



Full Length Article

Growth, Lipid Peroxidation and Antioxidant Enzyme Activities as a Selection Criterion for the Salt Tolerance of Maize Cultivars Grown under Salinity Stress

M.M. AZOOZ¹, A.M. ISMAIL[†] AND M.F. ABU ELHAMD[†]

Department of Biology, Faculty of Science, King Faisal University, P.O. Box: 380, Al-Hassa 31982, Saudi Arabia

[†]Department of Botany, Faculty of Science, South Valley University, Qena, Egypt

¹Corresponding author's e-mail: azzozm@yahoo.com

ABSTRACT

Different responses of three maize (*Zea mays* L.) cultivars SC 129, SC 13 and SC 155 (differently susceptible to salinity stress) in pot experiment were investigated. The rate of oxidative injury on growth, lipid peroxidation and activities of antioxidant enzymes in relation to NaCl-stress were tested. The cv. SC 129 tolerated NaCl up to 100 mM and cv. SC13 up to 50 mM, whereas cv. SC 155 was markedly affected even at the lowest salinity level used. Moreover, the cv. SC 155 died at a concentration higher than 200 mM NaCl. Concomitantly, the activity of antioxidant enzymes catalase, peroxidase, ascorbate, peroxidase and superoxide dismutase in the salt-tolerant cultivars (cv. SC 129 & cv. SC 13) increased markedly during salinity stress, while they were mostly decreased by salinity stress in the salt sensitive cultivar (cv. SC 155). Consequently, this led to a marked difference in the behavior of lipid peroxidation in the three maize cultivars. We concluded that the activities of antioxidant enzymes and lipid peroxidation were associated with the dry mass production and consequently with the salt tolerance of the three maize cultivars.

Key Words: Dry matter; Salt stress; *Zea mays*

Abbreviations: Ascorbate Peroxidase = APX; Catalase = CAT; Cultivars = cvs; Malondialdehyde = MDA; Nitroblue tetrazolium = NBT; Peroxidase = POD; Polyvinylpyrrolidone = PVP; Reactive oxygen species = ROS; Superoxide Dismutase = SOD; Trichloroacetic acid = TCA

INTRODUCTION

Plants in saline areas are often exposed to multiple abiotic stresses. High salinity is one of the most important abiotic stress factors limiting plant growth and productivity of a wide variety of crops (Flowers, 2004; Jaleel *et al.*, 2007; Athar *et al.*, 2008). Thus, increased soil salinity has become an increasingly important topic (Flowers & Flowers, 2005). High exogenous salt concentrations cause ionic imbalance in the cells resulting in ion toxicity and osmotic stress (Demiral & Turkan, 2005; Mandhania *et al.*, 2006).

The response of plants to excess salinity is complex and involves changes in their morphology, physiology and metabolism (Parida & Das, 2005). Morphologically the most typical symptom of saline injury to plant is reduction of growth (Azooz *et al.*, 2004; Jaleel *et al.*, 2008), which is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, photosynthetic efficiency, carbon allocation and utilization (Sultana *et al.*, 2002; Ismail, 2003; Taylor *et al.*, 2004; Yildirim *et al.*, 2006). Of the physiological and metabolic

changes possibly occurring as response to salinity stress are the production of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), singlet oxygen (O_1), hydroxyl radicals (OH) and concomitantly H_2O_2 (Misra & Gupta, 2006). ROS have potential to interact with many cellular components, causing significant damage to membrane and other cellular structures, and consequently growth inhibition (Verma & Mishra, 2005; Agarwal & Shaheen, 2007; Gao *et al.*, 2008). Some of the ROS are highly toxic and must be detoxified by cellular responses, if the plant is to survive and grow (Gratão *et al.*, 2005). ROS scavenging depends on the detoxification mechanism, which may occur as a result of sequential and simultaneous action of a number of antioxidant enzymes, including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX).

Plants under stress display some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high constitutive and induced antioxidant levels have better resistance to damage (Parida & Das, 2005). The scavenging of ROS is one among the common defense responses against abiotic stresses (Vranová *et al.*,

2002). The degree of damage by ROS depends on the balance between the product of ROS and its removal by these antioxidant scavenging systems (Demiral & Turkan, 2005; Khan & Panda, 2008).

