Europ.J.Hort.Sci., **71** (4). S. 169–172, 2006, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Ion and Lipid Peroxide Content in Sensitive and Tolerant Eggplant Callus Cultured under Salt Stress

F. Yasar[1)], S. Ellialtioglu[2)] and S. Kusvuran[1)]
([1)]Department of Horticulture, Faculty of Agriculture, University of Yuzuncu Yil, Van, Turkey and [2)]Institute of Biotechnology, University of Ankara, Diskapi-Ankara, Turkey)

Summary

The objective of present study was to determine difference in ion and MDA (malondialdehyde) content of tolerant and sensitive genotypes calli grown under salinity condition. For this purpose, two tolerant and two sensitive local Turkish eggplant varieties were used.

After 8 days of being cultured, callus growth of all genotypes showed a decrease in salted medium as compared to control medium. When grown in salted medium, sensitive lines produced less callus, accumulated lower K and Ca but higher Na and Cl than tolerant lines. Consequently, tolerant genotypes had higher K/Na and lower Na/Ca ratios than sensitive genotypes. K/Na and Na/Ca ratios were used as parameters determining salinity tolerance in eggplant.

The magnitude of lipid damage, as measured by MDA, was lower in the tolerant genotypes. MDA content of tolerant genotypes was two fold less than those of sensitive genotypes in NaCl treatment medium. MDA content of calli of the four eggplant varieties investigated in this study were also found as a significant indicator determining salt tolerant.

The present results demonstrated that the response of cultured calli of eggplant to salt stress was clearly genotype specific.

Key words. eggplant – ions – lipid peroxide – MDA amount – salt stress – tissue culture

Introduction

Salinity is one of the major abiotic stress factors affecting plant growth and productivity globally. Crop performance may be adversely affected by salinity-induced nutritional disorders. Elevated NaCl causes an increase in Na^+ concentration and a decrease in K^+ and Ca^{2+} concentration (Chartzoulakis and Loupassaki 1997; Munns et al. 2002). In addition, accumulation of Na^+ ions changes ion balance such as Na/Ca and K/Na ratio in plant cells under saline condition. A high Na/Ca ratio results in increased cell permeability (Levitt 1980). Ion disorders caused from salinity may also lead to changes in plant lipid metabolism (Kuiper 1985). When examining the effects of environmental stresses on plant membranes, many researchers have also measured the products of lipid peroxidation, such as malondialdehyde (MDA) and/or ethane (Wilson and McDonald 1986).

Eggplant is moderate sensitive to salinity (Savvas and Lenz 1996; Akıncı et al. 2004) but more attention to salinity is required in agricultural production with eggplant and its varieties. Some researcher have reported that vegetative growth of eggplant, i.e. shoot length, plant leaf area and dry weight were significantly reduced at salinities higher than 50 mM, and that leaf tissue concentration of Cl^- and Na^+ increased significantly with increasing salinity while K showed a trend to decrease.

In our previous study (Yasar 2003), 38 different eggplant genotypes grown under 150 mM NaCl were classified according to some growth parameters (i.e. shoot length, plant leaf area, leaf number, fresh and dry weight) as well as some antioxidant enzymes activities *in vivo* and *in vitro*. Most of these genotypes (four of them are registered open pollinated local cultivars and twenty-seven of them are landraces grown in different parts of Turkey) belong to *Solanum melongena* L.. The others belong to *S. sisymbriifolium, S. aethiobicum* and *S. torvum*. At the end of the study, two salt tolerant and two sensitive genotypes were selected according to growth parameters measured.

In present work, MDA and ions (Na, K, Ca and Cl) content in callus of the salt-tolerant ('Mardin Kızıltepe' and 'Burdur Bucak') and salt-sensitive ('Artvin Hopa' and 'Giresun') eggplant genotypes were studied for better understanding of their susceptibility to salt stress. Additionally, it is aimed to provide information on the ability of salt-tolerance eggplant genotypes to determine possible relationship between oxidative damage and ion accumulation in callus culture under salt stress.

