

Effect of Salt Stress on Relative Water Content, Lipid Peroxidation, Polyamines, Amino Acids and Ethylene of Two Wheat Cultivars

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ABSTRACT

The response of two wheat cultivars, Giza 168 and Gimeza 9, to NaCl stress (0-14 dSm⁻¹) was investigated. Changes in relative water content (RWC), polyamines (putrescine, PUT; Spermidine, Spd; Spermine, Spm), amino acids, ethylene and lipid peroxidation were determined in both cultivars in absence and presence of NaCl. NaCl stress reduced the RWC in both cultivars, the reduction was more pronounced in Giza 168. Lipid peroxidation was increased with salinity in both cultivars, more so in Giza 168. Salt stress increased Spd and Spm level in Gimeza 9 while the level of both polyamines was decreased in Giza 168. PUT was increased only by 2.1 dSm⁻¹ NaCl in Giza 168 whereas its level was decreased by all NaCl treatments in Gimeza 9. Amino acid content was increased in Gimeza 9, while the content was decreased in Giza 168 in all NaCl treatments. The predominant amino acids in both cultivars were glutamic acid and proline. Salt stress increased proline level in both cultivars; greater increase was obtained in Gimeza 9. Ethylene level was increased in Gimeza 9, while it was decreased in Giza 168 with increasing salt level. The results were discussed in relation to salt tolerance of wheat.

Key Words: Lipid peroxidation; Polyamines; Amino acids; Ethylene; Wheat

Abbreviations: putrescine = PUT; Spermidine = Spd; Spermine = Spm; relative water content = RWC

INTRODUCTION

Salinity is a complex environmental constraint that presents two main components: an osmotic component due to decrease in the external osmotic potential of the soil solution and an ionic component linked to the accumulation of ions which become toxic at high concentrations (mainly Na⁺ and Cl⁻). The accumulation of these elements leads to decrease the absorption availability of essential elements (e.g. potassium and calcium) (El-Bassiouny & Bekheta, 2001).

Salt stress induced increase in the endogenous polyamine contents has been reported in various plant species (Erdei *et al.*, 1996; Aziz *et al.*, 1999). Different stresses may influence polyamine metabolism in different manners and specific function under the stress condition (Zhou *et al.*, 1995). The differences in the endogenous polyamine responses under salt stress have been reported in different plant species. The endogenous levels of polyamines decreased in rice seedlings under salt stress (Prakash *et al.*, 1988), whereas Basu *et al.* (1988) reported an increase in the accumulation of polyamines in rice seedlings grown under salt stress. An increase in PUT level associated with low contents of Spd and Spm in salt-sensitive rice cultivars was reported, while the tolerant cultivars showed a little increase in PUT and high increase in Spd and Spm. Polyamines might therefore have modulating role in the salt tolerance mechanisms

(Krichnamurthy & Bahgwat, 1989). Moreover, Krichnamurthy (1991) reported that PUT accumulation could play a protective role in the plant cell under salt stress.

Ethylene (ETH) and polyamines occur ubiquitously in the plant kingdom and control many aspects of plant physiology processes such as plant growth, development and responses to stress (Bouchereau *et al.*, 1999; Locke *et al.*, 2000). Ethylene production by plants was increased under biotic and abiotic stress (Hyodo, 1991). In addition, Lutts *et al.* (1996) reported that under salt stress conditions the ethylene content in rice plants was higher in salt tolerant than in salt sensitive species. Since polyamines and ethylene synthesis are linked through the common precursor S-adenosyl methionine (SAM), polyamines and ethylene inhibits each other biosynthesis and action (Chin *et al.*, 2000).

