African Journal of Biotechnology Vol. 11(3), pp. 635-641, 10 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2119
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Antioxidative enzyme activities in the leaves and callus tissues of salt-tolerant and salt-susceptible melon varieties under salinity

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Accepted 1 December, 2011

The response of the antioxidant system to salt stress was studied in the leaves and callus tissues of 4 Turkish melon varieties (*Cucumis melo* L.) Besni, Yuva, Midyat, and Semame and a melon cultivar Galia C8. The antioxidant capability of the plants was determined by measuring superoxide dismutase (SOD) and catalase (CAT) activities. On the 8th day of the salt stressed callus culture, growth reductions were observed in the weight of the callus in the salty medium compared to the control in all varieties. Salt treatment increased enzyme activities in stress-tolerant Galia C8, Midyat, and Semame. On the other hand, salt treatment did not cause a significant increase in SOD activity in the callus tissues of salt-sensitive Yuva and moderately tolerant Besni. CAT activity increased in all of the genotypes grown under saline conditions compared with control calli. Increases in CAT activities were higher in salt-tolerant Galia C8 and Midyat than in all of the other varieties used in the callus culture. Data indicate that melon plants respond to salt-induced oxidative stress by increasing their enzymatic antioxidant defense systems. The results taken from the experiments with intact melon plants were obtained in the same manner as the callus culture.

Key words: Antioxidant, callus culture, *Cucumis melo*, enzyme, salt tolerance.

INTRODUCTION

Salinity stress, which usually occurs in arid and semiarid regions, is a major environmental constraint to crop productivity. Most of the crop plants are susceptible and cannot survive under conditions of high salinity. Plant species and cultivars within a crop species differ greatly in their response to salinity (Marschner, 1995; Dasgan et al., 2002). Since activated oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) can seriously disrupt normal metabolism through oxidative damage to lipids, protein, and nucleic acids, plants possess a number of antioxidant enzymes that protect them from these potential cytotoxic effects (Gossett et al., 1994a; Kusvuran et al., 2007; Li, 2009; Chookhampaeng, 2011). When plants are subjected to environmental stress such as temperature extremes, drought, herbicide treatment, and mineral deficiency, the balance between the production of reactive oxygen species and the quenching activity of antioxidants is upset, often resulting in oxidative damage.

Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Yasar, 2007; Dolatabadian et al., 2008; Amirjani, 2010; Siringam et al., 2011). The authors have reported a large variation among cotton varieties (Gossett et al., 1994b), tomato (Shannon et al., 1987; Cuartero and Fernandez-Munoz,

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Abbreviations: SOD, Superoxide dismutase; **CAT,** catalase.

1999), and melon genotypes (Kusvuran, 2010) in their response to salinity. Genetic variability within a species is a valuable tool for screening and breeding for higher salt tolerance.

Melon (*Cucumis melo* L.) is an important vegetable 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. However, it has been shown that salinity causes several types of damage such as yield and quality loses (Del Amor et al., 1999) and growth inhibition (Mendlinger, 1994; Kusvuran et al., 2007; Kusvuran, 2010). Furthermore, it has also been reported that the severity of salt damage is dependent on the cultivar (Carvajal et al., 1998; Yasar et al., 2006; Kusvuran 2010). Therefore, there is a need to develop new cultivars with higher salt tolerance.

Turkey is very rich in cucurbit genetic resources due to its diverse geographical and ecological situation. Turkey is one of the important diversity centers for cultivated cucurbits because of the adaptation to diverse ecological conditions as a result of natural selection and also the farmers' selection in accordance to their preference. Plant genetic resources are the most important sources for breeding new cultivars (Sari et al., 2008). Turkey has valuable genetic resources for melons (*cantalupensis*, *inodorous*, and *flexuosus*) (Küçük et al., 2002; Gomez-Guillamon et al., 2004; Sari and Solmaz, 2007).

Many reports suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems (Silvana et al., 2003; Yasar, 2007; Kusvuran, 2010). Studies with other non-halophytes (Smith and McComb, 1981, Gossett et al., 1994a) have shown that the degree of salt tolerance observed in the whole plant is also exhibited in callus tissue. Other research on squash and eggplant indicated that superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzyme activities in salt-tolerant genotypes are higher compared to salt-susceptible genotypes in both seedling and callus tissues (Yaşar, 2003; Sevengor, 2010). The aim of this study was to determine the activities of 2 antioxidative stress enzymes (example SOD, CAT) in some salt-sensitive or salt-tolerant melon varieties grown in hydroponic culture under salt-stress conditions. On the other hand, another aim of this study was to determine the presence of a relationship between antioxidant enzyme activity in the callus tissue and in the seedlings.

