ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/235950605

Determination of anti-oxidant activities in some melon (Cucumis melo L.) varieties and cultivars under salt stress

Article *in* Journal of Horticultural Science and Biotechnology · April 2006

DOI: 10.1080/14620316.2006.11512115

CITATIONS
33

READS
229

3 authors:



Fikret Yasar
Yuzuncu Yil University
33 PUBLICATIONS **343** CITATIONS
SEE PROFILE



Sebnem Kusvuran
Çankırı Karatekin Üniversitesi
68 PUBLICATIONS **235** CITATIONS
SEE PROFILE



Sebnem Ellialtioglu
Ankara University
52 PUBLICATIONS **242** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Determination of physiological and biochemical tolerance level in different pepper genotypes under salt and drought View project

All content following this page was uploaded by Sebnem Kusvuran on 10 February 2017.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.

Journal of Horticultural Science & Biotechnology (2006) **81** (4) 627–630

Determination of anti-oxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress

By FIKRET YASAR[1*], SEBNEM KUSVURAN[1] and SEBNEM ELLIALTIOGLU[2]
[1]Department of Horticulture, Faculty of Agriculture, University of Yuzuncu Yil, Zeve Campus, 65080 Van, Turkey
[2]Institute of Biotechnology, University of Ankara, Besevler-Ankara, Turkey
(e-mail: fyasar@yyu.edu.tr) *(Accepted 31 January 2006)*

SUMMARY
The response of the antioxidant system to salt stress was studied in leaves of seedlings of four Turkish melon (*Cucumis melo* L.) varieties 'Besni', 'Yuva', 'Midyat' and 'Şemame' and three melon cultivars 'Ananas', 'Galia C8' and 'Galia F1'. The anti-oxidant capability of plants was determined by measuring ascorbate peroxidase (APX) and glutathione reductase (GR) activities together with levels of the anti-oxidant, ascorbic acid (AA). Salt treatment increased APX activities in stress-tolerant 'Galia C8' and 'Galia F1' and in moderately tolerant 'Besni', 'Midyat' and 'Şemame'. These increases were greater in salt-tolerant cultivars. On the other hand, salt treatment did not cause a significant increase in APX activity in the salt-sensitive cvs. 'Yuva' and 'Ananas'. GR activity increased in all varieties and cultivars grown under saline conditions compared to control plants. Increases in GR activities were higher in salt-tolerant and moderately-tolerant varieties than in salt-sensitive ones. Likewise, AA contents increased with salt treatment in all varieties and cultivars except the salt-sensitive 'Ananas'. The data indicate that melon seedlings respond to salt-induced oxidative stress by increasing both their enzymatic and non-enzymatic anti-oxidant defense systems. These increases were most clear in the salt-tolerant cultivars.

Melon (*Cucumis melo* L.) is an important horticultural crop, often cultivated in arid and semi-arid regions of the World, where salinity threatens to become, or is already a problem. In general, melon is known to be moderately tolerant to salinity. It has been shown that salinity causes several types of damage such as growth inhibition (Franco *et al*., 1997; Mendlinger, 1994), metabolic disturbances (Mavrogianopoulos, 1999), and yield and quality losses (Del Amor, 1999). However, it has also been reported that the severity of salt damage is dependent on cultivar (Shannon *et al*., 1984; Carvajal, 1998). Therefore there is a need to develop cultivars with higher salt-tolerance.

Salt-tolerance in higher plants is the result of a number of physiological responses. There is evidence that high salt concentrations cause an imbalance in cellular ions, resulting in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS) which cause damage to DNA, lipids and proteins (Hernandez *et al*., 1993). Plants possess several anti-oxidant enzyme systems that protect their cells from the negative effects of ROS. These include non-enzymatic anti-oxidants such as ascorbic acid, glutathione and carotenoids, as well as anti-oxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR; Lee *et al*., 2002). It has been reported that APX activity is the major component of the plant response to oxidative stress and exerts a protective role in salt-tolerance (Gueta-Dahan *et al*., 1997). Silvana *et al*. (2003) have demonstrated that anti-oxidant enzyme activities [SOD, APX, GR, guaiacol peroxidase (GPOX) and glutathione dehydrogenase (DHAR)] were higher in salt-adapted sunflower calli than in control calli.