The effects of salt stress on the nitrogenous compounds are fairly well known (Mansour, 2000). Amino acids and polyamine accumulation in salt stress tolerant species has been studied by several investigators (Krichnamurthy & Bahgwat, 1989; Willadino *et al.*, 1996). Salt stress resulted in an elevation of amino acid levels in plants (Fougere *et al.*, 1991). Proline accumulation was proposed to be associated with tolerance to osmotic and saline stress (Aziz & Larher, 1995; Aziz *et al.*, 1999; Mansour, 2000). The endogenous polyamine and amino acid levels under salt stress conditions are directly related to the alterations in the enzymatic activities caused by feed

back and/or repressive mechanisms. PUT is synthesized in plants from arginine and/or ornithine through the action of the biosynthetic enzymes arginine decarboxylase and ornithine decarboxylase (Solcun & Weinstein, 1990).

This work aims to study the effect of different levels of salinity on the endogenous contents of diamine putrescine (PUT), triamine spermidine (Spd) and tetramine spermine (Spm), ethylene biosynthesis as well as amino acid contents in two wheat cultivars (Giza 168 & Gimeza 9). This was to address the physiological role of the changes occurred in the pervious compounds to wheat salt tolerance.

MATERIALS AND METHODS

Green house experiments were conducted in the National Research Centre (Cairo, Egypt) during two successive seasons (2002 & 2003). The caryopses of wheat cultivars (*Triticum aestivum* L.) Giza 168 and Gimeza 9 were obtained from the Agriculture Research Centre (Giza, Egypt). The caryopses were grown in pots containing equal amounts of clay and sand (ratio 1: 1). After 15 d of sowing, thinning of wheat seedlings was carried out and five uniform seedlings were left for each pot. After 21 d of sowing the caryopses were then subjected to different levels of salt treatments: 0 (tap water), 2000, 4000, 8000 and 12000 mg/L which equal to EC of 0.03, 2.1, 5.0, 9.0 and 14 dSm⁻¹, respectively. The saline solutions were prepared according to Strogonov equation (1962) (Table I). The seedlings were irrigated with equal volume (one liter/pot) of different salt solutions for two times, whereas tap water was used for the third one. The seedlings were left under the following natural growth conditions: 12h light period, 65%-70% relative humidity, day/night temperatures of 24/16°C. The plants were harvested after 60 day of sowing for different measurements: relative water content, lipid peroxidation, endogenous polyamines, ethylene and amino acid contents.

Relative water content. The relative water content was determined according to Henson *et al.* (1981) using the following formula

$$\text{RWC} = 100 \times (\text{fresh mass} - \text{dry mass}) / (\text{turgid mass} - \text{dry mass})$$

Turgid mass was determined after saturation of leaf

Table Ia. The components of salt mixture used for irrigation, expressed as % of total salt content

MgSO ₄	CaSO ₄	NaCl	MgCl ₂	CaCO ₃
10	1	78	2	9

Table Ib. Anions and cations in the salt mixture, expressed as % of total milliequivalent

Na ⁺	Mg ⁺²	Ca ⁺²	SO ₄ ⁻²	Cl ⁻	CO ₃ ⁻²
38	6	6	5	40	5

blades in distilled water in sealed glass tubes for 4 h at room temperature followed by over night storage at 5°C. The dry mass was determined after 48 h at 80°C.

Lipid peroxidation. The lipid peroxidation was measured by determining the level of malondialdehyde as indicator of lipid peroxidation. Malondialdehyde was assayed by Thiobarbituric acid reactive substance (TBARS) contents (Heath & Packer, 1968).

Polyamine analysis. The Polyamine contents were determined by using HPLC method (Agilent 1100 HPLC system, Germany). The shoots of wheat were frozen in liquid nitrogen and then homogenized with 5 % cold perchloric acid (1: 10 w/v). The homogenate was kept for 1 h at 2°C and then centrifuged at 15000 g for 20 min. The supernatant phase containing the free polyamine fraction was stored frozen. High HPLC and fluorescence spectrophotometry used to separate and quantify the free polyamines prepared as their dansyl derivatives (Flores & Galston, 1982) according to the method of Smith & Davies (1985). The samples were detected at 254 nm using a standard Spectrophotometer.

Amino acid contents. Identification and determination of the amino acid composition of the wheat shoots protein was carried out by using HPLC (Eppdraf, Germany) according to Millipore cooperative (1987).