A correlation between the antioxidant enzyme activity and salinity tolerance was demonstrated by comparison of tolerant cultivar with sensitive cultivar in several plants. The activities of these antioxidant enzymes were reported to increase under salinity stress and closely related to salt tolerance of many plants (Azevedo Neto *et al.*, 2006; Koca *et al.*, 2007; Athar *et al.*, 2008). Superoxide dismutase (SOD; EC 1.15.1.1) is located in various cell compartments and a major scavenger of superoxide radical (O_2^-). This enzyme converts O_2^- to H_2O_2 , which is eliminated by ascorbate peroxidase (POD; EC 1.11.1.7) at the expense of oxidizing ascorbate to monohydroascorbate (Lee *et al.*, 2001; Masood *et al.*, 2006). Hydrogen peroxide is also scavenged by catalase (CAT; EC 1.11.1.6) and peroxidase (POD) and converted into water and oxygen (Mittler, 2002; Chaparzadeh *et al.*, 2004).

The level of lipid peroxidation, measured as malondialdehyde (MDA) content, has been considered an indicator of salt-induced oxidation in cell membranes and a tool for determining salt tolerance in plants (Hernández & Almansa, 2002). Lipid peroxidation rate was found to increase with increase of salt stress especially in sensitive cultivars (Azevedo Neto *et al.*, 2006; Arora *et al.*, 2008).

Comparing the response of cultivars of the same species to salinity provides a convenient and useful tool for unveiling basic mechanisms involved in salt tolerance. Maize (*Zea mays* L.) is a main food and economical crop grown in the Mediterranean region and classified as a salt-sensitive (Maas & Hoffman, 1977). The response of maize cultivars antioxidant system to abiotic stress has been studied under drought (Mohammadkhani & Heidari, 2007) and salt stress (Azevedo Neto *et al.*, 2006; Nawaz & Ashraf, 2007; Arora *et al.*, 2008). However, studies related to the comparative analyses of NaCl-dependent antioxidant protection between tolerant and sensitive maize cultivars are still scarce.

The aim of this work was to study the comparative effects of different concentrations of salinity on growth, lipid peroxidation and antioxidant enzyme activities (e.g., CAT, POD, SOD & APX) of three maize cultivars to analyze the significance of these parameters in salinity stress tolerance and to assign the most tolerant of these cultivars to salinity stress. Comparison of these parameters in three maize cultivars differing in salt tolerance may be helpful in developing a better understanding and provide additional information on the mechanisms of salt tolerance.

MATERIALS AND METHODS

Plant material and growth conditions. Seeds of maize (*Zea mays* L.) cultivars (SC 129, SC 13 & SC 155) were obtained from the breeding program of Agricultural

Research Center, Dokky, Cairo, Egypt. Ten seeds of each cultivar were sown in weighed plastic pot (100 g) containing dried silt soil (1.5 kg). The pots were left to grow in a growth chamber maintained at 35/28°C day/night (12 h) temperature cycles and light intensity of 110 mol m⁻²s⁻¹ and irrigated with water up to seedling stage. After a week, the pots were watered with NaCl solution to the salinization levels: 0 (control), 50, 100, 150, 200 and 250 mM. Three replicates (pots) were prepared for each maize cultivar for each salt treatment. The growing plants were daily irrigated with water to reach the above desired salinity levels. After 15 days from NaCl treatment, the leaves were used in analysis of enzymes. To determine the dry matter yields, the freshly harvested organs (roots, stems & leaves) were dried in an aerated oven at 80°C to constant weight.

Enzyme extraction. The samples were prepared as described by Mukherjee and Choudhuri (1983). A leaf sample (0.5 g) was frozen in liquid nitrogen and finely ground by pestle in a chilled motor, the frozen powder was added to 10 mL of 100 mM phosphate buffer (KH₂PO₄/K₂HPO₄) pH 7.0, containing 0.1 mM Na₂EDTA and 0.1 g of polyvinylpyrrolidone (PVP). The homogenate was filtered through cheese cloth, centrifuged at 15000×g for 10 min at 4°C. The supernatant was recentrifuged at 18000×g for 10 min; the supernatant was stored at 4°C for catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APD; EC 1.11.1.11) and superoxide dismutase (SOD; EC 1.15.1.1) assays.

