# Effect of Salt Stress on Oxidative Parameters, Antioxidant Enzymes Activity and Gene Expression in Tomato Plants

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## ABSTRACT

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world. This study was conducted to investigate oxidative parameters (H2O2 and MDA levels), antioxidant enzyme activities (SOD, APX and CAT) and gene expression of these antioxidant enzymes in two tomato cultivars (Gabali and Mosyaf) under salt stress treatments (0, 50, 100 and 150 mM NaCl). Salt stress increased H2O2 and MDA contents, as well as the activities of SOD, APX and CAT. The expression of iron SOD (FeSOD) and manganese SOD (MnSOD) genes was upregulated by some concentrations of NaCl in Gabali cultivar, while it was decreased in Mosyaf cultivar at all NaCl concentrations. The expression of cytosolic APX (APXcyt) and thylakoid-bound APX (APXt) genes was increased by the most of NaCl concentrations in the two investigated cultivars. The expression of CAT1 and CAT2 genes was decreased at all NaCl treatments in both the studied cultivars. Our findings suggest that antioxidant enzymes and their gene expressions may be differentially or cooperatively involved in the defense mechanisms of tomato plants exposed to salt stress.

**Keywords**: enzymes, stress, Salinity, Tomato.

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# تأثير الإجهاد الملحي في معايير الأكسدة ونشاط الأنزيمات المضادة للأكسدة وتعبيرها المورثي في نباتات البندورة

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**الملخص**

يعد الإجهاد الملحي الإجهاد اللاأحيائي الرئيس الذي يقلل من إنتاج طيف واسع من المحاصيل في العالم. نفذ البحث بهدف دراسة تأثير الإجهاد الملحي في معايير الأكسدة ($H_2O_2$ وMDA)، والأنزيمات المضادة للأكسدة (SOD، APX وCAT)، والتعبير المورثي لهذه الأنزيمات في صنفين من البندورة (جبلي، مصياف)، تحت تأثير المعاملات الملحية (0، 50، 100 و150 ميلليمول من ملح كلوريد الصوديوم NaCl). أدى الإجهاد الملحي إلى زيادة المحتوى من H2O2 وMDA، وزيادة نشاط الأنزيمات المضادة للأكسدة (SOD وAPX وCAT) ، وقد إزداد نشاط أنزيمي سوبر أكسيد ديزموتاز الحديد (SlFe–SOD) والمنغنيز (SlMn–SOD) عند بعض التراكيز الملحية في الصنف جبلي، في حين إنخفض نشاط هذا الأنزيم في الصنف مصياف عند كافة التراكيز الملحية المدروسة. إزداد مستوى التعبير المورثي للمورثات المسؤولة عن أنزيمي

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أسكوربات بيروكسيداز ذات الأصل السيتوبلاسمي (SlAPXcyt)، والأسكوربات بيروكسيداز (SlAPXt) عند معظم التراكيز الملحية المدروسة في كلا الصنفين المدروسين، وقد إزداد التعبير المورثي لمورثات CAT1 وCAT2 عند كافة التراكيز الملحية المدروسة في كلا الصنفين المدروسين. تشير النتائج إلى أن الأنزيمات المضادة للأكسدة وتعبيرها المورثي قد تعمل معاً أو بشكل مستقل بعضها عن بعض في آليات الدفاع في نباتات البندورة تحت تأثير الإجهاد الملحي.

**الكلمات المفتاحية:** الأنزيمات ، الإجهاد ، الملحي، البندورة.

**Introduction**

Tomato (*Lycopersicones culentum* Mill.) is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in almost every country of the world (Abu-El-Heba *et al*., 2008). It is considered as the second vegetable crop world wild after potato (*Solanum tuberosum*) (Bhatia *et al*., 2004). Tomato is very multipurpose and grown either for fresh market or processing and it is rich in vitamins (A and C), fibers and free from cholesterol (Rao and Agarwal, 2000). Tomato is sensitive to moderate levels of salt in the soil solutions, and the response genotypes to salinity stress is genetic and species dependant (Mohammed *et al*., 2007).

Salinity has been defined as one of the major abiotic stresses that adversely affect seed germination, growth, productivity and fruit quality by inducing osmotic effects, ion-specific toxic effects and oxidative stress (Gorbani *et al*., 2011). Salt stress exerts its effect at least in part by causing oxidative damage, which is caused by increased production of reactive oxygen species (ROS) (Smirnoff, 1993).