Many reports suggest that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their anti-oxidant systems (Scandalio *et al*., 2001; Silvana *et al*., 2003). The aim of this study was to determine the activities of some anti-oxidative stress enzymes (e.g., APX, GR) and levels of the non-enzymatic anti-oxidant (AA) in some salt-sensitive or salt-tolerant melon varieties and cultivars grown in hydroponic culture under salt stress conditions. For this purpose, the salt-tolerant melon cultivars 'Galia C8' and 'Galia F1' (Franco *et al*., 1993; An *et al*., 2002), the moderately-tolerant varieties 'Midyat', 'Besni' and 'Semame', and the salt-sensitive varieties 'Yuva' and 'Ananas' (Kusvuran, 2004) were studied.

MATERIALS AND METHODS

Plant material and growth conditions

Four local Turkish melon varieties ('Besni', 'Yuva', 'Midyat' and 'Şemame') and three cultivars ('Ananas', 'Galia C8' and 'Galia F1') were used as plant material. All plants were grown under 280 μmoles m^{-2} s^{-1} of cool-white fluorescent light with a 16 h photoperiod in a controlled climate room at 28°/25°C day/night temperatures, and 70% relative humidity. Seeds were germinated in vermiculite moistened with distilled water. After 2 weeks, seedlings were transferred to plastic vessels filled with 4 l half-strength Hoagland's solution (Hoagland and Arnon, 1950). The solution in the vessels was replaced every week. Two weeks later, salt-treatment started and the NaCl concentration was increased by

*Author for correspondence.

50 mM d^{-1} until a final concentration of 100 mM was achieved. Non-salt-treated plants were kept as controls. Salt-stressed plants were subjected to 100 mM NaCl for 8 d and all plants, including controls, were then sampled.

Enzyme extractions and assays

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at –80°C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer, pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 × g for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C.

APX activity was determined by measuring the consumption of ascorbate by following absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 µmole ascorbate min^{-1} (Cakmak and Marschner, 1992).

GR activity was determined by measuring the enzyme-dependent oxidation of NADPH by following absorbance at 340 nm. One unit of GR activity was defined as the amount of enzyme that oxidised 1 µmole NADPH min^{-1} (Cakmak and Marschner, 1992).

Determining ascorbic acid content

Measurements of total ascorbic acid (AA) contents were carried out as described by Cakmak and Marschner (1992), as modified by Eker (2002). Approx. 0.5 g fresh leaf sample was extracted with 5 ml 5% *meta*-phosphoric acid and centrifuged at 4,000 × g for 30 min. A red colour resulted from the complex of bipyridine and Fe^{2+} caused by reduction of Fe^{3+} to Fe^{2+} by ascorbic acid and was measured at 525 nm.

All results were the means of three replicates, and each replicate consisted of ten plants Data were analysed statistically and treatment means were separated by Duncan's Multiple Range Test using SAS (1985) software.