MATERIALS AND METHODS

The plant materials used were 1 salt-tolerant melon cultivar, Galia C8 (Franco et al., 1993); 3 moderately-tolerant local Turkish varieties, Midyat, Semame, and Besni; and 1 salt-sensitive local Turkish melon cultivar, Yuva, whose salt tolerance situations were determined in our previous study (Kusvuran, 2004).

Hydroponic culture

All of the 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. The seeds were germinated in vermiculate moistened with distilled water. After 2 weeks, the seedlings were transferred to plastic vessels filled with 4 L of half-strength Hoagland's solution (Hoagland and Arnon, 1938). The solution in the vessels was replaced every week. Salt treatment started 2 weeks later and the NaCl concentration was increased by 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 days and all of the plants, including the controls, were then sampled.

Callus culture

Melon seeds were surface-sterilized for 20 min in a 25% sodium hypochloride solution containing 0.1% Tween 20, rinsed several times with distilled water, and germinated in MS basal medium, without hormones, under aseptic conditions. Calluses were initiated from the cotyledons of 3 week-old seedlings in a MS (Murashige and Skoog, 1962) medium to which 1.0 mg/L 2.4-D, 0.1 mg/L kinetin, 3% sucrose, and 0.7% agar were added, and incubated at 26 ± 2°C under conditions of continuous darkness. After 4 weeks, the calluses were separated from the explants and subcultivated in MS medium as described above. Added was 100 mM NaCl, and without salt for the control. Placed into each of the petri dishes was 1 g of each callus culture. For each treatment, 15 petri dishes were used. The calluses were left to develop in salted and unsalted conditions for 8 days. Then, the total weights of the calli were determined and the callus tissues were sampled for enzyme extractions.

Enzymes extraction and assay

Fresh leaf and callus samples were rinsed for 5 min in liquid nitrogen. The frozen leaves or callus were kept at -80°C for further analyses. Enzymes were extracted from 0.5 g of leaf or callus 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 15000 × g for 15 min and the supernatant fraction was used to assay for the enzymes. All of the operations for the preparation of enzymes extracts were performed at 4°C. SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. It was defined that 1 unit of SOD activity was the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT. CAT activity was determined by monitoring the disappearance of H_2O_2 according to the method of Cakmak and Marschner (1992).

All of the results were the means of 3 replicates, and each replicate consisted of 10 plants. Data were analyzed statistically and treatment means were compared by Duncan's multiple range test using SAS (1985) software.

RESULTS

Hydroponic culture

To determine the response of melon to salt-induced oxidative stress, SOD and CAT activities were measure in the leaves of seedlings grown with or without 100 mM NaCl.

Salinity decreased the fresh weight of melon seedlings.

Table 1. Effects of salinity on fresh weight in 5 varieties of melon seedlings.

Name of variety	Fresh weight (g/plant)		
	Control	NaCl	Decreasing ratio (%)
Galia C8	4.43[a]	4.06[a]	8.3
Midyat	4.24[a]	3.91[a]	7.9
Besni	3.62[ab]	2.85[b]	21
Semame	3.28[bc]	2.95[b]	9.8
Yuva	3.08[bc]	1.09[c]	64.4

Means followed by the same lowercase letter in the same column are not significantly different at the P = 0.05 probability level, based on Duncan's multiple range test.

Table 2. Effect of salinity on SOD and CAT enzyme activities in 5 varieties of melon seedlings.

Name of variety	Superoxide dismutase (SOD)(µmol min^{-1} mg^{-1} FW)		Catalase(CAT)(µmol min^{-1} mg^{-1} FW)	
	Control	NaCl	Control	NaCl
Galia C8	260.07[b]	359.88[a]	103.24[a]	436.64[a]
Midyat	347.72[a]	295.21[ab]	58.06[b]	167.52[b]
Besni	344.12[a]	358.29[a]	61.49[b]	178.37[b]
Semame	276.93[b]	265.85[b]	54.80[b]	151.77[b]
Yuva	351.73[a]	350.46[a]	113.94[a]	134.74[b]

Means followed by the same lowercase letter in the same column are not significantly different at the P = 0.05 probability level, based on Duncan's multiple range test.

