Acta Scientifica Balcanica, **2023, 4(2)**
DOI: 10.7251/ASB230402013P **UDC 633.368:[577.334:546.21**

Original scientific paper

ANTIOXIDATIVE RESPONSE OF MAIZE TO SALT-INDUCED STRESS

Biljana Pezić[1], Vanja Bjelić[1], Biljana Davidović-Plavšić[1]*, Biljana Kukavica[1]

[1]*University of Banja Luka, Faculty of Natural Sciences and Mathematics, Mladena Stojanovića 2, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina*
***Corresponding author:** biljana.davidovic-plavsic@pmf.unibl.org

Abstract

The aim of the work was to examine the influence of high concentrations of NaCl on protein concentration and the antioxidant system of maize roots and leaves. Maize plants (hybrid ZP 555) were treated with sodium chloride (NaCl) in concentrations of 50 and 150 mM for six days, hydroponically. Among the antioxidant parameters, the concentration of glutathione (GSH) and the activity of antioxidant enzymes: Class III peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APX EC 1.11.1.11), and catalase (CAT, EC 1.11.1.6) were determined. When treated with 50 mM NaCl, the concentration of proteins in the leaves and roots decreased while at 150 mM NaCl the concentration of proteins increased but only in the leaves. An increase in GSH concentration was detected at both concentrations of NaCl in the leaf, and in the root only at 50 mM NaCl, compared to the control. The activity of POX increased significantly only in the leaves treated with the higher concentration of NaCl, while at lower concentrations it decreased, in both leaves and roots. Five rPOX isoforms were detected by native gel electrophoresis in the control, while no rPOX5 isoform was detected in the treated samples. Two lPOX isoforms were detected in both control and treated samples. Native electrophoresis showed the presence of one CAT isoform only in leaves, in both control and treated samples. The highest CAT activity was measured at the lower NaCl concentration. Based on the obtained results, it can be concluded that salinity changes the antioxidant system in the leaves and roots of maize. Also, based on the measured parameters, it can be concluded that the ZP 555 hybrid has a moderate tolerance to the tested salinity levels.

Key words: salinity, ZP 555, proteins, GSH, POX, APX, CAT

INTRODUCTION

Salinity (higher NaCl concentration) is one of the most important types of abiotic stress that limits the growth and reduces the productivity of a large number of crops. The response of plants to increased salt concentrations is complex and includes changes in their morphology, physiology and metabolism (Azooz *et al*., 2009). Salinity reduces the activity of enzymes, the efficiency of photosynthesis and affects the structure of membranes, hormonal balance, water intake and the intake of nutrients in plants (Azooz *et al*., 2009). Increased concentrations of NaCl affect maize growth and development; however, the response of the plants varies depending on the intensity of the stress and the developmental stage. One of the consequences

of exposure of plant cells to increased concentrations of NaCl is oxidative stress that occurs as a result of increased production of reactive oxygen species (ROS: superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$)). ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids (de Azevedo Neto *et al.*, 2006). To mitigate the oxidative damage caused by ROS, plants have developed a complex antioxidant defense system, including low molecular weight antioxidants (glutathione (GSH), ascorbate, phenolic compounds) as well as antioxidant enzymes, such as superoxide dismutase (SOD), class III peroxidases (POX), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) (de Azevedo Neto *et al*., 2006; Hussain *et al*., 2019). Class III peroxidases (POX; EC 1.11.1.7) are oxidoreductases that catalyze the oxidation of a wide range of organic compounds including phenolic compounds, aromatic amines, indoles and sulfonates using H_2O_2 as an oxidant, and the products are water and a free radical (Gray and Montgomery, 2003). POX are involved in various vital processes of plant growth and development during the plant life cycle: cell wall metabolism, lignification, suberinization, ROS metabolism, auxin metabolism, fruit growth and ripening, defense against pathogens, etc. (Padney *et al*., 2017; Veljović Jovanović *et al*., 2018). Ascorbate peroxidase (APX, EC 1.1.11.1) plays an important role in controlling intracellular ROS levels. APX uses two molecules of ascorbate (AsA) to reduce H_2O_2 to water with the simultaneous formation of two molecules of MDHA (monodehydro ascorbate). APX is a member of class I heme peroxidase and its activity is regulated by redox signals and H_2O_2 (Sharma *et al*., 2012). Catalases (CAT; EC 1.11.1.6) are enzymes that catalyze the removal of H_2O_2 that occurs under physiological and stressful conditions (Štolfa *et al*., 2015). Catalase has a high Km for H_2O_2 compared to APX, thus CAT is more active at high H_2O_2 concentrations. The tripeptide glutathione (GSH, γ-glutamylcysteinylglycine) is localized in the cytoplasm, chloroplast, endoplasmic reticulum, vacuole and mitochondria (Villalpando-Rodriguez and Gibson, 2021). It is a cellular antioxidant and signaling molecule, and is also the main source of non-protein sulfur in plants (Foyer *et al*., 2001). Its central role in antioxidant defense is due to its ability to regenerate another powerful antioxidant, ascorbic acid via the ascorbate-glutathione cycle (Ahmad *et al*., 2010). The aim of this work was to examine the effect of higher NaCl concentrations (50 and 150 mM) on the antioxidant system of maize leaves and roots after treatment for six days compared to the control.