Material and Methods

Plant material and culture conditions

As plant material, two tolerant cultivars 'Mardin Kızıltepe' (MK) and 'Burdur Bucak' (B) and two sensitive cvs. 'Artvin Hopa' (AH) and 'Giresun' (G) local Turkish

eggplant cultivars, whose salt tolerance situations were determined in our previous study (YASAR 2003), were used.

Eggplant seeds were surface sterilized for 20 min in a 25 % sodium hypochlorite solution containing 0.1 % Tween 20, rinsed several time with distilled water and germinated in MS basal medium without hormones under aseptic conditions.

Calli were initiated from cotyledons of 3 week-old seedlings in MS (MURASHIGE and SKOOG 1962) medium to which 1.0 mg l^{-1} 2.4-D, 0.1 mg l^{-1} kinetin, 3 % sucrose and 0.7 % agar were added, and incubated at 26±2 °C under darkness. After 4 weeks, calli were separated from the explants and sub-cultivated on MS medium described above, added with 150 mM NaCl and without salt as control. 15 Petri dishes were used for each treatment. 1 g of each callus culture was put into each Petri dishe. The calli were left to develop in salted and unsalted conditions for 8 days. Then total weights of calli were determined.

Determination of ion content

For ions determination, fresh callus samples were extracted in concentrated 0.1 N nitric acid. N, K and Ca contents were determined by flame photometry in samples from calluses (TALEISNIK et al. 1997). For chloride determination, fresh callus material was extracted with 0.1 N HNO_3 in 10 % (v:v) acetic acid and Cl^- was determined by the silver ion-titration method with a automatic chloridometer (Buckhler–Cotlove chloridometer) according to BOZCUK (1970).

Lipid peroxide content (MDA)

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction (HEATH and PACKER 1968). Frozen samples were homogenized in a pre-chilled mortar with two volumes of ice-cold 0.1 % (w/v) tricloroacetic acid (TCA) and centrifuged for 15 min at 15000 x g. Assay mixture containing 1 ml aliquot of the supernatant and 2 ml of 0.5 % (w/v) thiobarbituric acid in 20 % (w/v) tricloroacetic acid (TCA) was heated to 95 °C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10000 x g for 10 min at 4 °C), the supernatant absorbance (532 nm) was read and values corresponding to non-specific absorption (600 nm) were subtracted. The MDA content was calculated according to the molar extinction coefficient of MDA (155 mM^{-1} cm^{-1}).

Table 1. Callus development of salt-sensitive and salt-tolerant eggplant genotypes.

Genotype	Callus weight (g FW)	
	Control	NaCl
'Mardin Kızıltepe' (MK)	1.308 b	1.144 c
'Burdur Bucak' (BB)	1.410 a	1.138 c
'Giresun' (G)	1.289 b	1.036 d
'Artvin Hopa' (AH)	1.371 ab	1.016 d

Mean values indicated by the same latter are not significant different (p≤0.01).
FW: fresh weight

All results reported were the means of three replicates. After data were analysed by analyses of variance, the means were compared by use and Duncan's multiple range test (P<0.01).

Results

The changes of the callus weights

In terms of callus weight, not only differences between control and salinity treatment but also differences between sensitive and tolerant genotypes were found to be significant. The callus weights of tolerant genotypes (MK and BB) were found to be higher than those of sensitive genotypes (AH and G) in saline media (p≤0.01) (Table 1).

Ion content

Saline treatment increased sodium (Na^+) in all of the callus lines compared with control groups. In the salt medium, the MK callus accumulated significantly less Na^+ than other callus lines. AH callus had the highest Na^+ accumulation in the salt medium. All of the callus lines showed a decreased K^+ content with salt treatment. Potassium contents of MK and BB callus lines were found to be higher than those of G and AH callus lines in the salt medium. Callus lines exposed to salt accumulated higher Cl^- levels than those maintained in control medium. Cl^- accumulation in AH callus was the very highest in four callus line.

