol.*, **38**: 325- 327.
- Alamgir, A. N. M., Musa, M. and Ali, M.Y. (2008) Some aspects of mechanisms of NaCl stress tolerance in the seedlings of four rice genotypes. *Bangladesh J Bot.*, **36**: 181- 184.
- Alcázar, R, Marco, F., Cuevas, J.C., Patron, M., Ferrando, A., Carrasco, A.P., Tiburcio, A.F. and Altabella, T. (2006) Involvement of polyamines in plant response to abiotic stress. *Biotechnol Letters*, **28**: 1867- 1876.
- Aldesuquy, H.S., Baka, Z .A. M., El-Shehaby, O. A. and Ghanem, H. E. (2012) Efficacy of seawater salinity on osmotic adjustment and solutes allocation in wheat (*Triticum aestivum*) flag leaf during grain filling. *Int J Plant Physiol and Biochem.*, **4**: 33-45.
- Aldesuquy, H.S., Haroun, S.A., Abo-Hamed, S.A. and Elsaied, A.A. (2011) Physiological studies of some polyamines on wheat plants irrigated with waste water. Osmolytes in relation to osmotic adjustment and grain yield. *Phyton*, **50**: 263-268.
- Alves, A.A.C. and Setter, T.L. (2004) Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. *Environ. and Exp Bot.*, **51**: 259- 271
- Amini, F. and Ehsanpour, A.A. (2005) Soluble proteins, proline, carbohydrates and Na^+/K^+ changes in two tomato (*Lycopersicon esculentimill*) cultivars under in vitro salt stress. *Amer. J Biochem and Biotechnol*, **1**: 212- 216.
- Ashraf, M. and Foolad, M .R. (2007) Roles of glycine betaine and proline in improving plant abiotic

- stress resistance. *Environ and Exp Bot.*, **59**: 206-216.
- Ashraf, M and Harris, P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plant. *Plant Sci.*, **166**: 3- 16.
- Barker, J. and Mapson, L .W. (1964). Studies on the respiratory and carbohydrate metabolism of plant tissues. *J Exp Bot.*, **15**: 272- 283.
- Bartels, D. and Sunkar, R. (2005) Drought and salt tolerance in plants. *CRC Critical Reviews in Plant Sci.*, **24**: 23- 58.
- Begum, F. and Karmoker, J. L. (1999) Effect of salinity on the accumulation and distribution of proline in wheat. *Rachis*, **18**: 22-25.
- Ben Khaled, L., Morte-Gomez, A , Honrubia, M and Oihabi, A. (2003). Effet du stress salin en milieu hydroponique sur le trèfle inoculé par le *Rhizobium*. *Agronomie*, **23**: 553- 560.
- Besford, R.T., Richardson, C. M., Campos, J. L. and Tiburcio, A. F. (1993) Effect of polyamines on stabilization of molecular complexes in thylakoid membranes of osmotically stress oat leaves. *Planta*, **189**: 201- 206.
- Blum, A. (1996) Crop response to drought and the interpretation of adaptation. *Plant Growth Regul.*, **20**: 135- 148.
- Bray, E.A., Bailey-Serres, J. and Weretilnyk, E (2000) Responses to abiotic stresses. In: *Biochemistry and Molecular Biology of Plants*. (eds. Gruissem, W., Buchanan, B. and Jonesm, R.). American Society of Plant Physiologists, Rockville, MD, pp 1158- 1249.
- Chapman, H.D. and Pratt, P. F. (1978) Methods of analysis for soils, plants and waters. *Univ. California, Div. Agric. Sci.*
- Chen, S.J. and Ching, H. K. (1996) Polyamines in relation to ammonium-inhibited growth in suspension-cultured rice cells. *Bot Bull Acad Sinica*, **37**: 197- 200.
- Duana, J., Lia, J., Guo, S. R. and Kanga, Y. (2007) Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *Plant Physiol.*, **165**: 1620- 1635.
- Ehltig, B., Dluzniewska, P., Dietrich, H. and Selle, A. (2007) Interaction of nitrogen nutrition and salinity in Grey poplar (*Populus tremula x alba*). *Plant Cell and Environ.*, **12**: 1- 16.
