Pak. J. Bot., 42(1): 559-565, 2010.

RESPONSE OF TWO GENETICALLY DIVERSE WHEAT CULTIVARS TO SALT STRESS AT DIFFERENT GROWTH STAGES: LEAF LIPID PEROXIDATION AND PHENOLIC CONTENTS

MUHAMMAD ARSLAN ASHRAF[1], MUHAMMAD ASHRAF[12*] AND QASIM ALI[1]

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan*
[2]*Second affiliation: King Saud University, Riyadh, Saudi Arabia.*

Abstract

The effect of root zone salinity on two hexaploid bread wheat (*Triticum aestivum* L.) cultivars (S-24, salt-tolerant; MH-97, salt-sensitive) was appraised at different growth stages. Grains of the two cultivars were sown in Petri-plates at two salt levels (0 and 150 mM of NaCl). After 8 days of germination, the seedlings were transplanted into plastic tubs containing either 0 or 150 mM of NaCl in full strength Hoagland's nutrient solution. Changes in growth, lipid peroxidation and phenolic contents were examined in the cultivars at different growth stages (vegetative, booting and reproductive) under salt stress. Higher MDA contents were observed in cv. MH-97 as compared to that in S-24 under saline regimes at different growth stages. Salt-induced effect in terms of lipid peroxidation was more pronounced at the booting and reproductive stages as compared with that at the vegetative stage in both cultivars, however, the accumulation of leaf total phenolics was higher at the booting stage as compared with that at the other stages. A significant variability in salt response was found among different growth stages in both cultivars. Correlations among growth and biochemical parameters showed a significant negative correlation between growth and MDA content but a positive correlation between growth and phenolic contents, which shows that phenolic compounds were involved in the mechanism of salt tolerance of the two cultivars by showing enhanced antioxidant activity which resulted in reduced membrane damage and hence improved growth.

Introduction

Salt tolerance in higher plants is regulated by a number of different physiological and biochemical processes. There is evidence that high levels of salt cause an unbalance of the cellular ions leading in both ion toxicity and osmotic stress (Ashraf & Harris, 2004), leading to the production of active O_2 species (AOS) such as superoxide (O_2^-), hydrogen-peroxide (H_2O_2) and hydroxyl radicals (OH^-) (Neill *et al*., 2002). The production of AOS creates oxidative stress in plants exposed to salinity or other stresses. For example, AOS have been shown to cause oxidative damage to DNA and proteins and peroxidation of lipid structures (Neill *et al*., 2002; Ashraf & Foolad 2007; Ashraf, 2009) as well as inactivation of antioxidant enzymes (Teisseire & Guy, 2000). Some reports suggest that resistance to oxidative stress is one of the prominent aspects of plant salt tolerance (Mittova *et al*., 2002; Badawi *et al*., 2004). It has been shown that under stress conditions, MDA (malondialdehyde) accumulation takes place in plants due to membrane lipid peroxidation. It is an effective means of assessing oxidative stress induced membrane damage (Shao *et al*., 2005) and cell membrane stability has been used an efficient criterion to discriminate among crop cultivars with respect to degree of salt tolerance (Meloni *et al*., 2003; Sairam *et al*., 2005).

*Corresponding author's e-mail: ashrafbot@yahoo.com

To reduce AOS-induced damage, plants have evolved an intricate antioxidative system, involving antioxidative enzymes, as well as low-molecular mass secondary metabolites such as ascorbate, glutathione, tocopherols, carotenoids and phenolic compounds (Posmyk *et al*., 2009). However a lot of research is being conducted these days to elucidate the role of various antioxidant metabolites in plant stress tolerance. Biological and antioxidant properties of phenolic compounds among other metabolites have been studied to a great extent (Tsai *et al*., 2002; Wang & Lin, 2000; Posmyk *et al*., 2009). Higher activity of phenolics could be due to the greater H-donating ability and radical stabilization than a variety of other antioxidant metabolites (Rice-Evans *et al*., 1996).

Different plant species and genotypes within a species respond differently to salt stress at different growth stages. So, the objective of the present work was to examine the pattern of accumulation of phenolic and MDA contents in the leaves of two wheat cultivars at different growth stages under salt stress, and the roles of these phenolic compounds in plant stress tolerance in terms of membrane lipid peroxidation, because it is known that different antioxidant compounds may act *In vivo* through different mechanisms, in plant stress tolerance.