Table 2. The effects of NaCl salinity on changes Na^+, K^+, Ca^{2+} contents in eggplant calli.

Genotype	Na^+ (μg mg FW^{-1})		K^+ (μg mg FW^{-1})		Cl^- (μg mg FW^{-1})		Ca^{2+} (μg mg FW^{-1})	
	Control	NaCl	Control	NaCl	Control	NaCl	Control	NaCl
'Mardin Kızıltepe' (MK)	0.370 a	2.263 d	1.513 a	0.870 a	0.427 a	1.353 c	1.713 a	1.057 a
'Burdur Bucak' (BB)	0.343 a	3.250 c	1.193 b	0.720 b	0.490 a	2.030 b	1.577 a	0.870 b
'Giresun' (G)	0.347 a	3.357 b	1.650 a	0.540 c	0.497 a	2.600 b	1.653 a	0.720 c
'Artvin Hopa' (AH)	0.270 b	4.147 a	1.070 b	0.460 d	0.403 a	4.040 a	1.643 a	0.700 c

Mean values indicated by the same latter in same column are not significant different (p≤0.01).
FW: fresh weight

Table 3. The effects of NaCl salinity on K^+/Na^+ and Na^+/Ca^{2+} ratios and amounts of MDA in eggplant calli

Genotype	K^+/Na^+		Na^+/Ca^{2+}		MDA (μmol g FW^{-1})	
	Control	NaCl	Control	NaCl	Control	NaCl
'Mardin Kızıltepe' (MK)	4.083 ab	0.384 a	0.216 a	2.164 d	2.595 a	2.697 c
'Burdur Bucak' (BB)	3.480 b	0.222 b	0.228 a	3.756 c	2.054 b	2.798 c
'Giresun' (G)	4.803 a	0.161 c	0.162 b	4.663 b	2.245 b	4.062 b
'Artvin Hopa' (AH)	4.046 ab	0.111 d	0.209 a	5.924 a	1.227 c	4.732 a

Mean values indicated by the same latter in same column are not significant different ($p \leq 0.01$).
FW: fresh weight

It is followed by those in G and BB and the lowest Cl^- contend was found in MK callus line Calcium (Ca^{2+}) accumulation of callus decreased with salt treatment in four genotypes. The decreases in MK and BB were lower than those in G and AH (Table 2).

K^+/Na^+ ratios of callus lines grown in salted medium were lower than those maintained in non salinity medium. From the aspect of K^+/Na^+ ratios, differences among the genotypes were significant. In salted medium, the highest K^+/Na^+ ratio was found in MK and the lowest ratio was acquired in AH. On the other hand, Na^+/Ca^{2+}ratios of callus lines grown in salted medium were higher than those grown in control medium. Under the salinity conditions, MK and BB calli had lower Na^+/Ca^{2+} than AH and G calli (Table 3).

Lipid peroxide content (MDA)

With regard to malonyldialdehyde (MDA), significant differences were found between genotypes both in control and salted medium. MDA amounts increased with salt treatment in all of the callus lines. These increases of MDA were very clear in G and AH. While being the lowest in control medium, MDA content of AH was the highest in salted medium. On the other hand, while being the lowest in salted medium, MDA content of MK was highest in control medium (Table 3).

Discussion

After 8 days of being cultured, callus growth of all genotypes showed a decrease in salted medium as compared to control medium. These decreases were found to be significant statistically. Likewise, many researchers reported that calli growth in salt-containing medium was less than that in control medium (Paek et al. 1988; Liu and Staden 2000).