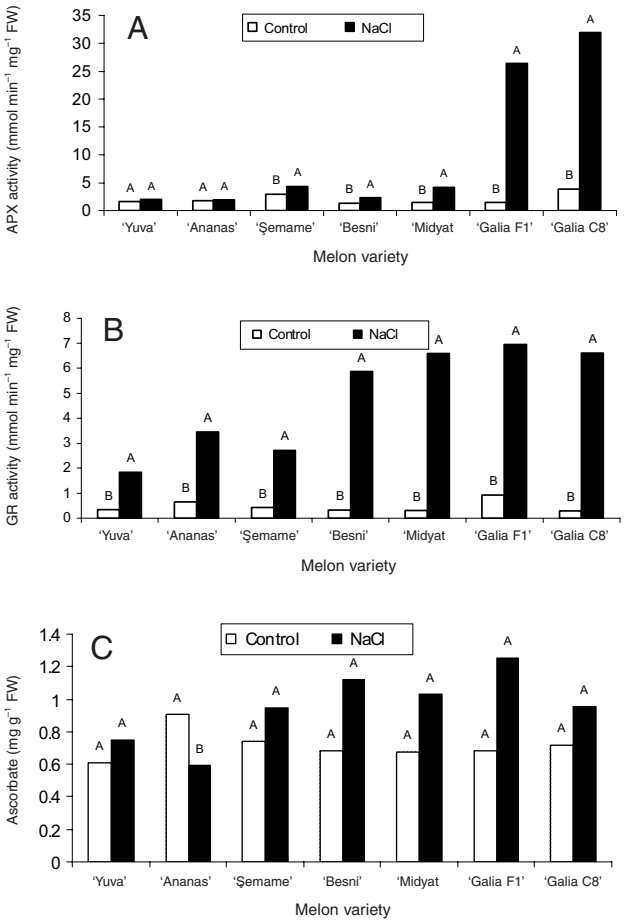


FIG. 1

Mean values (n = 30) of ascorbate per oxidase (APX; Panel A) and glutathione reductase (GR; Panel B) activities and ascorbic acid (AA; Panel C) concentrations in leaves of seven control (white columns) or 100 mM NaCl-treated (black columns) melon cultivars. Pairs of columns (mean values) with the same capital letter are not significantly different at P = 0.05, based on Duncan's multiple range test.

RESULTS

To determine the response of melon to salt-induced oxidative stress, APX and GR activities, and AA contents were measured in leaves of seedlings grown with or without 100 mM NaCl.

There were no significant differences in APX activity between varieties and cultivars, except for 'Galia C8' and 'Semame', grown under non-NaCl conditions. In non-salt control conditions, APX activities in 'Galia C8' and 'Semame' were found to be significantly higher than in other varieties and cultivars. Salt-stress caused an increase in APX activity in all treated varieties and cultivars, except 'Ananas' and 'Yuva'. This increase was highest in 'Galia C8' and 'Galia F1'. APX activities in 'Galia C8' and 'Galia F1', treated with 100 mM NaCl, were eight- and 17-fold higher, respectively than those measured in control plants (Table I; Figure 1).

Under non-salt control conditions, 'Galia F1' had the highest activity of GR among all cultivars and varieties studied. 'Galia F1' GR activity was followed by 'Ananas',

TABLE I

Effect of 100 mM NaCl on APX and GR enzyme activities and on AA content in seven varieties of melon seedlings

	Ascorbate Peroxidase (APX) (mmol min^{-1} mg^{-1} FW)		Glutathione Reductase (GR) (mmol min^{-1} mg^{-1} FW)		Ascorbic Acid (AA) (mg g^{-1} FW)	
Variety/cultivar	Control	NaCl	Control	NaCl	Control	NaCl
'Galia C8'	3.92 a	31.91 a	0.29 c	6.59 a	0.72 a	0.96 c
'Galia F1'	1.44 c	26.42 b	0.93 a	6.94 a	0.69 a	1.25 a
'Midyat'	1.42 c	4.22 c	0.31 c	6.59 a	0.67 a	1.03 bc
'Besni'	1.33 c	2.31 d	0.33 bc	5.86 a	0.68 a	1.12 b
'Semame'	2.88 b	4.37 c	0.42 bc	2.71 bc	0.74 a	0.95 c
'Ananas'	1.77 c	1.91 d	0.65 ab	3.46 b	0.90 a	0.59 e
'Yuva'	1.62 c	1.97 d	0.35 bc	1.82 c	0.61 a	0.75 d

Means followed by same lower-case letter in the same column are not significantly different at the $P = 0.05$ probability level, based on Duncan's Multiple Range Test. (n = 30)

'Semame', 'Yuva' and 'Besni', respectively. Salt-treatment increased GR activity in all cultivars and varieties, compared with their control groups. The increases in GR activities in 'Galia C8', 'Galia F1', 'Midyat' and 'Besni' were higher than those in 'Semame', 'Ananas' and 'Yuva'. GR activities in 'Galia C8', 'Galia F1', 'Midyat' and 'Besni' treated with salt were 23-, 7-, 21- and 17-fold higher, respectively, than those measured in control plants (Table I; Figure 1).