Materials and Methods

A study was carried out to appraise salt-induced adverse effects on growth and pattern of accumulation of leaf total phenolics and malondialdehyde, (MDA) as product of lipid per-oxidation, in two genetically diverse bread wheat cultivars (S-24, salt tolerant and MH-97, salt sensitive). Seeds of both wheat cultivars were sown on filter paper placed in Petriplates. The filter papers were moistened with 0 m*M* NaCl in Hoagland's nutrient solution or 150 mmol/L NaCl in nutrient solution. After 8 days of seed germination, 20 seedlings per replicate of each treatment were subjected to two salt levels (0 and 150 m*M* NaCl). The solution was consistently aerated daily for 8 h. The experiment was conducted under natural environmental conditions, in the net-house of the Botanical Garden of Department of Botany, University of Agriculture, Faisalabad. During the whole experimentation the average photosynthetically available radiation (*PAR*) measured at noon ranged from 794 to 1154 µmol $m^{-2}.s^{-1}$, average day/night R.H 35.1/75.1% and average day and night temperatures were 28.28 ± 3 ºC and 15.82 ± 2.6 ºC, respectively).The plants were harvested at different growth stages (vegetative, booting and reproductive) during the course of experimentation for the estimation of plant biomass, leaf MDA and total phenolic contents.

Estimation of MDA contents: MDA in the leaves was analyzed following Carmak & Horst (1991). This method is based on the reaction with thiobarbituric acid. Fresh leaves (1.0 g) were ground properly in 20 ml of 0.1% tri-chloroacetic acid solution and centrifuged for 10 min at 12000 *g*. One ml of the supernatant was reacted with 4 ml of 20% TCA solution comprising 0.5% thiobarbituric acid and then it was heated for 30 min., at 95ºC in a water bath and then immediately cooled on ice. After centrifugation for 10 min., at 12000 *g*, the absorbance of the supernatant was read at 532 and 600 nm. The contents of MDA were worked out using the extinction coefficient of 155/ (mM/cm) using the formula:

$$\text{MDA level (nmol)} = \Delta\ (\text{A } 532\text{nm-A } 600\text{nm})/1.56\times10^{5}$$

Total phenolics: Total phenolics were estimated following the method of Julkenen-Titto (1985). Fresh leaf samples (50 mg) were homogenized by adding 80% acetone. After centrifugation for 10 min at 10,000 *g*, the supernatant was separated. To 100 µl of the supernatant 2.0 ml of water and 1ml of Folin–Ciocalteau's phenol reagent was added. Then 5.0 ml of 20% Na_2CO_3 solution were added to the solution. Then the final volume was raised to 10 ml by adding distilled H_2O. After mixing thoroughly, the absorbance was read using a spectrophotometer (IRMECO U2020) at 750 nm.

Experimental design and statistical analysis: All the experimental units were in a completely randomized design (CRD) with three factor factorial arrangement and four replicates. ANOVA of the data for all vaiables was computed using the CoStat computer program (Version 6.303, PMB 320, Monterey, CA, 93940 USA). The LSD values were computed at 5% level of probability following Steel & Torrie (1986).

Results and Discussion

Of different indices of salt stress tolerance of crop plants, reduction in growth is one of the potential criteria, as indicated in some earlier reports (Munns 2002; Ruiz *et al*., 2005; Hichem *et al*., 2009). In the present invesigation, the effects of salinity on biomass production and changes in some bio-chemical attributes were recorded in two wheat cultivars differing in salt tolerance at different growth stages. Data show that imposition of salt stress to the growing medium significantly reduced the shoot fresh weight of both wheat cultivars measured at different growth stages Fig. 1. More reduction in shoot fresh weight due to salt stress was observed in cv. MH-97 as compared with that in cv. S-24 at all growth stages. On comparing the effects of salt stress at different growth stages it was observed that more reduction in shoot fresh weight of both cultivars took place at the reproductive stage as compared with that at the other growth stages, but this reduction was significantly more in cv. MH-97 as compared with that in S-24 (Fig. 1 and Table 1). Such growth reducing effects of salt stress have also been reported by Hichem *et al*., (2009) in different maize cultivars at different growth stages. Furthermore, growth reducing effects of different saline regimes in hydroponic culture system were also observed by Chen *et al*., (2007) in different bean cultivars and they reported that different cultivars showed differential response to salt stress.