When grown in salted medium, sensitive lines (AH and G) produced less callus than tolerant lines (MK and BB). While the tolerant line MK produced 12 % less callus, the sensitive line (AH) produced 25 % less callus in salt-containing medium with respect to control medium. Several researcher, using different plant species, have reported similar differences between sensitive and tolerant genotypes (Liu and Staden 2000; Garratt et al. 2002)

Accumulation of Na^+ and Cl^- is a common osmoregulatory response to NaCl stress (Levitt 1980). At the same time, NaCl treatment causes a decrease in concentration of K and Ca in plant (Chartzoulakis and Loupassaki 1997; Munns et al. 2002). Likewise, the result of the present study showed that NaCl treatment caused an increase in Na and Cl concentration, and a decrease in K^+ and Ca^{2+} concentration in all line. However, the increase in Na^+ and Cl^- concentration was higher in sensitive lines (G and AH) than in tolerant lines (MK and BB) and the decrease in K^+ and Ca^{2+} concentration was higher in sensitive than in tolerant lines. This situation caused the tolerant lines to have the higher K/Na ratio and a lower Na/Ca ratio compared to the sensitive ones. These ion ratios were reported to be associate with the relatively salt tolerant in many species, where tolerant lines had higher Ka/Na and lower Na/Ca ratios (Dvorak et al. 1994; Perez-Alfocea et al. 1996). A high K/Na ratio in the cytosol is essential for normal cellular functions of plants. Na^+ competes with K^+ uptake and may block the K^+ specific transporters under salinity. This results in toxic levels of Na^+ as well as insufficient K^+ concentration for enzymatic reaction and osmotic adjustment (Zhu 2003). In some of previous studies, it was observed that tolerant lines regulated the osmotic potential more effectively by avoiding the uptake of Na^+ and Cl^- and a simultaneous absorption of more essential ions like K^+ (Shanon and Noble 1995; Sivritepe et al. 2003). As has been seen in other species, in this study, K/Na and Na/Ca ratios appeared to determine salinity tolerance also in eggplant.

The generation of radical oxygen species is a common response to environmental stress conditions, such as salinity and drought (Luna et al. 1994; Gosset et al. 1994). Radical oxygen species cause membrane lipid peroxidation, reducing membrane fluidity and selectivity. Lipid peroxidation measured as MDA content is considered to be indicator of oxidative damage from stress (Dhindsa and Matowe 1981).

Our results show that salt stress elicits oxidative stress, measured as lipid peroxidation, as has been seen in other species (Dhindsa and Matowe 1981; Luna et al. 2000), and that the magnitude of lipid damage was lower in the tolerant genotypes MK and BB. In the control medium, difference between MDA content of BB (tolerant genotype) and G (sensitive genotype) was not found to be significant. Moreover, MDA content of MK (tolerant genotype) was higher than that of AH (sensitive genotype) in the control medium. On the other hand, MDA content of tolerant genotypes was two fold less than those of sensitive genotypes in NaCl treatment medium. Thus, MDA content of calli of the four eggplant varieties investigated in this study were also found as a significance indicator of salt tolerant.

References

AKİNCI, I.E., S.AKINCI, K. YILMAZ and H. DIKICI 2004: Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. New Zealand J. Crop and Hortic. Sci. **32**, 1993–2000.

BOZCUK, S. 1970: Water and salt relations of statice species with particular reference to the problem of halophytes. Ph.D. Thesis University of Sussex, UK.

CHARTZOULAKIS, K.S. and M.H. LOUPASSAKI 1997: Effects of NaCl salinity on germination, growth, gas exchange and yield of greenhouse eggplant. Agric. Water Management **32**, 215–225.

DHINDSA, R.S. and W. MATOWE 1981: Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. J. Bot. **32**, 79–91.

DVORAK, J., M.M. NOAMAN, S. GOYAL and J. GORHAM 1994: Enhancement of the salt tolerance of *Triticum turgidum* L. by the Kna-locus transferred from the *Triticum aestivum* L. chromosome 4D by homoeologous recombination. Theor. Appl. Genet. **87**, 872–877.