The AA contents of all control plants were similar, and no significant differences were found between varieties and cultivars. Salt-treatment changed the AA content of all varieties, and increased the AA content of six of the varieties used in this experiment. On the other hand, the only significant decrease in AA level was observed in 'Ananas' under salt- stress (Table I; Figure 1).

DISCUSSION

One consequence of salt-stress in plants is excessive generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Foyer *et al*., 1994; Mitler, 2002). Under normal growth conditions, the production of ROS in the cell is generally at low levels. However, under conditions of abiotic stress such as high salinity, cellular homeostasis is disrupted and leads to the production of relatively high levels of ROS (Polle, 2001; Mitler, 2002). These radicals can damage vital cellular macromolecules (e.g., *via* denaturation of proteins, mutation of DNA, and/or peroxidation of lipids). Plants have evolved both enzymatic and non-enzymatic mechanisms to scavenge ROS (Bowler and Van Montagu, 1992; Asada, 1999). Several reports have clearly demonstrated enhanced activity of various anti-oxidant enzymes under stressful conditions (Jiang and Zhang, 2002; Patsikka *et al*., 2002). However, ROS can also be scavenged non-enzymatically by anti-oxidants like AA.

Protecting against salt-stress damage, as in previous studies (Gossett *et al*., 1996; Karanlık, 2001), could be a result of higher levels of APX and GR anti-oxidative enzyme systems and increased AA concentration. In this experiment on melon seedlings, grown hydroponically, except for 'Galia C8' and 'Semame'. The APX activities in all other control plants were similar to one another. Salt treatment significantly increased APX activities in salt-tolerant cultivars ('Galia C8' and 'Galia F1') and in moderately-tolerant varieties ('Midyat', 'Besni' and 'Semame'). These increases were most dramatic in 'Galia C8' and 'Galia F1' which are reported to be tolerant to salt stress.

Shalata and Tal (1998) studied changes in the anti-oxidant enzyme activities in wild tomato species and in cultured tomatoes under salt-stress and reported that APX activity increased in salt-tolerant wild species and decreased in 'M82' which was a sensitive variety.

'Galia F1' had the highest GR activity among the control plants. Conversely, although 'Galia C8' was also reported to be salt-tolerant, it showed the lowest levels of GR activity among the control plants. Salt-treatment increased GR activities in all varieties and cultivars compared with control plants. This increase was clear in salt-tolerant 'Galia C8', 'Galia F1', and in moderately-tolerant 'Midyat' and 'Besni'. Likewise, previous studies reported that GR activities were higher in varieties of eggplant (Yasar, 2004) and rice (Vaidyanathan *et al*., 2003) with high salt-tolerance than in salt-sensitive genotypes.

In plants, one biochemical function of ascorbate is its anti-oxidative activity. Ascorbate reacts rapidly with superoxide, singlet oxygen and hydrogen peroxide and participates in the removal of these ROS which are generated during aerobic metabolism and during exposure to some stress conditions. It is generally believed that salt-treatment increases the AA content of plant, especially in salt-tolerant varieties.

In this study, the AA contents of control plants were similar and no significant differences were found between varieties and cultivars. Generally, NaCl-treatment increased the AA content of all varieties and cultivars used in this experiment. However, the only significant decrease in AA level was observed in the salt-sensitive cultivar 'Ananas' under salt stress. Under salt-treatment, the AA contents of the salt-sensitive varieties ('Ananas' and 'Yuva') were lower than those of the salt-tolerant ('Galia C8' and 'Galia F1') and moderately-tolerant varieties ('Midyat', 'Besni' and 'Semame'). Higher AA contents under salt-stress were also reported in salt-tolerant pepper species (Aktaş, 2002) and citrus species (Arbone *et al*., 2003).