ROS include a wide range of oxygen-radicals, such as superoxide anion ($O_2^{\cdot-}$ hydroxyl radical ($OH^{\cdot}$), perhydroxyl radical ($HO_2^{\cdot}$) and hydrogen peroxide ($H_2O_2$) (Gill and Tuteja, 2010). ROS are damageable for cellular structures and macromolecules, causing photoinhibition of the photosynthetic apparatus (Ahmad *et al*., 2011). Peroxidation of membrane lipids, inactivate metabolic enzymes and damage the nucleic acids leading to cell death (Mittler, 2002). The degree of damage depends on the balance between the formation of ROS and its removal by the antioxidant scavenging systems and it appears to represent an important stress-tolerance trait. Antioxidant defense systems in plants include both non-enzymatic antioxidants such as ascorbate, glutathione, carotenoids and some phenolic compounds, and enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Juan *et al*., 2005). SOD catalyzes the dismutation of the superoxide anion ($O_2^{\bullet-}$) to oxygen and hydrogen peroxide ($H_2O_2$) (Gomez *et al*., 2004), and $H_2O_2$ is converted into non-toxic water by CAT or APX (Asada, 2006).

**Objectives**

The aim of the present study is to investigate the effect of salt stress on some oxidative parameters and the antioxidant systems of two tomato cultivars (Gabali and Mosyaf).

**Materials and methods**

**1. Plant material and culture conditions:**

Two tomato cultivars (Gabali and Mosyaf) were used, which were selected among several important local cultivars depending on their tolerance to salinity stress. These two cultivars showed a different response to salinity stress during *in vitro* screening among the most important local tomato cultivars in Syria (data not shown).

Seeds of two tomato cultivars (Gabali and Mosyaf) were surface sterilized with 70% ethanol for 1 min then soaked in sodium hypochlorite (1%) for 7 min and thoroughly washed with sterile distilled water three times. Then seeds were incubated for germination in a ½ MS medium (the medium salt content was not considered in the salt treatments) (Murashig and Skoog, 1962) supplemented with 30 $g.l^{-1}$ sucrose and 5.5 $g.l^{-1}$ agar (pH 5.7±0.1). Cultures were incubated in a growth room, at 22 ± 2°C and a photoperiod cycle of 16/8 h as light/dark, provided by fluorescent tubes with light intensity of 30 $\mu Mm^{-2}. S^{-1}$. *In vitro* grown plants were propagated with a 4-week interval in order to obtain enough plant material (vegetative growth).

**2. Salt stress treatments:**

*In vitro* grown plants were cut into nodal sections consist of a single node and leaf. Salt stress was assessed by transferring nodal sections to MS medium containing 0, 50, 100 and 150 mM of sodium chloride (NaCl) with eight replicates per treatment. The plants were subjected to stress for a sufficient period (4 weeks) to stimulate long-term effects. *In vitro* grown plants were harvested after 4 weeks, the vegetative growth transferred and grinded in liquid nitrogen, then the samples were kept at -80°C for further analyses.

**3. Measurements:**

**Determination of the malonyldialdehyde content:**

The level of lipid peroxidation in plant tissues was measured by determination of MDA which is known to be the breakdown product of lipid peroxidation (Heath and Packer, 1969). Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction (Murshed *et al*., 2008a). Frozen samples powder (0.25 g)

was homogenized in 1 mL 0.1 % (w:v) TCA solution. The homogenate was centrifuged at 16,000 g for 15 min and 0.5 mL of the supernatant was added to 1 mL 0.5% (w:v) TBA in 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath, the absorbance was read at 532 nm. The amount of MDA–TBA complex (red pigment) was calculated using an extinction coefficient of 155 $mM^{-1}.cm^{-1}$.

**Determination of hydrogen peroxide content:**

$H_2O_2$ levels were determined as described by Murshed *et al*., (2008a). Frozen samples powder (0.25g) was homogenized in an ice bath with 1 mL 0.1 % (w:v) trichlor acetic acid (TCA) then centrifuged at 16,000 g for 15 min at 4C°. 0.5 mL of the supernatant was added to 250 µL of 10 mM potassium phosphate buffer (pH 7.0) and 500µL of 1M potassium iodide (KI). The mixture was briefly vortexed, incubated at room temperature for 30 min and the absorbance readings were taken at 390 nm. Commercial $H_2O_2$ was used to generate a standard curve, and the content of $H_2O_2$ was determined using the standard curve.