- El-Bassiouny, H.M., Mostafa, H. A., El-Khawas, S. A., Hassanein, R. A., Khalil, S. I. and Abd El-Monem, A. A. (2008) Physiological responses of wheat plant to foliar treatments with arginine or putrescine. *Aust J Basic and Applied Sci.*, **2**: 1390- 1403.
- El-Sawy, O.E.H. (2009) Protective effects of polyamines on wheat plants irrigated by seawater. *Ph. D. Thesis, Fac. Sci., Mans. Univ., Egypt*.
- El-Sharkawi, H.M.and Abdel Rahman, A .A. (1974) Response of olive and almond orchards to partial irrigation under dry farming practices in semi arid regions. II. Plant soil water relations in olive during the growing season. *Plant and Soil*, **41**: 13- 32.
- Ford, C.W. and Wilson, J.R. (1981) Change in levels of solutes during osmotic adjustment to water stress in leaves of four tropical pasture species. *Aust J Plant Physiol.*, **8**: 77- 91.
- Franco, A.C., Ball, E. and Lutttge, U. (1992) Differential effects of drought and light levels on accumulation of citric acid and malic acid during CAM in *Clusia*. *Plant, Cell and Environ.*, **15**: 821- 829.

- Friedman, T.E. and Haugen, G.E. (1943) Pyruvic acid. II. The determination of keto acids in blood and urine. *J Biol Chem.*, **147**: 415- 442.
- Gadallah, M.A.A. (1999) Effects of kinetin on growth, grain yield and some mineral elements in wheat plants growing under excess salinity and oxygen deficiency. *Plant Growth Regul.*, **27**: 63-74.
- Guo, L.Q., Shi, D. C. and Wang, D. L. (2009) The key physiological response to alkali stress by the alkali-resistant halophyte *Puccinellia tenuiflora* is the accumulation of large quantities of organic acids and into the rhizosphere. *J Agron and Crop Sci.*, **196**: 123- 135.
- Hansen, E.M. and Munns, D.N.(1988) Effect of CaSO₄ and NaCl on mineral content of *Leucaena leucocephala*. *Plant and Soil*, **107**: 101- 105.
- Hasaneen, M.N.A., Younis, M.E. and El-Saht, H.M. (1990) Plant growth, metabolism and adaptation in relation to stress conditions XII. Carbohydrates and acid accumulation in *Phaseolus vulgaris* and *Zea mays* stressed with sodium sulphate. *Qater Univ. Sci. Bull.*, **10**: 185- 197.
- Hassanein, R.A., Baraka, D. M. and Khalil, R. R. (2008) Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress. II. Changes in nitrogen constituents, protein profiles, protease enzyme and certain inorganic cations. *Aust J Basic and Appl Sci.*, **2**: 350- 359.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K. and Bohnert, H.J. (2000) Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. and Plant Mol Biol.*, **51**: 463- 499.
- He, L., Nada, K. and Tachibana, S. (2002) Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants (*Cucumis sativus* L.). *J Japan Soc Horticult. Sci.*, **71**: 490- 498.
- Hoque, M.D.A., Okuma, E. Banu, M.N.A., Nakamura, Y., Shimoishi, Y. and Y Murata, Y. (2007) Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *Plant Physiol.*, **164**: 553- 561.
- Humphries, E.C. (1956) Mineral components and ash analysis. In: Modern Method of Plant Analysis (eds. Pesch, K. and Tracey, M.V.). *Springer- Verlag, Berlin*.
- Hussein, M.M., EL-Geready, N.H.N. and EL-Desuki, M. (2006) Role of putrescine in resistance to salinity of pea plants (*Pisum sativum* L.). *J Appl Sci. Res.*, **2**: 598- 604.
- Ibraheem I.F. (1999) Physiological effects of some uride compounds on cultured *Phaseolus vulgaris* seedlings in vivo and in vitro. *M. Sc. Thesis, Fac. Sci., Mans. Univ., Mansoura, Egypt*.
- Ibrahim, A.H. (2004) Efficacy of exogenous glycine betaine application on sorghum plants grown under salinity stress. *Acta Bot. Hung.*, **43**: 307- 318.
- Iqbal, M. and Ashraf, M. (2007) Seed preconditioning modulates growth, ionic relations and photosynthetic capacity in adult plants of hexaploid wheat under salt stress. *J Plant Nutrition*, **30**: 381- 396.
