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# Antioxidative Responses of Some Eggplant Genotypes to Salinity Stress

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**Abstract:** The response of the antioxidant enzymes to salt stress was studied in the eggplant genotypes. In this study, two salt-tolerant Burdur Bucak (BB) and Mardin Kızıltepe (MK); two salt-sensitive Giresun (Gi) and Artvin Hopa (AH), four of them belong to *Solanum melongena* L. species are landraces grown different parts of Turkey, and a salt tolerant wild species *S. sisymbriifolium* (SS) were used. The antioxidant activities of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.1.1.11) and glutathione reductase (GR; EC 1.6.4.2) enzymes were investigated in these salt-tolerant and sensitive genotypes grown in hydroponics culture. At the end of the study, it was determined that all of the antioxidant enzymes were very effective on the salt tolerance, and salt-tolerant eggplant genotypes were capable of using their antioxidant enzyme systems more actively than the salt-sensitive ones.

**Key words:** Antioxidative enzymes, Hydroponic, Eggplant, Salt stress, Seedlings

## Bazı Patlıcan Genotiplerinin Tuz Stresine Antioksidatif Tepkileri

**Özet:** Patlıcanda antioksidant enzimlerin tuz stresine olan tepkileri çalışılmıştır. Çalışmada, Türkiyenin farklı bölgelerinde yetiştiriciliği yapılan *Solanum melongena* L türüne ait tuza tolerant olan Burdur Bucak (BB) ve Mardin Kızıltepe (MK) genotipleri ile tuza hassas Giresun (Gi) ve Artvin Hopa (AH) genotipleri ve yabani bir tür olan *S. sisymbriifolium* (SS) kullanılmıştır. Hidrofonik kültür ortamında tuz stresi altında genotiplerin, süperoksit dismutaz (SOD; EC 1.15.1.1), katalaz (CAT; EC 1.11.1.6), askorbat peroksidaz (APX; EC 1.1.1.11) ve glutatyon reduktaz (GR; EC 1.6.4.2) enzim aktiviteleri incelenmiştir. Araştırma sonucunda, antioksidant enzim aktivitelerinin tuza tolerans üzerinde çok etkili olduğu; tuzlu koşullarda yaşayabilen patlıcan genotiplerinin antioksidatif enzim sistemlerini duyarlı genotiplere göre çok daha aktif kullandıkları belirlenmiştir.

**Anahtar kelimeler:** Antioksidatif enzimler, Hidroponik, Patlıcan, Tuz stresi, Fide

## Introduction

Salt tolerance in high plants is the result of a number of physiological responses. There is evidence that high salt concentrations cause an imbalance of the 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 (Okuda et al. 1991; Asada 1994; Foyer et al. 1994; Cakmak 1994). Thus, oxidative stress is one of the major damaging factors in plants exposed to salinity (Hernandez et al. 1994; 1995). In plants, both enzymatic and non-enzymatic processes participate in ROS detoxification (Asada and Takahashi 1987). Several enzymes are involved in the detoxification of ROS. SOD converts superoxide to $H_2O_2$. Hydrogen peroxide is scavenged by CAT and different classes of peroxidases (Bowler et al. 1992). APX and GR plays a key role in the ascorbate-glutathione cycle by reducing $H_2O_2$ to water at the expense of oxidizing ascorbate to monodehydroascorbate (MDHA) (Cakmak et al. 1993; Asada 1994; Foyer et al. 1994; Gossett et al. 1994a). Plant tissues contain several enzymes scavenging ROS (superoxide dismutase {SOD; E.C. 1.15.1.1}, catalase {CAT; E.C. 1.11.1.6}, ascorbate peroxidase {APX; E.C. 1.1.1.11}, glutathione reductase {GR; E.C. 1.6.4.2}), and some other natural substances (Gossett et al. 1994b; Blokhina et al. 2003). Many reports have suggested that the proportion of oxidative cellular damage in

plants exposed to abiotic stress is controlled by the capacity of the antioxidant system (Scandalio et al. 2001; Silvana et al. 2003). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Dhindsa and Mathowe 1981; Wise and Naylor 1987; Spychalla and Desborough 1990). The aim of this study was to determine the activities of some anti-oxidative stress enzymes (SOD, CAT, APX and GR) in some sensitive and tolerant eggplant genotypes grown in hydroponics culture under salt stress conditions. For this purpose, SS, MK and BB salt-tolerant; AH and Gi reported to be salt-sensitive eggplant genotypes (Yaşar 2003) were used.