Ethylene biosynthesis. Ethylene biosynthesis was estimated on the wheat shoots according to Lutts *et al.* (1996). The flask were flushed with fresh air, sealed with rubber serum caps incubated at 22°C ± 2 on a rotating shaker (100 rev/min) for 6 h. A gas sample (1 mL) was then withdrawn from the headspace of the flask and ethylene was assayed using a gas chromatograph (Hewlett II HP 6890 series, Pack DRD, USA) equipped with an alumina column and aflame ionization detector.

Statistical analysis. Standard error was calculated and Student's t-test was carried out using SAS program (SAS, 1982).

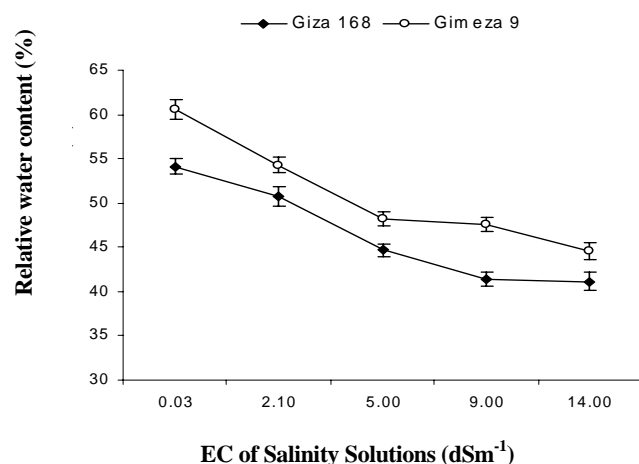
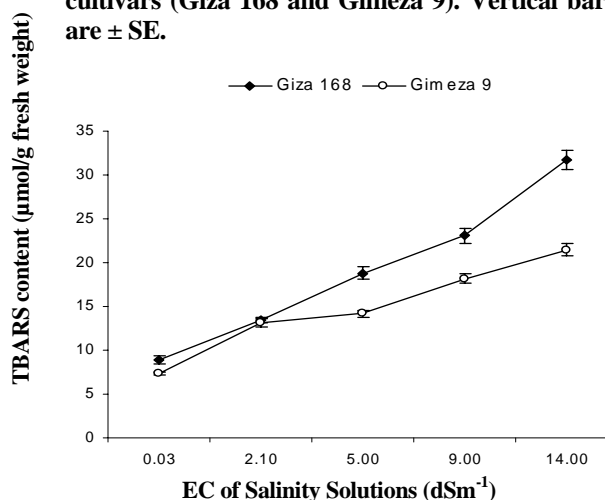
RESULTS AND DISCUSSION

Relative water content. Salt stress decreased the RWC of both cultivars, the effect increased with salinity level (Fig. 1). Giza 168 showed greater reduction in the RWC than Gimeza 9. The results suggest that Gimeza 9 had the ability to avoid the water stress induced by salinity than Giza 168. Our results are in agreement with those of Sairam *et al.* (2002) who report a greater reduction in the RWC of salt sensitive wheat cultivar as compared with tolerant one under salt stress. El-Bassiouny and Bekheta (2001) also found more growth reduction in Giza 168 than Gimeza 9 after salinity imposition.

Lipid peroxidation. Lipid peroxidation was increased with increasing the salinity level in both cultivars (Fig. 2), the effect was more pronounced in Giza 168. Shalata and Neumann (2001) similarly report that lipid peroxidation increased with salt level in tomato. The data of Sairam *et al.*

Table II. Effect of different levels of salinity on the amino acid contents of wheat shoots of cv. Giza 168 and Gimeza 9 (calculated as mg/ 100 g dry weight). Each value is the \pm SE.