Assays of Antioxidant Enzyme Activities

Assay of CAT activity. CAT activity was assayed in a reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 30% (w/v) H₂O₂ and 0.5 mL of enzyme extract (Aebi, 1984). The reaction was started by the addition of enzyme extract. The activity of catalase was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H₂O₂ consumed (Havir & McHale, 1987).

Assay of POD activity. CAT activity was determined according to (Maehly & Chance, 1954) by the oxidation of guaiacol in the presence of H₂O₂. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm (Klapheck *et al.*, 1990). The reaction solution was 3 mL containing 10 mM (KH₂PO₄/K₂HPO₄) pH 7.0, 10 mM H₂O₂, 20 mM guaiacol and 0.5 mL enzyme extract.

Assay of APD activity. The activity of APD was assayed according to (Chen & Asada, 1992). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.5 mM H₂O₂ and 0.1 mL enzyme extract. The reaction was started by the addition of H₂O₂. The activity of enzyme was assayed by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized.

Assay of SOD activity. SOD activity was measured according to the method of Dhindsa *et al.* (1981). Three mL of the mixture contained 13 mM methionine, 0.025 mM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and

0.5 mL enzyme extract. The reaction was started by adding 0.002 mM riboflavin and the tubes were shaken and placed under two 15-W fluorescent lamps. Illumination was started to initiate the reaction at 30°C. The reaction was allowed to proceed for 15 min, stopped by switched off the lights and covering the tubes with black cloth. The reaction medium without enzyme developed maximal color, while the non-irradiated reaction mixture served as blanks. Absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

All the enzyme activities were calculated and expressed as unit $\text{min}^{-1} \text{g}^{-1}$ fresh weight.

Determination of lipid peroxidation. The level of lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). A fresh leaf sample (0.5 g) was homogenized in 10 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at $15000 \times g$ for 10 min. To 2 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid in 20% TCA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath and centrifuged at $10,000 \times g$ for 10 min, the absorbance of supernatant was recorded at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA content was calculated using its molar extinction coefficient ($155 \text{ mM}^{-1} \text{ cm}^{-1}$) and the results expressed as nmol (MDA) g^{-1} fresh weight.

Statistical analysis. The data of all experiments were subjected to analysis by the least significance differences test (L.S.D) using SPSS program.

RESULTS

The dry matter production of the different organs (root, shoot & leaves) of the three maize cultivars differed in their response to salinity stress (Table I). Plants of cv. SC 129 and SC 13 tolerated salinity up to the level of 100 and 50 mM NaCl, respectively, and decreased gradually. However, cv. SC 155 displayed a highly significant reduction in dry matter of different organs at the most salinization levels as compared with the control. Generally, the growth of the salt-sensitive cultivar (SC 155) was extensively inhibited already at NaCl concentration lower than 250 mM NaCl. Moreover it was died at 250 mM NaCl. In contrast, the salt tolerant cultivars (SC 129 & SC 13) developed up to 250 mM NaCl.

In the salt tolerant cultivar (SC 129) CAT activity increased sharply in relative to the control (Table II). In the other salt tolerant cultivar (SC 13) this enzyme activity increased gradually up to the level of 200 mM NaCl, while at the highest level (250 mM NaCl), the activity reduced but still higher than the absolute control. On the other hand, all salinization levels induced a highly significant reduction in CAT activity in the salt sensitive cultivar (SC 155). POD activity in cv. SC 129 increased markedly at the most

salinization levels as shown in (Table II). Moreover, POD activity increased with increasing NaCl stress in cv. SC 13 leaves. However, in cv. SC 155, POD activity significantly decreased by the rise of salinity level.

The data indicated a highly significant increase in SOD activity in the salt-tolerant cultivars (SC 129 & SC13), while in the salt sensitive cultivar (SC 155), there is no visible change in the activity of this enzyme at the most salinization levels in comparing with control (Table III). Enzyme extract from the leaves of three maize cultivars was assayed for APX activity after exposure to different salinity concentrations (Table III). In cv. SC129, APX activity was non-significantly changed with increasing NaCl salinity in the soil; while in cv. SC13, APX activity increased markedly up to the level of 200 mM NaCl, then it reduced at the level of 250 mM NaCl, where the percent of reduction was 28.7% of the control. However, in cv. SC155 leaves, APX activity increased with increasing NaCl levels.