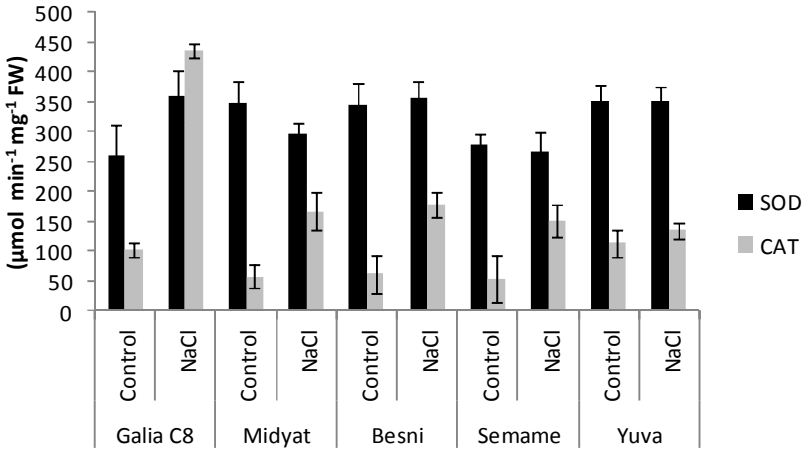

Figure 1. Mean values of SOD and CAT activities in leaves in 5 varieties of melon, control or 100 mM NaCl-treated.

The tolerant genotypes were protected and their fresh weight decreased by 7.9 and 8.3% in Midyat and Galia C8, respectively. However, the fresh weight drastically reduced from 21 and 64.4% in the sensitive genotypes, Besni and Yuva (Table 1).

There were no significant differences in SOD activity between varieties, except for Galia C8 and Semame, grown under non-NaCl conditions. In non-salt control conditions, SOD activities in these 2 genotypes were found to be significantly lower than in other varieties. Salt-stress caused an increase in the SOD activity in the Galia C8 and Besni varieties, but the other 3 varieties had some decreases. The increase in Galia C8 was higher than in Besni. The SOD activity in Galia C8 treated with 100 mM NaCl was approximately 1.5-fold higher than those measured in the control plants (Table 2 and Figure 1).

Under non-salt conditions, Yuva and Galia C8 had the highest CAT activities among the varieties used for this experiment. They were followed by Besni, Midyat, and

Table 3. Response of callus fresh weight of melon varieties on the 8th day of culture.

Name of variety	Callus fresh weight (g FW^{-1})		
	Control	NaCl	Decreasing ratio (%)
Galia C8	1.47^{ab}	1.35^{ab}	8.25
Midyat	1.49^{ab}	1.30^{b}	12.75
Besni	1.43^{ab}	1.37^{ab}	4.20
Semame	1.60^{a}	1.46^{a}	8.75
Yuva	1.37^{b}	1.34^{ab}	2.30

Means followed by the same lowercase letter in the same column are not significantly different at the P = 0.05 probability level, based on Duncan's multiple range test.

Semame, respectively. Salt-treatment increased CAT activity in all of the melon plants, compared with control groups. The increases in Galia C8, Midyat, Besni, and Semame were higher than those in Yuva. The activities of this enzyme in Galia C8, Midyat, Besni, and Semame treated with salt were 4- and 3-fold higher, respectively, than those measured in the control plants (Table 2 and Figure 1).

Changes in callus weights

Generally, significant differences in the weights of the callus tissues were not found among the genotypes on the 8th day of salt stress. However, the decreasing ratio in the callus weight was significantly different among varieties. Callus tissue differentiation, emerged from the cotyledon tissues, initiated in Yuva 4 days later than those of other genotypes. Callus development was greater in Galia C8 and the other tolerant genotypes (1.43 to 1.60 g) than Yuva (1.37 g) in the control petri dishes without salt addition.

The callus tissues of all of the genotypes cultured in the media including NaCl developed more slowly than the medium without salt addition, within 8 days. Salt-sensitive Yuva, which had the lowest callus development and callus weight in the control medium, had a callus weight decreased by salt treatment, as did other varieties used in the experiment. But this decreasing ratio (2.30%) was smaller than the others because of its first measured pure weight (1.37 g). The callus tissues of Besni, Galia C8, Midyat, and especially Semame, developed well in the MS medium. The 8 days of NaCl treatment under *in vitro* conditions affected callus development negatively and all of the genotypes had decreased callus weights. They had a higher decreasing ratio than sensitive variety Yuva, but the high value of the decreasing ratio in the salt-tolerant genotypes subjected to the salt medium was actually due to their faster and more robust development in the control medium, not because of less growth in the salt medium (Table 3).

It was observed that the callus cultures in the control media to which no NaCl was added were healthy and had a cream color. Weight increases reduced on the calluses in media containing salt. Especially in the salt-sensitive variety Yuva, the callus tissues were brownish.