Table 1. Mean squares from analyses of variance of the data for shoot fresh weight, leaf MDA contents and leaf phenolics when recorded at different growth stages of two wheat cultivar plants grown in salinized hydroponic system.

SOV	df	Shoot F.wt.	MDA	Leaf Phenolics
Main effects				
Salt (S)	1	1150.32 ***	480.72 ***	342.93 ***
Variety (V)	1	258.45 ***	6.98 ns	37.81 **
Growth Stage (GS)	2	1068.49 ***	13.06 **	2062.36 ***
Interaction				
S x V	1	70.85 ***	24.30 **	26.55 **
S x GS	2	203.90 ***	69.70 ***	364.06 ***
V x GS	2	66.19 ***	41.26 ***	42.85 ***
S x V x GS	2	21.17 *	0.13 ns	18.55 **
Error	36	5.369	2.08	3.38

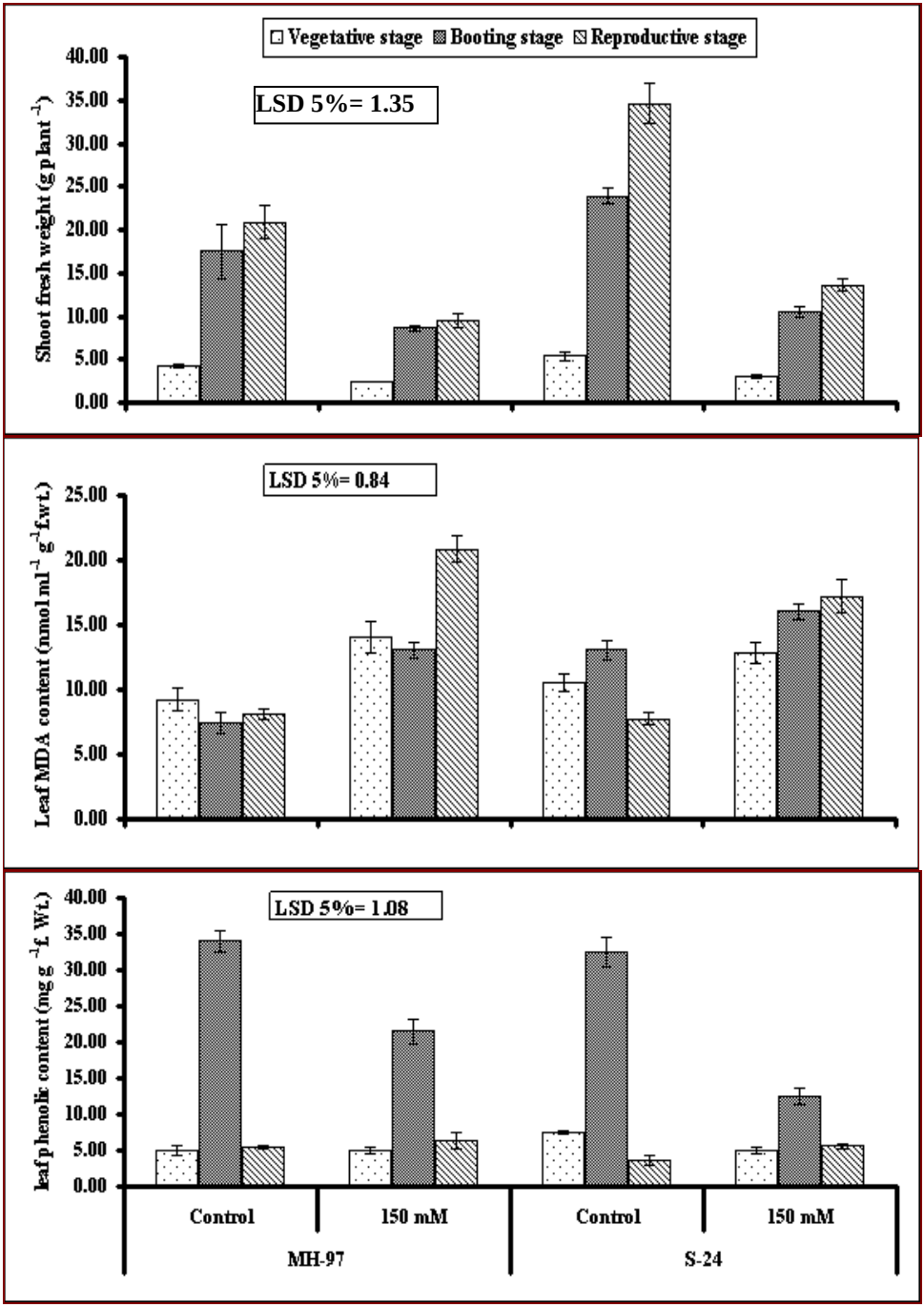

Fig. 1. Shoot fresh weight, leaf phenolic and MDA contents of two wheat cultivars when grown in salinized hydroponics system ($n=4$ ± S.E).