Lipid peroxidation as MDA content in the leaves of three maize cultivars exhibited variation among the tested maize cultivars (Table IV). There was a great variation among the three maize cultivars even at the level of control, indicating that the cultivars were genetically different. There was insignificant change in MDA content up to the level of 200 mM NaCl in cv. SC 129, but at the level of 250 mM NaCl, it reduced (about 10.2%) in comparing with the control. In cv. SC 13, MDA content markedly increased up to the level of 200 mM NaCl, then a significant reduction was recorded (about 6.1%) at 250 mM NaCl. On the other hand, salinity induced a highly significant increase in MDA content in cv. SC 155 leaves up to the level of 150 mM NaCl; thereafter a non-significant increase was recorded, but still was higher than the control.

DISCUSSION

The results obtained in the present work clearly demonstrated that the three maize cultivars (SC 129, SC 13 & SC 155) displayed distinct variation in salinity tolerance during vegetative growth stage. Accordingly, we ranked the three maize cultivars in order from most to least tolerant; SC 129 > SC 13 > SC 155. This reduction in growth might be due to toxicity of the ions or low osmotic potential as well as a decrease in wall extensibility (Grieve *et al.*, 2001; Haplerin & Lynch, 2003).

Tolerance to NaCl-stress in higher plants correlates to the levels of antioxidant systems and substrates (Jahnke & White, 2003; Koca *et al.*, 2007; Athar *et al.*, 2008). To overcome the effects of salinity-induced oxidative stress, plants make use of a complex antioxidant system. Relatively higher activities of ROS-scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stress. Thus, genotypes respond differently to various stresses as a result of variations in their antioxidant

Table I. Effect of salinity treatment on dry matter production (g plant⁻¹) of maize cvs. SC129, SC 13 and SC155

Maize cultivars	NaCl (mM)	Root	%	Stem	%	Leaves	%	Total	%
SC 129	0	0.272	100	0.160	100	0.266	100	0.698	100
	50	0.232	85.3	0.153	95.6	0.267	100.4	0.652	93.4
	100	0.225	82.7	0.131	81.9	0.234	88.0	0.590A	84.5
	150	0.152B	55.9	0.104B	65.0	0.167B	62.8	0.423B	60.6
	200	0.075B	27.6	0.078B	48.8	0.155B	58.3	0.308B	44.1
	250	0.068B	25.0	0.045B	28.1	0.097B	36.5	0.210B	30.1
L.S.D.	5%	0.048		0.025		0.070		0.090	
	1%	0.068		0.036		0.098		0.127	
SC 13	0	0.294	100	0.176	100	0.230	100	0.700	100
	50	0.261	88.8	0.162	92.0	0.230	100	0.653	93.3
	100	0.196B	66.7	0.148	84.1	0.182A	79.1	0.526B	75.1
	150	0.127B	43.2	0.098B	55.7	0.141B	61.3	0.365B	52.1
	200	0.105B	35.7	0.070B	39.8	0.130B	56.5	0.305B	43.6
	250	0.089B	30.3	0.067B	38.1	0.115B	50.0	0.271B	38.7
L.S.D.	5%	0.042		0.033		0.044		0.066	
	1%	0.059		0.046		0.062		0.092	
SC 155	0	0.178	100	0.129	100	0.222	100	0.529	100
	50	0.091B	51.1	0.098A	76.0	0.133B	59.9	0.322B	60.9
	100	0.091B	51.1	0.074B	57.4	0.112B	50.5	0.277B	52.4
	150	0.061B	34.3	0.062B	48.1	0.103B	46.4	0.226B	42.7
	200	0.043B	24.2	0.057B	44.2	0.089B	40.1	0.189B	35.7
	250	IE							
L.S.D.	5%	0.021		0.024		0.012		0.027	
	1%	0.031		0.035		0.018		0.038	

Means values in each column which are significantly different ($P = 0.05$) are followed by A letter and the highly significantly different ($P = 0.01$) are followed by B letter as compared with control (0.0 NaCl).