Enzyme activities in the callus tissue

SOD enzyme activity

The response of melon genotypes grown under salt-stress in terms of SOD enzyme activity was found to be different from one another. The highest SOD activity was found in the Galia C8, Midyat and Semame varieties (377.67, 357.00 and 350.00 µmol min^{-1} mg^{-1} FW, respectively) on the 8th day, after being transferred into the saline-media. Besni, with a value of 258.00 µmol min^{-1} mg^{-1} FW, took its place in the same group with Yuva, which showed the lowest SOD activity with 248.00 µmol min^{-1} mg^{-1} FW (Table 4 and Figure 2).

CAT enzyme activity

As demonstrated in Figure 2, CAT activity of the callus tissues grown on the NaCl medium showed a significant difference between the genotypes (P ≤ 0.01). The highest CAT activity occurred in Midyat with a value of 226.02 µmol/min^{-1} mg^{-1} FW. Galia C8 showed great performance in increasing the ratio of CAT enzyme activity under salt stress. It was followed by Yuva, Besni, and Semame.

DISCUSSION

Under normal growth conditions, the production of reactive oxygen species (ROS) in the plant cell is generally at low levels. These compounds, such as superoxide, hydrogen peroxide, and hydroxyl radicals can be responsible for cellular damage under stress conditions (Foyer et al., 1994; Mitler, 2002). High salinity is one of the most important abiotic stress conditions. Under salt stress, cellular homeostasis is disrupted and leads to the production of relatively high levels of ROS (Polle, 2001; Mitler, 2002; Huang et al., 2009). These radicals can damage vital cellular macromolecules

Table 4. SOD and CAT enzyme activities in the callus tissues of 5 melon varieties on the 8th day of salt stress.

Name of variety	Superoxide dismutase(SOD)(µmol min^{-1} mg^{-1} FW)		Catalase(CAT)(µmol min^{-1} mg^{-1} FW)	
	Control	NaCl	Control	NaCl
Galia C8	178.33^{c}	377.67^{a}	29.69^{c}	180.98^{b}
Midyat	195.33^{bc}	357.00^{a}	106.78^{a}	226.02^{a}
Besni	226.33^{bc}	258.00^{b}	53.86^{b}	134.77^{c}
Semame	244.33^{ab}	350.00^{a}	51.73^{b}	116.39^{c}
Yuva	279.67^{a}	248.00^{b}	57.28^{b}	138.65^{c}

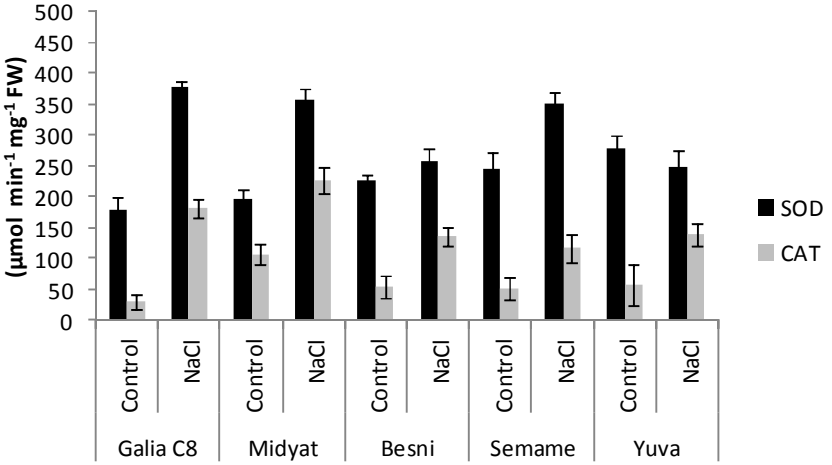

Figure 2. Mean values of SOD and CAT activities in callus tissues of 5 melon cultivars, control or 100 mM NaCl-treated.

(example via denaturation of proteins, peroxidation of lipids). Plants have evolved both enzymatic and nonenzymatic mechanisms to scavenge ROS (Asada, 1999; Kusvuran et al., 2007). Several reports have clearly demonstrated enhanced activity of various antioxidant enzymes under stressful conditions (Jiang and Zhang, 2002; Li, 2009; Huang et al., 2009).