## Material and Methods

### Plant material and culture conditions

As the plant material, two tolerant (MK and BB ) and two sensitive (AH and Gi) local Turkish eggplant varieties, belong to wild *Solanum melongena* species were used in the study, that salt tolerance situations were determined previously. In our previous study, three wild eggplant species (*S. aethiopicum*, *S. sisymbriifolium*, and *S. torvum*) and their native lines were tested to determine their tolerance levels against high salinity. *S. sisymbriifolium* lines were found as more tolerant than the other two wild species (Yaşar ve Ellialtıoğlu 2008). Because of these results, one of the *S. sisymbriifolium* lines was also used as salt-tolerant plant material in this research.

All the plants were grown under 280 µmol $m^{-2}$ $s^{-1}$ of cool white fluorescent light of 16 h photoperiod in a controlled climatic room at 25±1$^{o}$C day/night temperatures, and 70% relative humidity. Seeds were germinated in vermiculite moistened with distilled water. After 3 weeks, seedlings were transferred to plastic vessels filled with 4 L half-strength Hoagland's solution (Hoagland and Arnon 1938). The solution in the vessels was replaced every week. Two weeks later, salt-treatment started and the NaCl concentration was increased by increments of 50 mM $d^{-1}$ until a final concentration of 150 mM was achieved. Non-salt-treated plants were kept controls. Salt-stressed plants were the subjected to 150 mM NaCl for 10 d and all plants, including controls, were sampled for their analysis.

### Enzymes extraction and assay

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80 $^{o}$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 15000 x 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 $^{o}$C.

SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT. Catalase activity was determined by monitoring the disappearance of $H_2O_2$ according to the method of Cakmak and Marschner (1992). APX activity was determined by measuring ascorbate consumption by absorbance at 290 nm. One unit of APX was defined as the amount of enzyme required to consume 1 µmol ascorbate $min^{-1}$ (Cakmak and Marschner 1992). GR activity was determined by measuring the enzymatic-dependent oxidation of NADPH by absorbance at 340 nm. One unit of GR was defined as the amount of enzyme that oxidized 1µmol NADPH $min^{-1}$ (Cakmak and Marschner 1992). All results reported were the means of three replicates. The each repetition had 10 plants Data were analyzed statistically and the means of each treatment were separated by Duncan's Multiple Range Test(DMR) using SAS (1988) software.

## Results

Under salt stress at the 10$^{th}$ day significantly differences were found in the aspect of enzyme activities between salt-tolerant and salt-sensitive eggplant genotypes.

The response of eggplant genotypes under salt stress is the aspect of property of SOD enzyme activity is found to be different from each other. In non-salt conditions, in all genotypes were found took place in the same statistical group, but salt-stress, caused an increase in SOD, CAT, APX and GR enzymes

activity in the other treated genotypes except for Gi. The highest SOD activity was found in the wild species SS and MK local variety on the 10th day after salt treatment. BB took place in the same group with salt-sensitive AH variety and the lowest SOD activity was displayed in the salt-sensitive Gi variety (Table 1, Figure 1).

Table 1: Effect of salinity on SOD, CAT, GR and APX enzyme activities in seedlings of 5 eggplant genotypes

| Name of genotypes | Superoxide dismutase (U $min^{-1}$ $mg^{-1}$ FW) | | Catalase (µmol $min^{-1}$ $mg^{-1}$ FW) | | Glutathione Reductase (µmol $min^{-1}$ $mg^{-1}$ FW) | | Ascorbat Peroxidase (µmol $min^{-1}$ $mg^{-1}$ FW) | |
|---|---|---|---|---|---|---|---|---|
| | Control | NaCl | Control | NaCl | Control | NaCl | Control | NaCl |
| Gi | 211.7 $^{a*}$ | 168.3 $^{c}$ | 111.4 $^{ab}$ | 287.7 $^{d}$ | 276.0 $^{a}$ | 489.5 $^{cd}$ | 972.6 $^{c}$ | 2118.6 $^{d}$ |
| AH | 211.7 $^{a}$ | 250.0 $^{bc}$ | 121.8 $^{ab}$ | 158.0 $^{e}$ | 179.8 $^{c}$ | 422.2 $^{d}$ | 632.5 $^{c}$ | 3139.6 $^{c}$ |
| BB | 176.7 $^{a}$ | 330.7 $^{b}$ | 83.8 $^{b}$ | 317.0 $^{c}$ | 210.2 $^{bc}$ | 1141.7 $^{b}$ | 745.8 $^{c}$ | 5760.6 $^{b}$ |
| MK | 204.0 $^{a}$ | 535.3 $^{a}$ | 79.4 $^{b}$ | 374.6 $^{b}$ | 223.7 $^{bc}$ | 645.1 $^{c}$ | 1393.1 $^{b}$ | 5968.0 $^{b}$ |
| SS | 154.3 $^{a}$ | 597.7 $^{a}$ | 152.0 $^{a}$ | 631.6 $^{a}$ | 237.3 $^{ab}$ | 1502.5 $^{a}$ | 2241.7 $^{a}$ | 9557.6 $^{a}$ |

*Means followed by same latter in same column are not significantly different at 0.01 probability level, based on DMR

From the catalase activity point of view, it is found out difference between genotypes at the level of $p \leq 0.01$ is significant. Salt-treatment increased CAT activity in all genotypes compared with control groups, and the highest catalase activity occurred in wild genotype (SS). It was followed by MK, BB, AH genotypes. Gi and AH had the lowest CAT activity in the salt treatment (Table 1, Figure 1).