Salinity levels (dSm ⁻¹)	0		2.1		5		9		14	
	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9
Amino acids										
Asparatic acid	10.11 \pm 0.380	1.46 \pm 0.060	8.11 \pm 0.170	2.80 \pm 0.060	5.52 \pm 0.140	3.06 \pm 0.050	4.86 \pm 0.110	5.93 \pm 0.080	4.57 \pm 0.070	7.97 \pm 0.200
Threonine	5.78 \pm 0.140	0.98 \pm 0.030	4.61 \pm 0.140	1.16 \pm 0.040	3.29 \pm 0.050	1.21 \pm 0.040	2.69 \pm 0.060	2.33 \pm 0.050	1.96 \pm 0.060	3.54 \pm 0.080
Serine	6.56 \pm 0.120	1.36 \pm 0.050	5.19 \pm 0.140	1.53 \pm 0.030	3.64 \pm 0.100	1.60 \pm 0.040	2.25 \pm 0.020	1.82 \pm 0.060	2.41 \pm 0.080	4.38 \pm 0.110
Glutamic acid	24.74 \pm 0.160	5.46 \pm 0.110	26.18 \pm 0.330	12.00 \pm 0.170	26.15 \pm 0.14	12.33 \pm 0.310	20.53 \pm 0.080	24.43 \pm 0.84	19.17 \pm 0.340	33.64 \pm 1.010
Proline	5.02 \pm 0.120	2.44 \pm 0.070	8.57 \pm 0.160	6.56 \pm 0.150	14.14 \pm 0.200	7.18 \pm 0.050	11.06 \pm 0.080	8.94 \pm 0.090	9.08 \pm 0.160	13.71 \pm 0.110
Glycine	6.56 \pm 0.120	1.95 \pm 0.060	4.79 \pm 0.140	1.19 \pm 0.070	4.67 \pm 0.140	1.38 \pm 0.030	2.74 \pm 0.060	2.65 \pm 0.070	2.00 \pm 0.060	3.83 \pm 3.390
Alanine	7.07 \pm 0.130	2.40 \pm 0.080	5.65 \pm 0.110	3.77 \pm 0.080	4.18 \pm 0.120	3.91 \pm 0.090	5.59 \pm 0.190	8.075 \pm 0.120	6.18 \pm 0.180	10.56 \pm 3.130
Valine	6.02 \pm 0.140	1.73 \pm 0.050	4.66 \pm 0.090	2.46 \pm 0.060	3.63 \pm 0.070	2.52 \pm 0.070	3.56 \pm 0.050	4.57 \pm 0.120	3.66 \pm 0.070	6.91 \pm 3.180
Methionine	0.18 \pm 0.010	0.24 \pm 0.005	0.06 \pm 0.003	0.33 \pm 0.005	-	0.32 \pm 0.007	0.44 \pm 0.002	0.313 \pm 0.005	0.43 \pm 0.010	2.25 \pm 2.002
Leucine	3.68 \pm 0.120	1.17 \pm 0.020	2.90 \pm 0.060	1.44 \pm 0.004	2.14 \pm 0.080	1.48 \pm 0.030	2.23 \pm 0.070	2.83 \pm 0.070	2.36 \pm 0.050	4.27 \pm 0.080
Isoleucine	6.79 \pm 0.110	3.26 \pm 0.090	4.97 \pm 0.110	4.46 \pm 0.100	4.10 \pm 0.100	4.70 \pm 0.120	6.81 \pm 0.140	9.03 \pm 0.210	7.24 \pm 0.150	12.94 \pm 0.230
Phenylalanine	1.84 \pm 0.070	1.160 \pm 0.04	1.45 \pm 0.030	1.68 \pm 0.050	1.05 \pm 0.030	1.83 \pm 0.040	2.63 \pm 0.070	3.46 \pm 0.100	2.91 \pm 0.060	4.73 \pm 0.080
Tyrosine	4.59 \pm 0.100	0.83 \pm 0.020	3.45 \pm 0.060	0.20 \pm 0.001	2.79 \pm 0.08	0.07 \pm 0.00	0.58 \pm 0.010	4.57 \pm 0.060	0.13 \pm 0.010	0.88 \pm 0.020
Histidine	3.56 \pm 0.140	1.12 \pm 0.030	2.68 \pm 0.070	0.84 \pm 0.020	2.14 \pm 0.080	0.78 \pm 0.020	1.21 \pm 0.030	1.87 \pm 0.040	1.32 \pm 0.050	2.11 \pm 0.040
Lysine	7.67 \pm 0.110	1.83 \pm 0.030	5.85 \pm 0.070	0.80 \pm 0.020	4.60 \pm 0.120	0.73 \pm 0.020	1.18 \pm 0.060	1.74 \pm 0.030	1.07 \pm 0.020	2.54 \pm 0.060
NH ₄	11.95 \pm 0.260	4.93 \pm 0.110	11.47 \pm 0.230	4.74 \pm 0.100	8.41 \pm 0.160	4.98 \pm 0.120	7.37 \pm 0.040	10.68 \pm 0.180	8.13 \pm 0.180	12.10 \pm 0.350
Arginine	6.90 \pm 0.120	0.91 \pm 0.020	4.96 \pm 0.090	0.87 \pm 0.020	4.50 \pm 0.130	0.80 \pm 0.020	0.98 \pm 0.020	1.03 \pm 0.030	0.88 \pm 0.020	2.46 \pm 0.090
Cystine	1.96 \pm 0.050	-	0.69 \pm 0.030	0.010 \pm 0.000	0.71 \pm 0.010	0.005 \pm 0.000	0.01 \pm 0.000	-	-	-
Total amino acids	120.97 \pm 2.260	33.23 \pm 1.040	106.24 \pm 2.170	46.85 \pm 1.210	95.65 \pm 1.730	48.87 \pm 1.420	76.71 \pm 1.160	90.24 \pm 2.280	73.50 \pm 1.410	126.82 \pm 2.930 \pm