The SOD enzyme destroys the superoxide radical; however, as a result of that it creates hydrogen peroxide, which also has high toxic properties. It was stated by other researchers that SOD activity increases with salt application (Gossett et al., 1994b; Lin and Kao, 2000). Shalata and Tal (1998), who examined the changes of antioxidative enzyme activity in wild and cultured tomatoes under salt-stress, stated that SOD activities increased in the wild variety which was salt-tolerant, and decreased in salt-sensitive genotype M82. Investigated for the aspect of salt tolerance were 120 different pepper genotypes. In the salt-tolerant wild pepper genotype, SOD activity was found to be much higher that it was salt-sensitive Pazarcık-3 genotype (Aktas, 2002). In this experiment on melon seedlings, grown hydroponically except for Galia C8, the SOD activities in all of the other control plants were similar to one another. Salt treatment caused a significant increase in activity and was higher in Galia C8 then in the other varieties. The changes in SOD activities in the other genotypes were different. The SOD activities of moderately-tolerant Midyat and Semame and salt-sensitive Yuva decreased slightly by the salt treatment. The significant increase in SOD activity could increase the ability of the leaves to scavenge O_2^- radicals, which could cause membrane damage. Increases in SOD activity and differential varietal salt susceptibility have also been reported in salt-treated wheat (Sairam et al., 2002), rice (Khan and Panda, 2008), and cucumber (Baysal and Tipirdamaz, 2010). Zaefyzadeh et al. (2009) reported that SOD production is one of the stress confrontation systems under oxidative stress that is activated in drought and salinity conditions.

CAT eliminates H_2O_2 by decomposing it directly to water and oxygen (Yasar et al., 2006; Amirjani, 2010). Salt treatment increased CAT activities in all of the varieties compared with the control plants. However, Galia C8 had the highest CAT activity among the plants under salt stress. This increase was clear in salt-tolerant Galia C8, and in moderately-tolerant Midyat, Besni, and Semame. The increase of CAT activity was very low in salt-sensitive Yuva. Likewise, previous studies reported that CAT activities were higher in varieties of wheat (Karanlik, 2001), wheat (Sairam et al., 2002), eggplant

(Yasar, 2003), and pumpkin (Sevengor, 2010) with salt-tolerance than in salt-sensitive genotypes. Similar results have been reported by Perez-Lopez et al. (2009). They concluded that CAT activity is important for the elimination of H_2O_2 under salinity, and that Iranis barley cultivar might be the more salt-tolerant cultivar because of its higher constitutive SOD and CAT activities.

The results obtained from the measuring of enzyme activities made on the 8th day after the formation of salt-stress in the callus culture in melons, generally showed a similarity to the results obtained from experiments made with the intact plant (Yasar, 2003; Yasar et al., 2006; Sevengor, 2010; Niknam et al., 2011). But the callus culture gave more useful parameters to screen the plant for salt-tolerance. For example, SOD activities were increased in callus tissues of all of the melon genotypes, except salt-sensitive Yuva on the 8th day of salt-treatment. The fact that the callus culture can be used very easily in experiments made for enzyme analyses, therefore it provides a great facility in the preparation of homogeneous samples without residues (Yasar, 2003; Sevengor, 2010). On the other hand, in the callus culture, all of the conditions were optimized such as growth media and continued darkness. Despite the fact that the calluses became brown in the media to which NaCl was added, in the salt-sensitive variety Yuva, on the 8th day of the salt application, there was a significant difference in color compared to the controls and this was not observed in the genotypes determined to be salt-tolerant. The change in the color of the callus gives an idea about the status of salt-tolerance. However, for determination of tolerance, the callus weight and development may be misleading because of development in cases where there are different melon varieties in the control conditions, so the decreasing ratio is different in stress conditions.

CAT activity was increased in the calli of all of the melon varieties after 8 days of salt treatment. A significant increase of CAT activity was obtained from the salt-tolerant Galia C8 cultivar and the Midyat variety, which had more tolerance than the other moderately-tolerant varieties Besni and Semame (Kusvuran, 2004; Yasar et al., 2006; Kusvuran et al., 2007).

In conclusion, the antioxidative enzyme activities play a protective role against salt-stress, and that antioxidative defense mechanism was effective in providing tolerance to salt stress in melon seedlings grown hydroponically. These results suggest that the salt-tolerant genotypes exhibit a better protection mechanism against salt stress in callus culture by maintaining a higher inherited and induced activity of antioxidant enzymes than the sensitive genotypes. Callus culture could be a useful screening method for determining the salt tolerance level of melon genotypes. The change in callus fresh weight under salt stress was not found to be useful for detecting the salt tolerant melon variety. For this purpose, the increasing ratio in CAT activity, and moreover, SOD activity in the callus tissues of melon after salt treatment could be meaningful for selecting salt-tolerant genotypes.

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