Table 2. Correlation coefficient (r) between different growths and biochemical attributes.

	Fresh weight	MDA	Phenolics
Fresh weight	1		
MDA	-0.356**	1	
Phenolics	0.242**	-0.138*	1

Leaf malondialdehyde (MDA) content, the product of lipid peroxidation, is a prominent indicator of membrane impairment in plants exposed to saline regimes (Katsuhara *et al*., 2005). It increased in both wheat cultivars due to root zone salinity. Both cultivars responded differently to leaf MDA contents under salt stress. For example, more increase in leaf MDA contents due to salinity was observed in cv. MH-97 as compared with that in cv. S-24. A significant difference among growth stages was observed with respect to leaf MDA contents under salt stress, which, in fact, increased with increase in plant age. Maximum production of MDA contents in leaves was observed at the reproductive stage in both wheat cultivars as compared to that at other growth stages (Fig. 1; Table 1). Furthermore, a negative correlation (-0.356**) was observed in plant biomass production and leaf MDA contents in both wheat cultivars under salt stress (Table 2), indicating that low lipid peroxidation resulted in increased biomass production i.e., growth. Such accumulation of MDA contents coupled with reduced plant growth under salt stress is strongly in agreement with the studies of Li (2009) and Koca *et al*., (2007) in tomato and sesame, respectively. They reported that growth reduction under salt stress in different cultivars is closely associated with increased lipid peroxidation levels. Furthermore, they also reported that salt tolerant cultivars accumulated less MDA as compared with salt sensitive cultivars at high salinity levels. Reduced contents of MDA is an important indicator of stress tolerance as shown in some earlier studies e.g., in salt tolerant cultivars of barley (Liang *et al*., 2003), sorghum (Brankova *et al*., 2005) and tobacco (Ruiz *et al*., 2005).

Leaf phenolic contents are important protective components of plant cells. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 (Rice-Evans *et al*., 1997). The synthesis of phenolics is generally affected in response to different biotic/ abiotic stresses including salinity (Parida *et al*., 2004) and reduced phenolic contents were observed in *Cynara cardunculus* leaves under saline conditions (Falleh *et al*., 2008). Similarly, in the present, study the phenolic contents were also affected significantly due to salt stress. Although root zone salinity significantly decreased the leaf phenolic contents in both wheat cultivars, this reduction in phenolic contents was only observed at the booting stage. At the other growth stages, such inhibitory effect of salt stress on leaf phenolic contents was not observed in both cultivars. Such differential response of plants in phenolic accumulation at different growth stages may have been due to the reason that the accumulation of phenolics depends on plant growth stage (Choi *et al*., 2006; Barros *et al*., 2007). Perhaps high accumulation of phenolics at the reproductive stage occurs due to their putative role in reproduction (Bravo, 1998). Furthermore, Hichem *et al*., (2009) reported that such variation in concentration of leaf phenolics within a plant under salt stress in relation to leaf age, may be due to the reflection of different requirements for counteracting abiotic stresses at different growth stages.

Correlation coefficients presented in Table 2 show that a significant positive correlation (0.242**) was observed between plant growth and leaf phenolic contents and a significant negative correlation (-0.138*) between leaf MDA and phenolic contents in both wheat cultivars under salt stress.

From the results, it can be concluded that high salt tolerance of cv. S-24 in terms of high biomass production may be due to low production of MDA content. This reduction in MDA contents may be due to reduced production of AOS hence decreased oxidative stress. A negative correlation in MDA and phenolic contents indicates the role of phenolics as antioxidant in scavenging AOS and there by decreasing MDA contents. Furthermore, different growth stages of the crop shows differential response to salt stress in different wheat cultivars in terms of production of MDA and phenolic contents.