In this and rest of the tables, **IE** is Injurious effects; plants failed to survive

systems (Sairam *et al.*, 2000; Mohammadkhani & Heidari, 2007; Nawaz & Ashraf, 2007). In the the present study, SOD activity increased sharply in the salt tolerant cultivars (SC 129 & SC 13), whereas, there was no visible increase in this enzyme activity in the salt sensitive cultivar (SC 155). SOD is the key enzyme in the active oxygen scavenger system and considered to be the first line of defense against ROS (Hamilton & Heckathorn, 2001). The significant increase observed in SOD activity in both salt tolerant cultivars (SC 129 & SC 13) suggested that the enzyme may function as a ROS scavenger by converting O_2^- to H_2O_2 (Alscher *et al.*, 2002; Costa *et al.*, 2005). This implied that the tolerance of cv. SC 129 and SC 13 may be at least partially associated with increased SOD activity.

The CAT and POD destroy the H_2O_2 produced by SOD and other reactions (Foyer *et al.*, 1994). In this study, CAT and POD activities increased markedly in the salt tolerant cultivars (SC 129 & SC 13), while they reduced in the sensitive one (SC 155). This showed that plants of cvs. SC 129 and SC 13 were more efficient scavenger of H_2O_2 , which may result in better protection against H_2O_2 . POD is thought to be involved in various plant processes, including lignification (Hendriks *et al.*, 1991), oxidation of phenolics (Largrimini, 1991), regulation of cell elongation (Fry, 1986) and detoxification of toxic compounds such as H_2O_2 , which are produced as a result of oxidative stress (Chaparzadeh *et al.*, 2004). Moreover, increasing body of evidence suggests that high salinity levels induce oxidative stress (Savouré *et al.*, 1999). The present results showed that at the highest salinization level (250 mM NaCl), CAT decreased but still

Table II. Effect of salinity on catalase and peroxidase activities (unit min⁻¹ g⁻¹ fresh weight) of leaves of maize cvs. SC 129, SC 13 and SC 155

NaCl (mM)	Catalase (CAT)			Peroxidase (POD)		
	SC 129	SC 13	SC 155	SC 129	SC 13	SC 155
0	2.33	2.48	3.81	2.43	2.09	4.60
50	6.16B	2.74	1.72B	2.85	3.60B	3.92
100	6.53B	2.88	1.70B	3.41B	3.60B	3.24A
150	6.58B	4.42B	1.64B	3.57B	4.60B	3.21A
200	6.64B	5.19B	1.58B	4.49B	5.38B	2.52B
250	7.70B	3.00	IE	2.84B	5.69B	IE
L.S.D at 5%	1.53	0.97	0.55	0.68	0.67	1.24
L.S.D. at 1%	2.15	1.37	0.79	0.95	0.94	1.77

Means values in each column which are significantly different ($P = 0.05$) are followed by A letter and the highly significantly different ($P = 0.01$) are followed by B letter as compared with control (0.0 NaCl).

higher than the control in cv. SC 13. Also, POD activity revealed the same trend in cv. SC 129. Moreover, in the salt sensitive cultivar, CAT and POD activities were inhibited, which might have resulted in H_2O_2 accumulation, which react with (O_2) to produce hydroxyl-free radicals (OH^{\cdot}) via the Herbert-Weiss reactions (Elstner, 1982; Bowler *et al.*, 1992). The tolerance of some genotypes to environmental stresses has been associated with higher activities of antioxidant enzymes. For example, the wild NaCl-tolerant species *Lycopersicon pennellii* had higher activities of SOD, POD and CAT than the cultivated species *L. esculentum* (Shalata & Tal, 1998). Costa *et al.* (2005) suggested that a strong correlation between salt tolerance and POD activity in sorghum genotypes. Agarwal and Shaheen (2007)

Table III. Effect of salinity on superoxide dismutase and ascorbate peroxidase activities (unit min⁻¹ g⁻¹ fresh weight) of leaves of maize cvs. SC 129, SC 13 and SC 155

NaCl (mM)	Superoxide dismutase (SOD)			Ascorbate peroxidase (APX)		
	SC 129	SC 13	SC 155	SC 129	SC 13	SC 155
0	1.08	2.48	3.29	2.36	1.57	0.84
50	4.94B	2.72B	3.62	2.26	1.72	1.23
100	5.25B	4.82B	3.57	2.44	2.30	1.62
150	5.48B	4.92B	3.52	2.63A	2.74A	1.78A
200	4.17B	5.81B	3.56	2.43	3.81B	1.94A
250	3.28B	5.48B	IE	2.02	1.12	IE
L.S.D at 5%	0.53	0.83	0.36	0.26	0.81	0.93
L.S.D. at 1%	0.74	1.16	0.52	0.36	1.41	1.38

Means values in each column which are significantly different ($P = 0.05$) are followed by A letter and the highly significantly different ($P = 0.01$) are followed by B letter as compared with control (0.0 NaCl).