**Extraction of enzymes:**

Enzymes extraction was performed according to the method of Murshed *et al*., (2008b). Frozen samples powder (0.25 g) was homogenized in 1 ml of 50 mM 2-N-morpholino-ethanesulfonic acid buffer (MES/KOH) (pH 6.0), containing 40 mM potassium chloride (KCl), 2 mM calcium chloride ($CaCl_2$), and 1 mM ascorbate (AsA). Extracts were centrifuged at 4 °C for 15 min at 16000g. Supernatants were analyzed immediately for enzyme activities. Protein was quantified using Bradford's method (Bradford, 1976).

**Enzyme assays:**

All enzyme activities were measured in kinetic reactions at 25 °C, using a spectrophotometer. SOD, APX and CAT activities were measured as described in the method of Murshed *et al*., ($2008_b$).

SOD activity was assayed in a 1500 µl reaction mixture containing 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 100µL of sample supernatant and 2 µM riboflavin. Tubes were briefly vortexed then placed under a light source for 5 min. The reaction was stopped by switching off the light and covering the tubes. Absorbance readings were taken at 560 nm. Commercial SOD was used to generate a standard curve. SOD in the

extract inhibited the photochemical reduction of NBT to blue formazan. Activity was expressed in units of SOD, an enzyme unit is the amount of enzyme that converts one micromole of the substrate per minute under standard conditions, from the standard curve of activity units versus absorbance. This method was modified from that of Dhindsa and Mathowe (1981).

APX activity was measured according to Yoshimura *et al*., (2000). Reaction complex consisted of 50 mM phosphate buffer solution (pH 7), 0.25 mM AsA, 5 mM $H_2O_2$ and 100 µl enzyme solution. Changes in samples absorption were recorded at a 290 nm wave length in 3 minutes and enzyme activity was measured by using the 2.8 $mM^{-1}cm^{-1}$ extinction coefficient.

The activity of CAT enzyme was measured according to Aebi (1984). Reaction complex consisted of 50 mM potassium phosphate buffer (pH 7), 15 mM $H_2O_2$ and 100 µl enzyme solution. Reaction started by adding $H_2O_2$. Activity was determined by measuring the decrease in the reaction rate at 240 nm and calculated using a 43.6 $M^{-1}.cm^{-1}$ extinction coefficient.

**RNA extraction, cDNA synthesis and quantitative RT-PCR detection:**

Total RNA was isolated from 50 mg of frozen samples powder using RNEASY MINI KIT (Qiagen, Cat no: 74104) following the manufacturer's instructions. RNAs were quantified spectrophotometrically by measuring the absorbance at 260 and 280 nm. RNA integrity was verified on 1 % agarose gels.

For RT-PCR experiments, first-strand cDNA was synthesized from 100 ng of total RNA, and then the quantitative assessment of mRNA levels was performed using Power Sybr Green RNA-to-$C_T^{TM}$ 1-Step Kit (Applied Biosystems) following the manufacturer's instructions. The cDNA synthesis and the quantification of the accumulation of the target transcript relative to the actin (SlActin) transcript, taken as the control gene for the experimental conditions, were performed with specific primers pairs for each gene. Specific primers pairs of SlActin, SlFe–SOD, SlMn–SOD, SlAPXcyto, SlAPXt, SlCAT1 and SlCAT2 were designed based on sequences present in the GenBank database (http://www.ncbi.nlm.nih.gov) using DNAMAN software (Table 1).

Three independent RNA isolations, with two replications for each of the RNA isolations, were performed for each treatment of salt stress.

**Table 1**: Sets of primers used to amplify gene-specific regions, corresponding size and accession number of the amplified product

| PCR fragment | Primersequence (5′→3′) | Size (bp) | Accession number |
|---|---|---|---|
| SlCAT1 | F:CTAATTGAGAAGCTCGCGACATTTG<br>R: ATACCAGGGACAATATGTCCAGGG | 861 | NM_001247898 |
| SlCAT2 | F:CCATTTGGTGGAGAAACTTGCCAAC<br>R:ACACCTGGAACCACAATAGAAGGG | 862 | AY128694 |
| SlFe-SOD | F: GGGAAGTATCACAGGGCGTA<br>R:TAATTCACCGGATGGCTCTC | 212 | NM_001246860 |
| SlMn-SOD | F: AGATGAACGCAGAAGGTGCT<br>R: GCGTGTTCCCAAACGTCTAT | 168 | XM_004240820 |
| SlAPXt | F: TTCACCCAATGACTTCCCT<br>R: TATCATTTAGTCCCATTCTGT | 700 | FJ532352 |
| SlAPXcyt | F: GTTGAAGGTCGCTTGCCG<br>R: CCAAGGTATGGGCACCAG | 118 | FJ532353 |
| SlActin | F: ATGACTCAAATCATGTTTGAG<br>R: TACCTTAATCTTCATGCTGCT | 633 | FJ532351 |

**4. Statistical analysis:**

The experiment was repeated two times, and the presented values are the means of the two experiments. Statistical significance was determined by analysis of variance ($p<0.05$) using MINITAB Release 14 Statistical Software.