- Iqbal, M., Ashraf, M. and Jamil, A. (2006) Seed enhancement with cytokinins: Changes in growth and grain yield in salt-stressed wheat plants. *Plant Growth Regul.*, **50**: 29- 39.
- Izzo, R., Incerti, A and Bertolla, C. (2008) Seawater irrigation: Effects on growth and nutrient

- uptake of sunflower plants. In: Biosaline Agriculture and High Salinity Tolerance. pp 61-69.
- Jimenez-Bremont J.F., Becerra-Flora, A., Hernandez-Lucero, E., Rodriguez-Kessler, M., Costa-Gallegos, J. A. and Ramirez-Pimentel, J.G. (2006) Proline accumulation in two bean cultivars under salt stress and the effect of polyamines and ornithine. *Biologia Plant.*, **50**: 763- 766.
- Jin, Z.M., Wang, C.H., Liu, Z.P. and Gong, W.J. (2007) Physiological and ecological characters studies on *Aloe vera* under soil salinity and seawater irrigation. *Process Biochem.*, **42**: 710- 714.
- Kader, M.A. and Lindberg, S. (2005) Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice (*Oryza sativa* L.) determined by the fluorescent dye SBFI. *J Exp Bot.*, **56**: 3149- 3158.
- Kakkar, R.K. and Nagar, P.K. (1996) Polyamines and senescence of maintenance foliage of tea, *Camellia sinensis* L. *Biologia Plant.*, **38**: 153- 157.
- Kakkar, R.K., Nagar, P.K., Ahuja P.S. and Rai, V.R. (2000) Polyamines and plant morphogenesis. *Biol. Plant.*, **43**: 1-11.
- Kasinathan, V. and Wingler, A. 2004. Effect of reduced arginine decarboxylase activity on salt tolerance and on polyamine formation during salt stress in *Arabidopsis thaliana*. *Physiol Plant.*, **121**: 101- 107.
- Kasukabe, Y., He, L., Nada, K., Misawa, S., Ihara, I. and Tachibana, S. (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. *Cell Physiol.*, **45**: 712- 22.
- Kavi, K.P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S. and Naidu, K .R. (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current Sci.*, **88**: 424- 438.
- Khan, D. and Ahmad, R. (2007) Effects of irrigation with amended dilutions of seawater on germination, growth and ionic distribution in *Pennisetum divisum* (GMEL.) HENR - An arid psammophytic perennial graminoid. *Inter J Biol and Biotechnol.*, **4**: 347- 355.
- Khan N.A., Syeed, S. A. Masood, Nazar, R. and Iqbal, N. 2010. Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. *Inter. J. Plant Biol.*, **1**: 1-8.
- Khorshidi, M.B., Yarnia, M. and Hassanpanah, D. (2009) Salinity effect on nutrients accumulation in alfalfa shoots in hydroponic condition. *J. Food Agric. Environ.*, **7**: 787- 790.
- Kohler, J., Hernández, J.A., Caravaca, F. and Roldán, A. (2009) Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environ and Exp Bot.*, **65**: 245- 252.
- Koyro, H.W. (2006) Effect of salinity on growth, photosynthesis, water relations and solute composition of potential cash crop halophyte *Plantago coronopus* L. *Environ and Exp Bot.*, **56**: 136- 146.
- Koyro, H.W., Lieth, H. and Said, S. (2008) Salt tolerance of *Chenopodium quinoa* Willd., grains

- of the Andes: Influence of salinity on biomass production, yield, composition of reserves in the seeds, water and solute. In: *Mangroves and Halophytes: Restoration and Utilisation. Part III*, pp 133- 145.
- Kuznetsov, V.V. and Shevyakova, N.I. (1997) Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. *Physiol Plant.*, **100**: 320- 326.
- Lecoeur, J., Wery, J. and Turc, O. (1992) Osmotic adjustment as a mechanism of dehydration postponement in chick pea (*Cicer arietinum* L.) leaves. *Plant and Soil*, **144**: 177- 189.
- Lee, D.W., Keon, S.H., Sun, H.I. and Tian, J.H. (1994) Effects of methylglyoxal bis-(gunylhydrazone) and polyamines on carbohydrate metabolism during adventitious root formation in soybean cotyledons. *J Plant Biol.*, **37**: 195- 201.