Table IV. Effect of salinity on malondialdehyde content (nmol g⁻¹ fresh weight) of leaves of maize cvs. SC 129, SC 13 and SC 155

NaCl (mM)	Malondialdehyde content (MDA)		
	SC 129	SC 13	SC 155
0	104.4	135.2	33.6
50	105.1	154.2B	56.8B
100	109.8	156.0B	54.2B
150	105.7	160.9B	43.9B
200	101.5	146.5A	38.7
250	93.7A	126.9	IE
L.S.D at 5%	5.6	10.3	5.9
L.S.D. at 1%	7.9	14.4	8.3

Means values in each column which are significantly different ($P = 0.05$) are followed by A letter and the highly significantly different ($P = 0.01$) are followed by B letter as compared with control (0.0 NaCl).

reported that a higher CAT activity in *Monordica charantia* was associated with tolerance of plant to NaCl.

The APX also destroyed the H₂O₂. It is worthy to mention that APX reversed the above criteria of CAT and POD in SC 155. This implied that the chemical defense might be by APX in this cultivar. In contrast, the activity of APX did not affect in plant resistance to oxidative stresses in the two tolerant cultivars (SC 129 & SC 13).

Malondialdehyde is a product of peroxidation of unsaturated fatty acids in phospholipids, and the level of lipid peroxidation has been used as an indicator of free radical damage to cell membranes under stress conditions. Therefore, MDA has been widely used as selection to assess salt injury as criterion in various plants (Jain *et al.*, 2001; Katsuhara *et al.*, 2005; Jaleel *et al.*, 2007). Lipid peroxidation can occur in both chloroplasts and mitochondria (Elstner, 1982; Bowler *et al.*, 1992). Our results indicated a great variation in the three maize cultivars even at the level of control, substantiating that the three cultivars were genetically different. The increase in the MDA content at all salinity levels in cv. SC 155 may be due to oxidative damage affecting both organelles (chloroplasts & mitochondria). The lower MDA level at the highest salinization level in salt tolerance cultivars (SC 129 & SC 13) than the control is important in terms of salt tolerance as

represented in different studies (Ruiz *et al.*, 2005; Koca *et al.*, 2007; Jaleel, *et al.*, 2007), and confirming with their tolerance to salinity stress at the highest level (250 mM NaCl) compared to salt sensitive (SC 155), which died at this level. Thus, it is likely that MDA plays important role in salt tolerance of maize cultivars as reported by Azevedo Neto *et al.* (2006), Khan and Panda (2008).

CONCLUSION

The maize cultivars in this study showed differential responses in the activities of the four enzymes measured. It seems that the scavenging system in salt tolerant cultivars (SC 129 & SC 13) depended often on CAT, POD and SOD activities, whilst in the salt sensitive cultivar (SC 155), the chemical defense might be achieved by APX activity. Thus the salt tolerance of these maize cultivars seems to be linked to the activities of these antioxidant enzymes. The salt tolerance of maize cultivars could induce antioxidative enzyme system more efficiently, resulting in growth suppression and lower lipid peroxidation under salinity stress.