**5. Results**

**Malonyldialdehyde and hydrogen peroxide contents:**

Results showed that the MDA content in the plants of both tomato cultivars (Gabali and Mosyaf) was significantly increased by increasing the NaCl concentration as compared to the control (Table 2).

The $H_2O_2$ accumulation in Gabali cultivar was significantly higher than that in Mosyaf cultivar (Table 2). Moreover, the content of $H_2O_2$ was significantly increased as compared to control by increasing the NaCl concentration in both studied tomato cultivars.

Salt stress is known to result in extensive lipid peroxidation, which is an effective indicator of salt-induced oxidative damage at the cellular level. As a result of lipid peroxidation some products are formed, one of them is malonyldialdehyde (MDA). Many researchers reported that MDA content increased under several abiotic stress factors (Ertuk *et al*., 2007; Sivritepe *et al*., 2008; Shri *et al*., 2009).

In our study, the MDA content was also increased under salt stress (Table 2), indicating that antioxidant systems were not efficient enough to remove the whole ROS and counter the oxidative damage caused by salt stress. These results do not agree with those of previous studies, where a lower lipid peroxidation resulted from elevated activities of antioxidants under salt stress as reported in rice (*Oryza sativa* L.) (Dionisio and Tobita, 1998), sugar-beet (*Beta vulgaris* L.) (Bor *et al*., 2003) and tomato (Shalata *et al*., 2001). Increased $H_2O_2$ levels as a result of salt stress have been reported in different plant species (Agarwal and Pandey, 2004; Sairam *et al*., 2005: Kukreja *et al*., 2005). Our results are in line with Mittova *et al* (2002) who found that salt stress increased the $H_2O_2$ content in tomato. The $H_2O_2$ accumulation under high salinity conditions may be a signal for adaptive response to the stress (Van Breusegem *et al*., 2001), Mittler (2002) proposed that membrane damage might be caused by high $H_2O_2$ levels, which could accelerate the Haber–Weiss reaction, resulting in hydroxyl radical ($OH^{\bullet}$) formation and thus lipid peroxidation.

**Table 2**: Effect of different sodium chloride concentrations (mM) on $H_2O_2$ and MDA content (µmol. $g^{-1}$ FW) in two tomato cultivars.

| NaCl concentration | $H_2O_2$ | | MDA | |
|---|---|---|---|---|
| | Gabali | Mosyaf | Gabali | Mosyaf |
| 0 | $98.33^{d}$ | $28.21^{c}$ | $38.52^{d}$ | $34.19^{d}$ |
| 50 | $102.22^{c}$ | $45.91^{b}$ | $41.55^{c}$ | $38.64^{c}$ |
| 100 | $111.11^{b}$ | $89.58^{a}$ | $45.42^{b}$ | $42.10^{b}$ |
| 150 | $115.81^{a}$ | $89.72^{a}$ | $50.85^{a}$ | $46.57^{a}$ |
| $LSD_{5\%}$ | 2.60 | 1.29 | 1.15 | 0.37 |

Different letters within columns indicate significant differences ($P<0.05$).

**Antioxidant enzymes activities and gene expression**

Under salinity conditions, the activity of SOD was increased in Mosyaf cultivar at all NaCl treatments as compared to the control, meanwhile SOD activity in Gabali cultivar was increased only at the concentration of 100 mM and decreased at the other studied concentrations (Table 3). SlFe-SOD transcript level was increased at the concentrations of 50 and 150 mM of NaCl in Gabali cultivar, but it was decreased at all salt stress treatments in Mosyaf cultivar (Table 3). SlMn-SOD transcript level was increased at concentration of 50mM of NaCl in

Gabali cultivar, while it was decreased at all salt stress treatments in Mosyaf cultivar (Table 3). APX activity was increased under salinity conditions in Gabali cultivar without significant deference with the control, while in Mosyaf cultivar the increasing was significant at 150 mM of NaCl, while it was not significant at 50 and 100 mM of NaCl as compared to the control (Table4). The SlAPXcyto transcript level in Gabali cultivar was increased by most salt stress treatments except at 100 mM of NaCl, while in Mosyaf cultivar the SlAPXcyto transcript level was increased only at 50 mM of NaCl and decreased at all other concentrations of NaCl (Table 4). SlAPXt transcript level was increased by all the salt stress treatments in Gabali cultivar, while it was increased only at150 mM of NaCl in Mosyaf cultivar.