- Liu, H., Yu, B.J., Zhang, W. and Liu, Y. (2005) Effect of osmotic stress on the activity of H⁺-ATPase and the levels of covalently and noncovalently conjugated polyamines in plasma membrane preparation from wheat seedling roots. *Plant Sci.*, **168**: 1599-1607.
- Liu, J., Wu, X. , He, T. and Zhang, W. (2004) Study of ultrastructure of *Phragmites communis* mesophyll cell under salt stress. *Acta Bot Boreali-occidentalia Sinica*, **24**: 1035- 1040.
- Maggio, A., Migazaki, S.P., Veronese, T., Fujita, H.I., Ibeas, B., Damsz, M.L., Navasimhan, P.M. Joly R.A. and Bressan, R .A. (2002) Does proline accumulation play an active role in stress induced growth reduction? *The Plant J.*, **31**: 169- 712.
- Maiale, S., Sanchez, D.H., Guirado, A., Vidal, A. and Ruiz, O.A. 2004. Spermine accumulation under salt stress. *Plant Physiol.*, **161**: 35- 42.
- Martínez, J.P., Kinet, J.M. , Bajji, M. and Lutts, S. (2005) NaCl alleviates polyethylene glycol induced water stress in the halophyte species (*Atriplex halimus* L.). *J Exp Bot.*, **56**: 2421- 2431.
- McKell, C.M. and Goodin, J. K. (1984) A brief overview of the saline lands of the United States. Research and development seminar on forage and fuel production from salt-affected Wasteland, Western Australia.
- Misra, N. and Saxena, P. (2009) Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.*, **177**: 181- 189.
- Munns, R. (2005) Genes and salt tolerance: Bringing them together. *New Phytol.*, **167**: 645- 663.
- Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Ann Rev Plant Biol.*, **59**: 651- 681.
- Murakeozy, E.P., Nagy, Z. Duhaze, C., Bouchereau, A and Tuba, Z. (2003) Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *Plant Physiol.*, **160**: 395- 401.
- Murphy, L.R., Kinsey, S.T. and Durako, M.J. (2003) Physiological effects of short-term salinity changes on *Ruppia maritima*. *Aquatic Bot.*, **75**: 293- 309.
- Neocleous, D. and Vasilakakis, M. (2007) Effects of NaCl stress on red raspberry (*Rubus idaeus* L. and *Autumn Bliss* L.). *Scientia Horticult.*, **112**: 282- 289.
- Oliveira, H., Barros, A.S., Delgadillo, I., Coimbra, M.A. and Santosa, C. (2009) Effects of fungus inoculation and salt stress on physiology and biochemistry of in vitro grapevines: Emphasis on sugar composition changes by FT-IR analyses. *Environ and Exp Bot.*, **65**: 1- 10.

- Ottow, E.A., Brinker, M., Teichmann, T., Fritz, E., Kaiser, W., Brosché, M., Kangasjärvi, J., Jiang, X. and Polle, A. (2005) *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf succulence under salt stress. *Plant Physiol.*, **139**: 1762- 1772.
- Pandy, S. and Srivastava, H. S.(1995) Stimulation of growth and nitrate assimilation in *Leucaena leucocephala* seedlings in response to spermidine supply. *Biol Plant.*, **37**: 153- 157.
- Pine, N.W. (1955) Proteins. In: Modern methods of plant analysis. (Peack, K. and Tracey, M. V., eds) IV, 23, Springer Verlage, Berlin.
- Pospisilova, J., Synková, H. and Rulcová, J. (2000) Cytokinins and water stress: *Minireview*. *Biol Plant.*, **43**: 321- 328.
- Pérez-Pérez, J.G., Robles, J.M., Tovar, J.C. and Botia, P. (2009) Response to drought and salt stress of lemon 'Fino 49' under field conditions: water relations, osmotic adjustment and gas exchange. *Scientia Horticult.*, **122**: 83- 90.
- Rabie, G.H. (2005) Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. *Mycorrhiza*, **15**: 225- 230.
- Ramoliya, P.J., Patel, H.M. and Pandey, A.N. (2004) Effect of salinisation of soil on growth and macro- and micro-nutrient accumulation in seedlings of *Acacia catechu* (Mimosaceae). *Annals Appl Biol.*, **144**: 321- 332.