REFERENCES

- Aebi, H., 1984. Catalase *in vitro*. *Methods Enzymol.*, 105: 121–126
- Agarwal, S. and R. Shaheen, 2007. Stimulation of antioxidant system and lipid peroxidation by abiotic stress in leaves of *Momordica charantia*. *Brazilian J. Plant Physiol.*, 19: 149–161
- Alscher, R.G., N. Erturk and L.S. Heath, 2002. Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.*, 53: 1331–1341
- Arora, N., R. Bhardwaj, P. Sharma and H.K. Arora, 2008. 28-Homobrassinolide alleviates oxidative stress in salt-treated maize (*Zea mays* L.) plants. *Brazilian J. Plant Physiol.*, 20: 153–157
- Athar, H., A. Khan and M. Ashraf, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.*, 63: 224–231
- Azevedo Neto, A.D., J.T. Prisco, J. Enéas-Filho, C.E.B. Abreu and E. Gomes-Filho, 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *J. Environ. Exp. Bot.*, 56: 87–94
- Azooz, M.M., M.A. Shaddad and A.A. Abdel-Latef, 2004. Leaf growth and K⁺/Na⁺ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. *Acta Agron. Hungarica*, 52: 287–296
- Bowler, C., M. Van Montagu and D. Inze, 1992. Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 43: 83–116
- Chaparzadeh, N., M.L. D'Amico, R.A. Khavari-Nejad, R. Izzo and F. Navari-Izzo, 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant Physiol. Biochem.*, 42: 695–701
- Chen, G. and K. Asada, 1992. Inactivation of ascorbate peroxidase by thiols requires hydrogen peroxide. *Plant Cell Physiol.*, 33: 117–123
- Costa, P.H.A., A.D.A. Neto, M.A. Bezerra, J.T. Prisco and E. Gomes-Filho, 2005. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. *Brazilian J. Plant Physiol.*, 17: 353–361
- Demiral, T. and I. Turkan, 2005. Comparative lipid peroxidation, antioxidant defense system and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot.*, 53: 247–257
- Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorne, 1981. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93–101
- Elstner, E.F., 1982. Oxygen activation and oxygen toxicity. *Annu. Rev. Plant Physiol.*, 33: 73–96