In our study, CAT activity was increased by all salt stress treatments in both studied cultivars. The increase was significant at 150 mM of NaCl in Gabali cultivar and at 100 and 150 mM of NaCl in Mosyaf cultivar (Table 5). Our results also showed changes in SlCAT1 and SlCAT2 transcript levels according to the salt stress treatments (Table 5).

Numerous studies indicate that acquisition of salt tolerance may be a consequence of improving resistance to oxidative stress (Hernandez *et al*., 2001: Shalata and Tal, 1998). Most of these studies showed a positive correlation between resistance to salt stress and the efficiency of the antioxidant systems. Plants employ both enzymatic and non-enzymatic antioxidant defense systems against oxidative damage (Murshed *et al*., 2013). The mechanisms that confer protection against oxidative stress were also investigated. For this purpose, the activities of antioxidant enzymes: SOD, APX and CAT, as well as the relative transcript levels of some genes encoding these enzymes were determined under different salt stress treatments using real-time quantitative RT-PCR.

Among the antioxidant enzymes, SOD is the first line of defense against oxidative stress in plants (Salin, 1987). It plays an important part in determining the concentration of $O_2^{\cdot -}$ and $H_2O_2$ , it causes the dismutation of $O_2^{\cdot -}$ to produce $H_2O_2$ (Bowler *et al*., 1992).

Numerous studies reported an increase in SOD activity during PEG and NaCl stress (Gomez *et al*., 2004; Fadzilla *et al*., 1997). Our results agree with those reported by Gómez *et al*., (2004), who found an increase in all SOD isoenzymes of pea under salt stress. Shalata *et al*., (2001) reported increased SOD activity in salt-tolerant *Lycopersicon Pennelli* L.

This increasing in SOD activity can increase the ability of the plant to scavenge $O_2^{\cdot-}$ radicals, which might lessen membrane damage, this induction of SOD activity was suggested as a reason for improved tolerance to salinity in these cases (Gossett *et al*., 1994; Mittova *et al*., 2004). The changes in SOD activity are not directly related to changes in SlFe-SOD and SlMn-SOD genes expression, suggested a post-transcriptional regulation or the existence of other genes coding for SOD (Murshed *et al*., 2013).

Our results showed an increasing in APX activity at all NaCl concentrations in Gabali and Mosyaf cultivars. These results agree with those reported by several studies that showed a large increasing in APX activity under salinity stress (Gossett *et al*., 1994; Hernandez *et al*., 2000; Mittova *et al*., 2004). The increasing in APX activity was suggested as an adaptation to remove the excess $H_2O_2$ generated due to the increasing in SOD activity under salt stress. The changes in APX activity were not correlated with that in APXcyto and APXt transcripts, this suggests a post-transcriptional regulation or the existence of other genes coding for APX (Murshed *et al*., 2013).

CAT is involved in scavenging $H_2O_2$ during salt stress and other abiotic stress conditions (Kaya *et al*., 2013). Our results showed an increase in the activity of CAT by salt stress in Gabali and Mosyaf cultivars, this increasing in the activity of CAT upon salt stress was often related to the enhanced tolerance to salt stress (Mittova *et al*., 2004). The changes in CAT activity were not correlated with those of SlCAT1 and SlCAT2 genes expression, a post-transcriptional regulation or the existence of other genes coding for CAT (Murshed *et al*., 2013).

From these results, it can be concluded that there is a relationship between salt stress and oxidative stress in tomato plants. An evidence was found for changes in ROS scavenging enzymes in plant sunder salt stress conditions. Results also confirmed that different salinity stress levels caused different antioxidant responses. The results obtained in this investigation underline the important role of some antioxidant enzymes in protecting cellular apparatus during salt stress conditions and may be important for the selection for salinity tolerance in tomato.