Fig. 1. Effect of different salinity levels on relative water content (RWC) of two wheat cultivars (Giza 168 and Gimeza 9). Vertical bars are \pm SE.

Fig. 2. Effect of different salinity levels on lipid peroxidation (TBARS content) of two wheat cultivars (Giza 168 and Gimeza 9). Vertical bars are \pm SE.


(2002) support our results, where they report more level of lipid peroxidation in salt sensitive wheat relative to the tolerant cultivar. The possible role of the cell membranes in plant salt tolerance, therefore, was suggested (Mansour & Salama, 2004).

Polyamine levels. Salt stress decreased the PUT level in Gimeza 9 and Giza 168 (except 2.1 dSm⁻¹) (Fig. 3A). The value of Spd was increased in Gimeza 9 and decreased in Giza 168 (except 2.1 dSm⁻¹) by salinity (Fig. 3B). The maximum increase in Spd level (41%) induced by salinity was reached at 9 dSm⁻¹ in Gimeza 9. Salt imposition up to 9

dSm⁻¹ (83%) increased in Spm level in Gimeza 9 whereas a decrease in Spm level of Giza 168 was observed (Fig. 3C).

The increase in PUT level at 2.1 dSm⁻¹ in Giza 168 is in agreement with the finding of Lefevre *et al.* (2001) who found more PUT accumulation in rice salt cultivar than in tolerant one. The response of Spd and Spm to salinity in both cultivar reported in this study agree with the results of Krishnamurthy and Bhagwat (1989) who indicate that salt tolerant rice cultivars accumulated more Spd and Spm than sensitive ones under salt stress. They also report greater PUT accumulation in sensitive rice than in tolerant one under salinity. Erdei *et al.* (1996) indicate that Spd and Spm

Fig. 3. Effect of different salinity levels on polyamine contents (Put, Spd, Spm) of two wheat cultivars (Giza 168 and Gimeza 9). Vertical bars are \pm SE.