- Flowers, T.J., 2004. Improving crop salt tolerance. *J. Exp. Bot.*, 55: 307–319
- Flowers, T.J. and S.A. Flowers, 2005. Why does salinity pose such a difficult problem for plant breeders? *Agric. Water Manag.*, 78: 15–24
- Foyer, C.H., P. Descourvieres and K.J. Kunert, 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant Cell Environ.*, 17: 507–523
- Fry, S.C., 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annu. Rev. Plant Physiol.*, 37: 165–186
- Gao, S., S. Ouyang, S. Wang, Y. Xu, L. Tang and E. Chen, 2008. Effect of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant Soil Environ.*, 54: 374–381
- Gratão, P.L., A. Polle, P.J. Lea and R.A. Azevedo, 2005. Making the life of heavy metal-stressed plants a little easier. *Funct. Plant Biol.*, 32: 481–494
- Grieve, C.M., J.A. Poss, D.L. Suarez and D.A. Dierig, 2001. Lesquerella growth and selenium uptake affected by saline irrigation water composition. *Ind. Crops Prod.*, 13: 57–65
- Hamilton, E.W. and S.A. Heckathorn, 2001. Mitochondrial adaptations to NaCl: Complex I is protected by antioxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. *Plant Physiol.*, 126: 1266–1274
- Haplerin, S.J. and J.P. Lynch, 2003. Effects of salinity on cytosolic Na⁺ and K⁺ in root hairs of *Arabidopsis thaliana*: *in vivo* measurements using the fluorescent dyes SPFI and PBFL. *J. Exp. Bot.*, 54: 2035–2043
- Havir, E.A. and N.A. McHale, 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol.*, 84: 450–455
- Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, 125: 189–198
- Hendriks, T., H.J. Wijsman and L.C. Van Loon, 1991. Petunia peroxidase a: Isolation, purification and characteristics. *European J. Biochem.*, 199: 139–146
- Hernández, J.A. and M.S. Almansa, 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol. Plant.*, 115: 251–257
- Ismail, A.M., 2003. Effect of salinity on the physiological responses of selected lines/variety of wheat. *Acta Agron. Hungarica*, 51: 1–9
- Jahnke, L.S. and A.L. White, 2003. Long-term hyposaline and hypersaline stresses produce antioxidant responses in the marine algae *Dunaliella tertiolecta*. *J. Plant Physiol.*, 160: 1193–1202
- Jain, M., G. Marthur, S. Koul and N.B. Sarin, 2001. Ameliorating effects of proline on salt stress – induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). *Plant Cell Rep.*, 20: 463–468
- Jaleel, C.A., R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneerselvam, 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South African J. Bot.* 73: 190–195
- Jaleel, C.A., G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 2008. Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. *South African J. Bot.*, 74: 126–132
- Katsuhara, M., T. Otsuka and B. Ezaki, 2005. Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in *Arabidopsis*. *Plant Sci.*, 169: 369–373
- Khan, M.H. and S.K. Panda, 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiol. Plant*, 30: 81–89
- Klapheck, S., I. Zimmer and H. Cosse, 1990. Scavenging of hydrogen peroxide in endosperm of *Ricinus communis* by ascorbate peroxidase. *Plant Cell Physiol.*, 31: 1005–1013
- Koca, H., M. Bor, F. Ozdemir and I. Turkan, 2007. Effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.*, 60: 344–351
- Largirimi, L.M., 1991. Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. *Plant Physiol.*, 96: 509–516
- Lee, D.H., Y.S. Kim and C.B. Lee, 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J. Plant Physiol.*, 158: 737–745
- Maehly, A.C. and B. Chance, 1954. The assay of catalase and peroxidase. *Meth. Anal. Biochem.*, 1: 357–424
- Mandhanian, S., S. Madan and V. Sawhney, 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol. Plant*, 227: 227–231
- Masood, A., N.A. Shah, M. Zeeshan and G. Abraham, 2006. Differential response of antioxidant enzymes to salinity stress in two varieties of Azolla (*Azolla pinnata* and *Azolla filiculoides*). *Environ. Exp. Bot.*, 58: 216–222
- Maas, E.V. and G.J. Hoffman, 1977. Crop salt tolerance current assessment. *J. Irrig. Drain Div. ASCE*, 103: 115–134
- Misra, N. and A.K. Gupta, 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. *J. Plant Physiol.*, 163: 11–18
- Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7: 405–410
- Mohammadkhani, N. and R. Heidari, 2007. Effects of drought stress on protective enzyme activities and lipid peroxidation in two maize cultivars. *Pakistan J. Biol. Sci.*, 10: 3835–3840
- Mukherjee, S.P. and M.A. Choudhuri, 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Plant Physiol.*, 58: 166–170
- Nawaz, K. and M. Ashraf, 2007. Improvement in salt tolerance of maize by exogenous application of glycinebetain: growth and water relations. *Pakistan J. Bot.*, 39: 1647–1653
- Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants. *Ecotoxicol. Environ. Safety*, 60: 324–349
- Ruiz, J.M., B. Blasco, R.M. Rivero and L. Romero, 2005. Nicotine-free and salt tolerant tobacco plants obtained by grafting to salinity-resistant rootstocks of tomato. *Physiol. Plant.*, 124: 465–475
- Sairam, R.K., G.C. Srivastava and D.C. Saxena, 2000. Increased antioxidant activity under elevated temperatures: A mechanism of heat stress in wheat genotypes. *Biol. Plant*, 43: 245–251
- Savouré, A., D. Thorin, M. Davey, X.J. Hua, S. Mauro, M. Van Montagu, D. Inze and N. Verbruggen, 1999. NaCl and CuZnSO₄ treatments trigger distinct oxidative defense mechanism in *Nicotiana plumbaginifolia* L. *Plant Cell Environ.*, 22: 387–396
- Shalata, A. and M. Tal, 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiol. Plant.*, 104: 169–174
- Sultana, N., T. Ikeda and M.A. Kashem, 2002. Effect of seawater on photosynthesis and dry matter accumulation in developing rice grains. *Photosynthetica*, 40: 115–119
- Taylor, N.L., D.A. Day and A.H. Millar, 2004. Targets of stress-induced oxidative damage in plant mitochondria and their impact on cell carbon/nitrogen metabolism. *J. Exp. Bot.*, 55: 1–10
- Verma, S. and N. Mishra, 2005. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defence system. *J. Plant Physiol.*, 162: 669–677
- Vranová, V., D. Inzé and F. Van Breusegem, 2002. Singal transduction during oxidative stress. *J. Exp. Bot.*, 53: 1227–1236
- Yildirim, E., A.G. Taylor and T.D. Spittler, 2006. Ameliorative effects of biological treatments on growth of squash plants under salt stress. *Sci. Hort.*, 111: 1–6

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