**Table 3**: Effect of different sodium chloride concentrations (mM) on the activity of superoxide dismutase (SOD; U.mg$^{-1}$ protein) and the levels of mRNA of chloroplasts SOD (SlFe-SOD) and mitochondria SOD (SlMn-SOD) genes in two tomato cultivars.

| NaCl concentration | SOD Activity | | SlFe-SOD | | SlMn-SOD | |
|---|---|---|---|---|---|---|
| | Gabali | Mosyaf | Gabali | Mosyaf | Gabali | Mosyaf |
| 0 | 69.39$^{b}$ | 55.57$^{d}$ | 1.00$^{c}$ | 1.00$^{a}$ | 1.00$^{b}$ | 1.00$^{a}$ |
| 50 | 47.06$^{d}$ | 64.21$^{b}$ | 1.66$^{b}$ | 0.68$^{b}$ | 1.56$^{a}$ | 0.66$^{b}$ |
| 100 | 71.70$^{a}$ | 57.15$^{c}$ | 2.06$^{a}$ | 0.62$^{c}$ | 0.34$^{d}$ | 0.1$^{d}$ |
| 150 | 65.66$^{c}$ | 70.34$^{a}$ | 0.59$^{d}$ | 0.28$^{d}$ | 0.74$^{c}$ | 0.37$^{c}$ |
| $LSD_{5\%}$ | 0.99 | 0.46 | 0.09 | 0.04 | 0.20 | 0.06 |

Different letters within columns indicate significant differences ($P<0.05$).

**Table 4**: Effect of different sodium chloride concentrations (mM) on the activity of ascorbate peroxidase (APX; µmol.min$^{-1}$.mg$^{-1}$ protein) and the levels of mRNA of cytosolic APX (SlAPXcyto) and thylakoid-bound APX (SlAPXt) genes in two tomato cultivars.

| NaCl concentration | APX Activity | | SlAPXcyto | | SlAPXt | |
|---|---|---|---|---|---|---|
| | Gabali | Mosyaf | Gabali | Mosyaf | Gabali | Mosyaf |
| 0 | 200.71$^{a}$ | 119.66$^{b}$ | 1.00$^{d}$ | 1.00$^{a}$ | 1.00$^{d}$ | 1.00$^{a}$ |
| 50 | 203.58$^{a}$ | 120.41$^{b}$ | 2.23$^{a}$ | 1.09$^{b}$ | 3.23$^{b}$ | 0.68$^{b}$ |
| 100 | 205.75$^{a}$ | 125.63$^{b}$ | 0.57$^{c}$ | 0.23$^{c}$ | 3.39$^{a}$ | 0.59$^{b}$ |
| 150 | 215.58$^{a}$ | 162.96$^{a}$ | 1.79$^{b}$ | 0.12$^{d}$ | 2.58$^{c}$ | 1.11$^{a}$ |
| $LSD_{5\%}$ | 19.24 | 11.59 | 0.09 | 0.06 | 0.07 | 0.09 |

Different letters within columns indicate significant differences ($P<0.05$).

**Table 5**: Effect of different sodium chloride concentrations (mM) on the activity of catalase (CAT; μmol.min$^{-1}$.mg$^{-1}$ protein) and the levels of mRNA of catalase 1 (SlCAT1) and catalase 2 (SlCAT2) genes in two tomato cultivars.

| NaCl concentration | CAT Activity | | SlCAT1 | | SlCAT2 | |
|---|---|---|---|---|---|---|
| | Gabali | Mosyaf | Gabali | Mosyaf | Gabali | Mosyaf |
| 0 | 34363.33$^{b}$ | 11906.67$^{c}$ | 1.00$^{a}$ | 1.00$^{a}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| 50 | 35620.00$^{b}$ | 13840.00$^{bc}$ | 0.33$^{c}$ | 0.40$^{c}$ | 0.49$^{c}$ | 0.89$^{b}$ |
| 100 | 38533.33$^{a}$ $^{b}$ | 16916.67$^{ab}$ | 0.43$^{b}$ | 0.90$^{b}$ | 0.57$^{c}$ | 0.90$^{b}$ |
| 150 | 42430.00$^{a}$ | 18486.67$^{a}$ | 0.13$^{d}$ | 0.27$^{d}$ | 0.70$^{b}$ | 0.77$^{c}$ |
| $LSD_{5\%}$ | 4757.45 | 3250.40 | 0.07 | 0.06 | 0.07 | 0.08 |

Different letters within columns indicate significant differences ($P<0.05$).

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