We conclude that anti-oxidative enzyme activities play a protective role against salt-stress, and that anti-oxidative defense mechanisms were effective in providing resistance to salt-stress in melons grown hydroponically.

REFERENCES

AKTAS, H. (2002). *Selection and Physiological Characterization of Pepper for Resistance to Salinity Stress*. Ph.D. Thesis. Institute of Applied and Natural Science, University of Cukur Ova, Turkey. 105 pp. (In Turkish).

ARBONA, V., FLORS, V., JACAS, J., GARCÍA-AGUSTÍN, P. and GÓMEZ-CADENAS, A. (2003). Enzymatic and non-enzymatic antioxidant responses of Carrizo citrange, a salt-sensitive citrus rootstock, to different levels of salinity. *Plant Cell Physiology*, **44,** 388–394.

ASADA, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50,** 601–639.

BOWLER, C., VAN MONTAGU, M. D. and INZE, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43,** 83–116.

CAKMAK, I. (1994). Activity of ascorbate-dependent H_2O_2 scavenging enzymes and leaf chlorosis are enhanced in magnesium and potassium deficient leaves, but not in phosphorus deficient leaves. *Journal of Experimental Botany*, **45,** 1259–1266.

CAKMAK, I. and MARSCHNER, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiology*, **98,** 1222–1226.

Carvajal, M., Del Amor, F. M., Fernandez-Ballester, G., Martínez, V. and Cerdá, A. (1998). Time-course of solute accumulation and water relations in muskmelon plants exposed to salt during different growth stages. *Plant Science*, **138,** 103–112.

Del Amor, F. M., Martinez, V. and Cerda, A. (1999). Salinity duration and concentration affect fruit yield and quality, and growth and mineral composition of melon plants grown in perlite. *Horticultural Science*, **34,** 1234–1237.

Eker, S. (2002). *An Investigation on the Effects of Follar Nitrogen Applications on Low Temperature Stress in Lemons and Mandarins with Respect to Anti-oxidative Defense Mechanism*. Ph.D. Thesis. Institute of Applied and Natural Science, University of Cukur Ova, Turkey. 147 pp. (In Turkish).

Flowers, T. J. and Yeo, A. R. (1995). Breeding for salinity resistance in crop plants. *Australian Journal of Plant Physiology*, **22,** 875–884.

Foyer, C. H., Lelandais, M. and Kunert, K. J. (1994). Photo-oxidative stress in plants. *Plant Physiology*, **92,** 696–717

Franco, J. A., Esteban, C. and Rodriguez, C. (1993). Effect of salinity on various growth stages of muskmelon cv. 'Revigal'. *Journal of Horticultural Science*, **68,** 899–904.

Franco, J. A, Fernandez, J. A., Banon, S. and Gonzalez, A. (1997). Relationship between the effects of salinity on seedling leaf area and fruit yield of six muskmelon cultivars. *Horticultural Science*, **34,** 642–644.

Gossett, D. R., Millhollon, E. P., Lucas, M. C. and Marney, M. M. (1993). Antioxidant enzymes and salt stress in cotton. *Beltwide Research Conference National Cotton Council*, Memphis, TN, USA. 1262–1266.

Gossett, D. R., Millhollon, E. P., and Lucas, M. C. (1994a). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science*, **34,** 706–714.

Gossett, D. R., Millhollon, E. P., Lucas, M. C., Marney, M. M. and Banks, S. W. (1994b). The effects of NaCl on anti-oxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.). *Plant Cell Reports*, **13,** 498–503.

Gossett, D. R., Baris, S. W., Millhollon, E. P. and Lucas, M. C. (1996). Antioxidant response to NaCl stress in a control and an NaCl tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. *Plant Physiology*, **112,** 803–809.