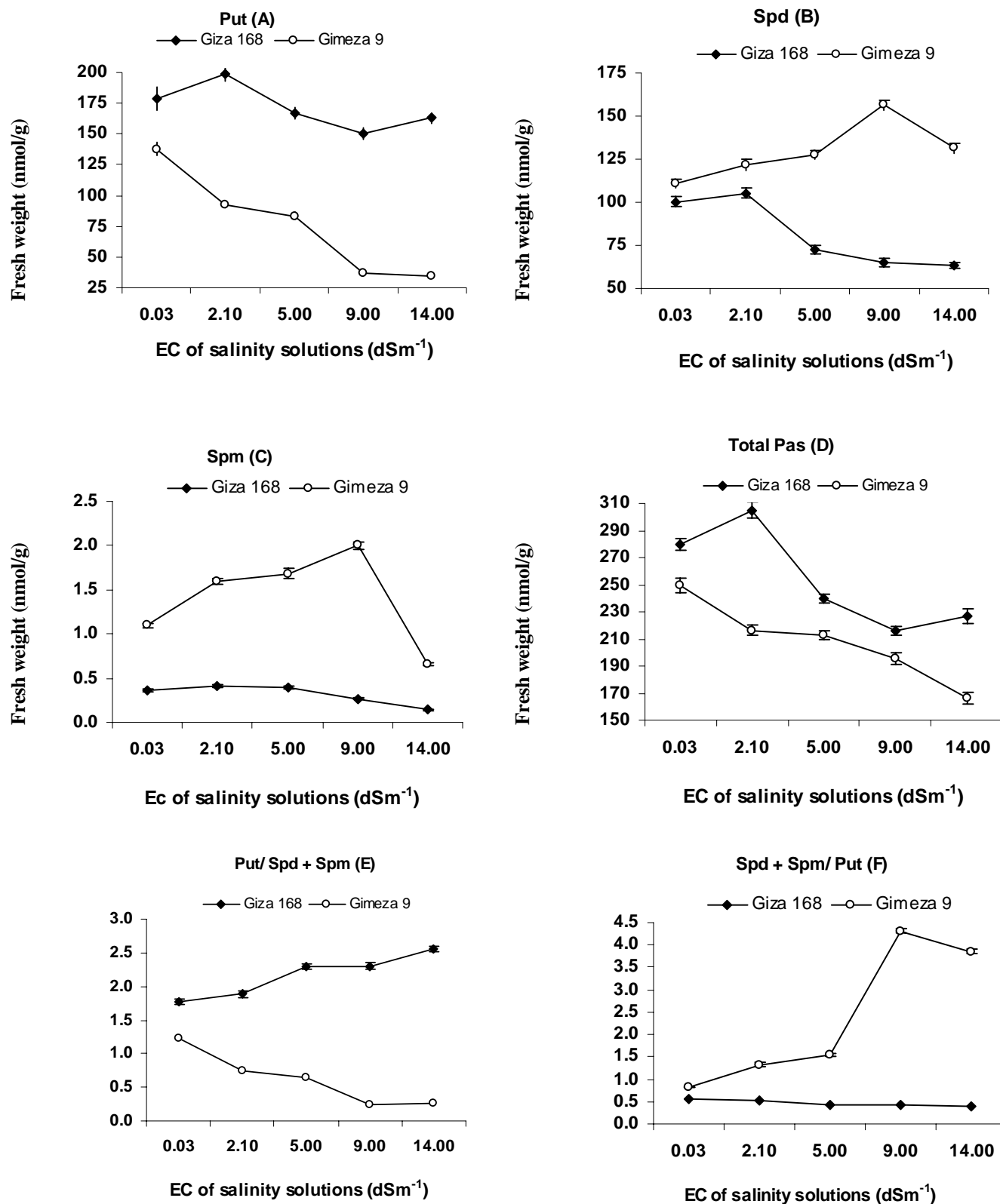
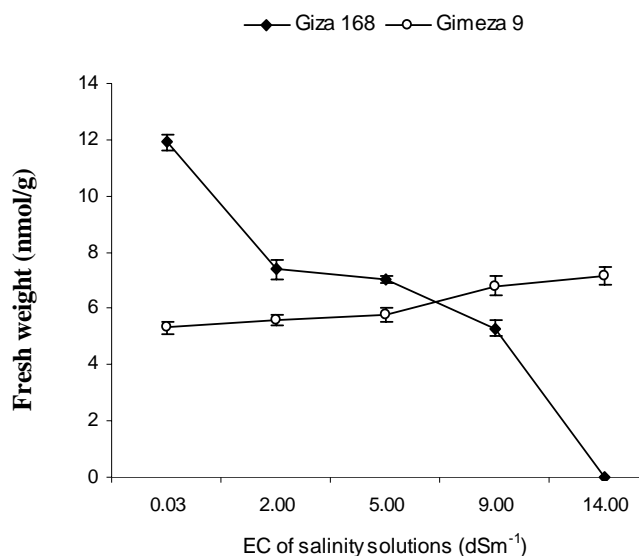