APX enzyme activity is other important enzyme that is effective in ascorbat-glutathion cycle. APX activities showed on increase in the all eggplant genotypes under salt treatments compared with control groups. Under non salt treatment, SS and MK genotypes had the highest APX enzyme activity respectively, it was very low in Gi, BB and AH genotypes and those were found similar. In all genotypes had different of APX enzyme activity in statistical aspects under salt stress, except for MK and BB. APX activity was the highest found in SS, MK and BB, the lowest found in Gi and AH genotypes. Salt-tolerant MK and BB of turkish local eggplant varieties showed great performance in increasing ratio APX enzyme activity under salt stress (Table 1, Figure 1).

Salt-treatment changed the GR enzyme activities of all genotypes compare with control groups. Under non-salt conditions, Gi had the highest activity of GR among their other genotypes used for this experiment. It was followed by SS, MK, BB and AH, respectively. From the point of view of GR activity, significantly differences were found between salt-tolerant (SS, MK, BB) and salt-sensitive (Gi, AH) genotypes grown under NaCl stres. Wild species SS and BB genotypes had the highest GR enzyme activities under salt stress, but salt-tolerant MK and salt-sensitive Gi genotypes were took place in same statistical group. On the other hand Gi and AH types gave low values to this enzyme (Table 1, Figure 1).

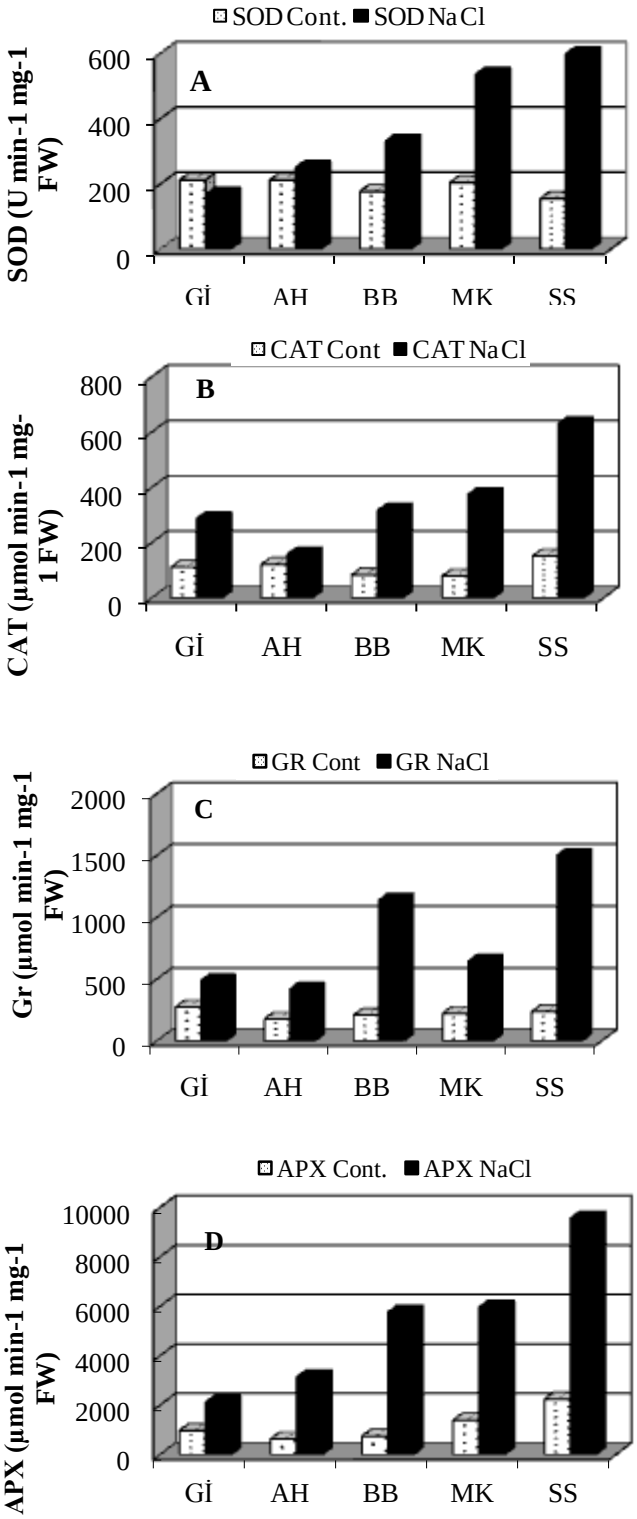


Figure1. Comparison of control and NaCl treated eggplant genotypes for superoxide dismutase (SOD;Panel A) activity, catalase (CAT; Panel B) activity, glutathione reductase (GR; Panel C) activity and ascorbate peroxidase (APX ; Panel D) activity (Means followed by same latter are not significantly different at 0.01 probability level, based on DMR)