Gueta-Dahan, Y., Yaniv, Z., Zilinskas, B.A. and Ben-Hayyim, G. (1997). Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in *Citrus*. *Planta*, **203,** 460–469.

Hernandez, J. A., Corpas, F. J., Gomez, M., Del Rio, L. A. and Sevilla, F. (1993). Salt-induced oxidative stres mediated by activated oxygen species in pea leaf mitochondria. *Physiologia Plantarum*, **89,** 103–110.

Jiang, M. and Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany*, **53,** 2401–2410.

Karanlik, S. (2001). *Resistance to Salinity in Different Wheat Genotypes and Physiological Mechanisms Involved in Salt Resistance*. Ph.D. Thesis. Institute of Natural and Applied Science, University of Cukur Ova, Turkey. 122 pp. (In Turkish).

Kusvuran, S. (2004). *Possibilities of Using of Antioxidant Enzyme Activity and Lipid Peroxidation for Determination of Salt Tolerance in Melon (*Cucumis melo *L.)*. M.Sc. Thesis. Graduate School of Natural and Applied Sciences, Department of Horticulture, Ankara University, Turkey. 110 pp. (In Turkish)

Lee, C., Garratt, B. S., Janagoudar, K. C., Lowe, P., Anthony, J., Power, B. and Davey, M. R. (2002). Salinity tolerance and antioxidant status in cotton cultures. *Free Radical Biology and Medicine*, **33,** 502–511.

Lin, C. C. and Kao, C. H. (2000). Effect of NaCl stress on H_2O_2 metabolism in rice leaves. *Plant Growth Regulation*, **30,** 151–155

Mavrogianopoulos, G. N., Spanakis, J. and Tsikalas, P. (1999). Effect of carbon dioxide enrichment and salinity on photosynthesis and yield in melon. *Scientia Horticulturae*, **79,** 51–63.

Mendlinger, S. (1994). Effect of increasing plant density and salinity on yield and fruit quality in muskmelon. *Scientia Horticulturae*. **57,** 41–49.

Mitler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, **7,** 405–410.

Patsikka, E., Kairavuo, M., Sersen, F., Aro, E. M. and Tyystjarvi, E. (2002). Excess copper predisposes photosystem II to photoinhibition *in vivo* by out-competing iron and causing a decrease in leaf chlorophyll. *Plant Physiology*, **129,** 1359–1367.

Perez-Alfocea, F., Estan, M. T., Santa, C. A. and Bolarin, M. C. (1993). Effects of salinity on nitrate, total nitrogen, soluble protein and free amino acid levels in tomato plants. *Journal of Horticultural Science*, **68,** 1021–1027.

Polle, A. (2001). Dissecting the superoxide dismutase-ascorbate-glutathione pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. *Plant Physiology*, **126,** 445–462.

Scandalio, L. M., Dalurzo, H. C., Gómez, M., Romero-Puertas, M. C. and Del Río, L. A. (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, **52,** 2115–2126.

SAS (1985). *SAS/State User's Guide*. 6.03 Edition. SAS Institute, Cary, NC, USA.

Shalata, A. and Tal, M. (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*. *Physiology Plantarum*, **104,** 169–174.

Shannon, M. C., John, G. W. and McCreight, J. D. (1984). Salt tolerance among muskmelon genotypes during seed emergence and seedling growth. *Horticultural Science*, **19,** 828–830.

Silvana, B. D., Gallego, S. M., Benavides, M. P. and Tomaro, M. L. (2003). Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. *Plant Growth Regulation*, **40,** 81–88.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. *Plant Science*, **165,** 1411–1418

Yasar, F. (2003). *Investigation of Some Antioxidant Enzyme Activities in Eggplant Genotypes Grown Under Salt Stress* in vitro *and* in vivo. Ph.D. Thesis. Institute of Natural and Applied Science, University of Yuzuncu Yil, Turkey. 139 pp. (In Turkish).

View publication stats