Fig. 4. Effect of different salinity levels on ethylene biosynthesis of two wheat cultivars (Giza 168 and Gimeza 9). Vertical bars are \pm SE.



accumulation in sorghum is one of the adaptive responses to salt stress. Willadino *et al.* (1996) and Chattopadhyay *et al.* (2002) proposed that accumulation of Spd and Spm may contribute to stress tolerance, while PUT accumulation may have no positive effect under salinity stress. It could be proposed that this may be the case in our study.

Salt stress induced a total polyamine (PUT, Spd, Spm) decrease in Gimeza 9 and Giza 168 (except 2.1 dSm⁻¹) (Fig. 3D). These results agree with that of Prakash and Prathapseman (1988) and Das *et al.* (1995) who found decreased levels of PA in rice seedlings and brassica under NaCl stress. The ratio of PUT/Spd+Spm (diamine/polyamine) was decreased in Gimeza 9 and increased in Giza 168 in response to salinity (Fig. 3E). However, Polyamine/diamine ratio was increased and decreased under salinity in Gimeza 9 and Giza 168, respectively (Fig. 3F). These results are in agreement with those obtained by Santa-Cruz *et al.* (1997) in tomato. The authors report that polyamine/diamine ratio increased in salt tolerant tomato under salinity, comparing with sensitive tomato.

Amino acid contents. Salinity increased the total amino acid contents in Gimeza 9 while it decreased this content in Giza 168 (Table II). The increase in total amino acids may result from PUT conversion into different amino acids (Kishnamurthy, 1991). This proposal based on the fact that amino acids and polyamines are related in their metabolic pathways and affected by alteration in enzymatic levels caused by salinity (Flores & Filner, 1985; Solcum & Weinstein, 1990). Our results are in agreement with the previous finding, where the level of amino acids increased and PUT content decreased and vice versa. Similar results were reported by Aziz *et al.* (1999) and Santa-Grúz *et al.*

(1999), where NaCl decreased the PA level and increased proline content in tomato leaves. Amino acid accumulation (e.g. arginine and proline) may be considered as a detoxification mechanism of the ammonium produced in plants subjected to stress (Solcum & Weinstein, 1990).

Among accumulated amino acids, proline may be of special interest because of its proposed role in plant salt tolerance (Mansour, 2000). Salt imposition resulted in proline accumulation in both cultivars, more so in Gimeza 9 (Table II). Wiladino *et al.* (1990) and Aziz *et al.* (1998) indicate greater proline accumulation in tolerant maize and tomato, which was interpreted by them to be associated with salt tolerance. Proposed functions for proline accumulation under salinity are presented by Mansour (2000) and Solcum & Weinstein (1990).

Ethylene biosynthesis. Salt stress increased ethylene synthesis in Gimeza 9, while it decreased its synthesis in Giza 168 (Fig. 4). Lutts *et al.* (1996) reported that ethylene production was greater in rice salt tolerance cultivars than sensitive ones, which agree with our results.

Polyamines and ethylene biosynthesis share a common intermediate S-adenosyl methionine (SAM) (Chin *et al.* 2000; Tari & Csizzar, 2003). Fig. 4 shows that decreased ethylene biosynthesis associated with putrescine increase (Fig. 3). Similar results obtained by Turano *et al.* (1997) who demonstrated that inhibition of ethylene biosynthesis increased PUT accumulation in soybean leaves.

In conclusion, less reduction in RWC, lower lipid peroxidation, increased Spd, Spm and proline accumulation as well as increased ethylene production in Gimeza 9 relative to Giza 168 may contribute to salt tolerance mechanism in Gimeza 9.

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