## Discussion

Reactive oxygen species (ROS) can cause oxidative damage to many cellular components including membrane lipids, proteins, and nucleic acids (Halliwell and Gutteridge 1989). Several defense systems and anti-oxidant molecules are then induced and involves in the detoxification of ROS. In plants, both enzymatic and non-enzymatic processes participate in ROS detoxification (Shalata et al. 2001).

In these study, the anti-oxidant enzyme activities (SOD, CAT, APX and GR) were constitutively higher in the salt-tolerant (MK, BB) genotypes and wild species SS as compared to the salt-sensitive (Gi and AH) genotypes.

The main anti-oxidant activity is SOD enzyme that catalyzes the conversion of superoxide radical into hydrogen peroxide (Gossett et al. 1994b; Lin and Kao 2000). Salinity caused a significant increase of SOD activity in the salt-tolerant Tukish local genotypes (MK and BB) as well as in belong to wild a species *Solanum sisymbriifolium* (SS) compared with control plants (Table 1, Figure 1).This elevated constitutive level of the anti-oxidant system has been related to a higher resistance to salt stress by other authors, as shown by Shalata and Tal (1998) in tomato; Sreenivasulu et al. (2000) in seedlings of foxtail (*Setaria italica*). Tolerance to high NaCl levels involves an increase in the antioxidant capacity of the plant to detoxify reactive oxygen species.

To avoid hydrogen peroxide accumulation, a compound even more damaging than the superoxide radical, two enzyme activities, CAT and APX act detoxifying this compound and yielding water and oxygen. The expression of both enzymes seems to be induced by oxidative products (Lopez et al. 1996; Jiang and Zhang 2002; Lingqiang and Scandalios 2002). CAT activity is higher in cotton and tomato and the kinds those have high resistant against salt then sensitive genotypes. Shalata and Tal (1998) in tomato, Sreenivasulu et al. (2000) in *Setaria sp*., Karanlık (2001) in wheat stated that after the salt application CAT enzyme activity become higher in the kind strong against salt than sensitive types. Similarly, in our research under salt stress, salt-treatment increased CAT activity in all genotypes, compared with control groups and in the aspect of CAT enzyme activities, between high tolerant eggplant genotypes (SS, MK and BB) and sensitive genotypes (Gi and AH) are observed important clear and statistical differences.

In eggplant genotypes plants subjected to salt stress, APX and GR seemed to be the enzymes that remove the excess of $H_2O_2$ formed. This is consistent with the results found by the others authors like in tomato (Shalata and Tal 1998); in carrot (Lopez et al. 1996); in Arabidopsis (Tsugane et al. 1999) and in foxtail (Sreenivasulu et al. 2000) subjected to salt stress. Lopez et al. (1996) have reported that salt stress induced APX activity in *Raphanus sativus* plants. Hernandez et al. (1995), have reported that salt stress was increased APX activity in salt-tolerant bean varieties, but did not salt-sensitive varieties. In this experiment on eggplant seedlings grown hydroponically, except for wild species SS and MK, the APX activities of the other control plants were similar to one another. On the other hand, salt treatment increased significantly the APX activities all of genotypes. But, APX activity were much increased in tolerant genotypes (MK and BB) and wild species SS.

GR and APX enzymes are participate together with in the ascorbate-glutathione cycle. Both enzymes catalyze the reaction that maintain the large pool of glutathione and ascorbate, that are essential for the appropriate functioning of the antioxidant system in plants (Gosset et al. 1994a; Hernandez et al. 1995). In our study showed that GR activity, significantly differences were found between salt-tolerant (SS, MK, BB) and salt-sensitive (Gi, AH) eggplant types grown under NaCl stres. SS and BB had the highest GR enzyme activities under salt stress, but salt-tolerant MK and salt-sensitive Gi genotypes had low GR enzyme activity and were took place in same statistical group. Likewise, it was reported from previous studies that the GR activities in varieties of rice (Vaidyanathan et al. 2003) with high salt resistance were determined to have higher values compared to the sensitive genotypes.

The results of this study of eggplant show that wild species (SS) and salt-tolerant genotypes (MK and BB) have a high hereditary and induced capability under salinity which provides to it a better protection from oxidative damage caused by salt treatment. This protection might be resulted by significantly higher constitutive activities of SOD and constitutive and induced activities of APX, CAT and GR in the leaves of wild species (SS) and salt-tolerant (BB and MK) genotypes.

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