Acknowledgment

The work presented in this manuscript is a part of research work being conducted by PhD scholar Muhammad Arslan Ashraf (PIN No. 074-0188-Bm4-056) whose study is funded by the Higher Education Commission through Indigenous Ph.D. Scheme.

References

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech. Advan*., 27: 84-93.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env. Exp. Bot*., 59: 206-216.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166: 3-16.

Badawi, G.H, Y. Yamauchi, E. Shimada, R. Sasaki, N. Kawano and K. Tanaka. 2004. Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Sci*., 66: 919-28.

Barros, L., P. Baptista and I.C.F.R. Ferreira. 2007. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food Chem. Toxicol*., 45(9): 1731-1737.

Brankova, L., S. Ivanov, V. Alexieva and E. Karanov. 2005. Salt induced alteration in the levels of some oxidative parameters and unspecific defence compounds in leaves of two plant species (cotton and bean) with different sensitivity to salinity. *Compt. Rend. De I`Acad. Bulgare des Sci*., 58(11): 1307-1312.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutr. Rev*., 56: 317-333.

Carmak, I. and J.H. Horst. 1991. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*., 83: 463-468.

Chen, C., C. Tao, H. Peng and Y. Ding. 2007. Genetic analysis of salt stress responses in asparagus bean (*Vigna unguiculata* (L.) ssp. *sesquipedalis* Verdc.). *J. Hered.*, 98(7): 655-665.

Choi, Y., S.M. Lee, J. Chun, H.B. Lee and J. Lee. 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of shiitake (*Lentinus edodes*) mushroom. *Food Chem.*, 99: 381-387.

Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba and C. Abdelly. 2008. Phenolic composition of *Cynara cardunculus* L., organs and their biological activities. *C. R. Biologies*, 331: 372-379.

Hernandez, 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.

Hichem, H., D. Mounir and E.A. Naceurc. 2009. Differential responses of two maize (*Zea mays* L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages. *Ind. Crops Prod*., 30: 144-151.

Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Agric. Food Chem*., 33: 213-217.

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.

Koca, H., M. Bor, F. Özdemir, I. Türkan. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Env. Exp. Bot*., 60: 344-351.

Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. *Modern Appl. Sci.*, 3: 171-176.

Liang, Y., Q. Chen, Q. Liu, W. Zhang and R. Ding. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol*., 160 (10): 1157-1164.

Meloni, D.A, M.A. Oliva, C.A. Martinez and J. Cambraia. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Env. Exp. Bot*., 49: 69-76.

Mittova, V., M. Guy, M. Tal and M. Volokita. 2002. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt dependent oxidative stress: increased activities of antioxidant enzymes in root plastids. *Free Radic. Res*., 36: 195-202.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Env*., 25: 239-250.

Neill, S.J., R. Desikan, A. Clarke, R.D. Hurst and J.T. Hancock. 2002. Hydrogen peroxide and nitric oxide as signalling molecules in plants. *J. Exp. Bot*., 53: 1237-1247.

Parida, A., A.B. Das, Y. Sanada and P. Mohanty. 2004. Effects of salinity on biochemical components of the mangrove *Aegiceras corniculatum*. *Aquat. Bot*., 80: 77-87.

Posmyk, M.M., R. Kontek and K.M. Janas. 2009. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Env. Safety*, 72: 596-602.

Rice-Evans, C., N.J. Miller and G. Paganga. 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci*., 2: 152-159.

Rice-Evans, C.A., N.J. Miller and G. Paganga. 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Medi*., 20: 933-956.

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, S. Agarwal and R.C. Meena. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol. Plant.*, 49: 85-91.

Shao, H.B., Z.S. Liang and M.A. Shao. 2005. Changes of some anti-oxidative enzymes under soil water deficits among 10 wheat genotypes at maturation stage. *Colloids Surf. B: Biointerfaces*., 45: 7-13.

Steel, R.G.D. and J.H. Torrie. 1986. *Principles and procedures of statistics*. McGraw Hill Book Co., Inc. New York, NY. 1986.

Teisseire, H. and V. Guy. 2000. Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*). *Plant Sci*., 153: 65-72.

Tsai, P. J., J. Mc-Instosh, P. Pearce, B. Camden and B.R. Jordan. 2002. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. *Food Res. Int*., 35: 351-356.

Wang, Y. and N. Nil. 2000. Changes in chlorophyll, ribulose biphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hort. Sci. Biotechnol*., 75: 623-627.

(Received for publication 17 July 2009)