- Rayle, D.L., Ross, C.W. and Robinson, N. (1982) Estimation of osmotic parameters accompanying zeatin-induced growth of detached cucumber cotyledons. *Plant Physiol.*, **70**: 1634- 1636.
- Reed, A.J. and Hageman, R.H. (1980) Relationship between nitrate uptake, flux and reduction and accumulation of reduced nitrogen in maize (*Zea mays* L). II. Effect of nutrient nitrate concentration. *Plant Physiol.*, **66**: 1184- 1189.
- Snedecor, G.W. and Cochran, W.G. (1976) *Statistical Methods*. 6th Ed. Oxford IBH Publishing Co. New Delhi.
- Snell, F.D. and Snell, C.T. (1949) *Colorimetric methods of analysis*. Volume II. D. Van Nostrand Co. Inc., New Yourk.
- Snell, F.D. and Snell, C.T. (1954) *Colorimetric methods of analysis*. Volume IV. D. Van Nostrand Co. Inc., New Yourk.
- Soares GMB, MTP Amorim, R Hrdina and M Costa-Ferreira, 2002. Studies on the biotransformation of novel disazo dyes by laccase. *Process Biochem.*, **37**: 581- 587.
- Subbarao, G.V., Nam, N.H., Chauhan, Y.S. and Johansen, C. 2000. Osmotic adjustment, water relations and carbohydrate remobilization in pigeon pea under water deficits. *Plant Physiol.*, **157**: 651- 659.
- Taji, T., Ohsumi, C. , Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K. .and Shinozaki, K. (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *The Plant J*, **29**: 417- 426.
- Tawfik, M.M., Bahr, A.A. and Salem, A.K.M. (2006) Response of kallar grass (*Leptochloa fusca* L.) to biofertilizer inoculation under different levels of seawater irrigation. *J Appl Sci Res.*, **2**: 1203- 1211.
- Thomas, J.C., McElwain, E.F. and Bohnert, H.J. (1992) Convergent induction of osmotic stress-response. Abscisic acid, cytokinin, and the

- effects of NaCl. *Plant Physiol.*, **100**: 416- 423.
- Thomas, J.C., Smigocki, A.C. and Bohnert, H.J. (1995) Light-induced expression of ipt from *Agrobacterium tumefaciens* results in cytokinin accumulation and osmotic stress symptoms in transgenic tobacco. *Plant Mol. Biol.*, **27**: 225-235.
- Tipirdamaz, R., Durusoy, M. and Bozcuk, S. (1995) Effect of exogenous polyamines on alpha-amylase activity during seed germination under salt stress. *Turkish J. Bot.*, **19**: 411- 416.
- Türkan, I. and Demiral, T. (2009) Recent developments in understanding salinity tolerance. *Environ and Exp. Bot.*, **67**: 2- 6.
- Walter, H. (1949) Grundlagen der flenzenverliertung. Eintubring, in di pflanzengeographie- fur studierends der hocholen, standorstlehre: Stuttgart Ulmer.
- Wang, S., Yongxia, J., Guo, S. and Zhou, G. (2007) Effects of polyamines on K⁺, Na⁺ and Cl⁻ content and distribution in different organs of cucumber (*Cucumis sativus* L.) seedlings under NaCl stress. *Frontiers of Agric in China*, **1**: 430-437.
- Widodo, J.H.P., Patterson, J.H., Newbigin, E.D., Tester, M., Bacic, N. and Roessner, U. (2009) Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J Exp Bot.*, **60**: 4089- 4103.
- Xuan, L. and Catherine, G. (2009) Accumulation of chiro-inositol and other non-structural carbohydrates in Limonium species in response to saline irrigation waters. *J Amer. Soc. Horticult. Sci.*, **134**: 329- 336.
- Yadav, N., Gupta, V. and Yadav, V.K. (1997) Role of benzyladenine and gibberellic acid in alleviating water-stress effect in gram (*Cicer arietinum*). *Indian J Agric. Sci.*, **67**: 381- 387.
- Zhifang, G. and Loescher, W.H. (2003) Expression of a celerymannose 6-phosphate reductase in *Arabidopsis thaliana* enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimmer. *Plant Cell and Environment.*, **26**: 275- 283.