GARRATT LEE, C., B.S. JANAGOUDAR, K.C. LOWE, P. ANTHONY and J. BRIAN POWER 2002: Salinity tolerance and antioxidant status in cotton cultures. Free Radical and Medicine, **33**, 502–511.

GOSSETT, D.R., P. MILLHOLON and C. LUCAS 1994: Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. **34**, 706–714.

HEATH, R. and L. PACKER 1968: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. **196**, 385–395.

KUIPER, P.J.C. 1985: Enviromental chance of lipid metabolism of higher plants. Physiol. Plant. **64**, 118–122

LEVITT, J. 1980: Responses of plants to environmental stresses. Vol. II, 2nd ed. Academic Press, New York, pp. 607.

LIU, T. and J. VAN STADEN 2000: Selection and characterization of sodium chloride-tolerant callus of *Glycine max* (L.) Merr. cv. 'Acme'. Plant growth regulation **31**, 195–207

LUNA, C., C. GONZALEZ and V. TRIPPI 1994: Oxidative damage caused by an excess of copper I oat leaves . Plant Cell Physiol. **35**, 11–15.

LUNA, C., L. GARCIA SEFFINO, C. ARIAS and E. TALEISNIK 2000: Oxidative stress indicators as selection tools for salt tolerance in *Choloris gayana*. Plant Breeding **119**, 341–345.

MUNNS, R., S. HUSAIN, A.R. RIVELLI, R.A. JAMES, A.G. CONDON, M. P. LINDSAY, E.S. LAGUDAH, D.P. SCHACHTMAN and R.A. HARE 2002: Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant and Soil. **247**, 93–105.

MURASHIGE, T. and F. SKOOG 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473–497.

PAEK, K.Y., F. STEPHEN and A.T. TREVOR 1988: Physiological effects of Na_2SO_4 and NaCl on callus cultures of *Brassica compestris* (Chinese cabbage). Physiol. Plant. **72**, 160–166.

PEREZ-ALFOCEA, F., M.E. BALIBREA, A. SANTA CRUZ and M.T. ESTAN 1996: Agronomical and physiological characterization of salinity tolerance in a commercial tomato hybrid. Plant Soil **180**, 251–257.

SAVVAS, D. and F. LENZ 1996: Influence of NaCl concentration in the nutrient solution on mineral composition of eggplants grown in sand culture. Angew. Bot. **70**, 124–127.

SIVRITEPE, N., H.O. SIVRITEPE and A. ERIS 2003: The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. Sci.. Hort. **97**, 229–237.

SHANON, M.C. and C.L. NOBLE 1995: Variation in salt tolerance and ion accumulation among subterranean clover cultivars. Crop. Sci. **35**, 789–804.

TALEISNIK, E., G. PEYRANO and C. ARIAS 1997: Response of *Chloris gayana* cultivars to salinity. 1. Germination and early vegetative growth. Trop. Grassl. **31** 232–240.

WILSON, D.O. and M.B. JR. MCDONALD 1986: The lipid peroxidation model of seed aging. Seed Sci. Tech. **14**, 269–300.

YASAR, F. 2003: Investigation of some antioxidant enzyme activities in eggplant genotypes grown under salt stress *in vitro* and *in vivo*. Ph.D. Thesis, Yuzuncu Yıl Univ. Fen Bil. Enst.(In Turkish) Van. 139 p.

ZHU, J.K. 2003: Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. **6**, 441–445.

Received December 15, 2004 / Accepted September 14, 2005

Addresses of authors: Fikret Yasar and Sebnem Kusvuran, Department of Horticulture, Faculty of Agriculture, University of Yuzuncu Yil, Zeve Campus, 65080, Van, Turkey, and Sebnem Ellialtioglu, Institute of Biotechnology, University of Ankara, Diskapi-Ankara, Turkey, e-mail: yasarfikret@yahoo.com.