MATERIALS AND METHODS

Plant material and cultivation conditions. For the experiments we used the ZP 555 maize hybrid which originates from the Maize Research Institute “Zemun Polje”. The maize seeds were soaked in distilled water and left for 24 hours in water, then they were germinated on wet filter paper, covered with foil and left to germinate for five days. After that, the seedlings were transferred to hydroponics on tap water and grown under controlled laboratory conditions (temperature of 25 °C, with constant aeration) (Figure 1). After seven days, a portion of the plants was treated with NaCl solutions, in concentrations of 50 mM and 150 mM, for a period of six days. The remainder of the plants was not treated and was marked as control.

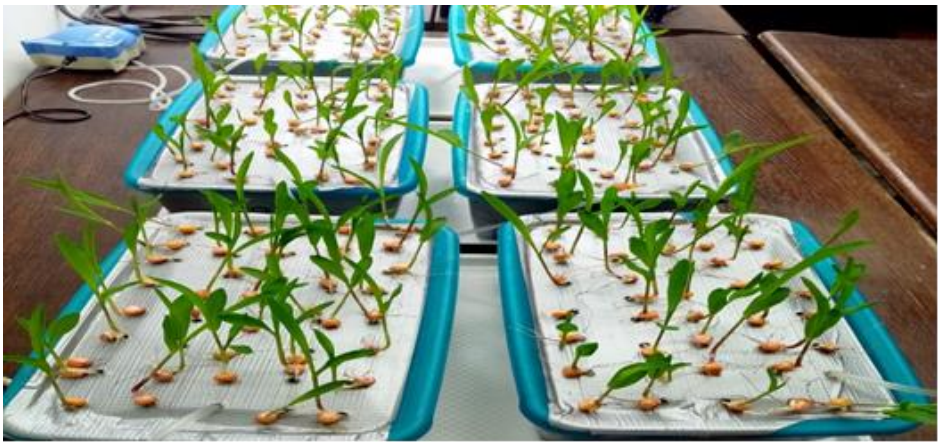

Figure 1. Maize plants grown hydroponically under controlled conditions (Source: personal archive)

The samples were labeled as follows: LC (leaf control), L50 (leaf treated with 50 mM NaCl), L150 (leaf treated with 150 mM NaCl), RC (root control), R50 (root treated with 50 mM NaCl), R150 (root treated with 150 mM NaCl). Mixed samples obtained by combining at least five plants were used for control and treated leaf and root samples.

Extraction of soluble proteins. Plant tissue was pulverized with liquid nitrogen and homogenized with extraction buffer I (0.1 M Na-phosphate buffer pH 6.4 containing 1 mM PMSF and 0.1% TWEEN) to determine protein and GSH concentrations, POX and CAT activities, and isoforms. To determine APX activity, extraction buffer II (90 mM Na-Pi buffer pH 7.8 containing 1 mM PMSF, 8% glycerol, 1 mM EDTA, 5 mM ascorbic acid (added before extraction)) was used. The samples were transferred to eppendorf tubes and centrifuged for 10 minutes at 10,000 revolutions/minute at 4°C and then the supernatant was separated and used for further analyses.

Determination of protein concentration. Lowry's method (Lowry *et al*., 1951) was used to determine the total protein concentration. The protein concentration was determined based on the equation of the calibration curve constructed using standard solutions of bovine serum albumin (BSA). The protein concentration was expressed in milligrams per gram of fresh weight (mg/gFW).

Determination of total glutathione concentration. Ellman's method was used to determine the total concentration of glutathione (Eyer *et al*., 2003). Absorbance was measured at 412 nm. Glutathione concentration was determined based on the equation for the calibration curve (glutathione range 50-250 μmol/L). The glutathione concentration was expressed in milligrams per gram of fresh weight (mg/gFW).

Determination of peroxidase class III activity. POX activity in the protein extract of leaves and roots was determined spectrophotometrically in a reaction mixture containing the Na-phosphate buffer pH 6.4, 1 M pyrogallol, 1 M H_2O_2 and sample (100 μL leaf extract, 50 μL root extract). The reaction was initiated by adding hydrogen peroxide, while the increase in absorbance at 430 nm was monitored within one minute (Kukavica *et al*., 2012). POX activity was expressed in $\mu mol/mg_{proteins}$*min based on the extinction coefficient for purpurigalin (12 $mM^{-1}cm^{-1}$).

Determination of ascorbate peroxidase activity. Ascorbate peroxidase activity was determined spectrophotometrically as described by Miyake and Asada (1992). The reaction

mixture contained: 0.5 mM ascorbate (ε=2.8 $mM^{-1}cm^{-1}$), 0.1 mM hydrogen peroxide, 50 mM Na-Pi buffer pH 7.0, and leaf extract. The decrease in absorbance at 290 nm was measured during one minute. Ascorbate peroxidase activity was expressed in $\mu mol/mg_{proteins}$ *min.

Determination of catalase activity. To determine CAT activity, the reaction mixture contained: 10 mM NaPi buffer pH 6.8 3% H_2O_2 solution (ε=43.10 $mM^{-1}cm^{-1}$), and 100 µL of the sample. The change in absorbance at 240 nm was measured during one minute (Aebi *et al*., 1974). Catalase activity was expressed in $\mu mol/mg_{proteins}$*min.

Native electrophoresis. Isoforms were detected by native polyacrylamide gel electrophoresis, using 5% concentration gel, while the separation gels for POX and CAT were 10% and 8%, respectively. The amounts of proteins applied to the gels for POX were: 30 µg (leaf samples) and 13 µg (root samples); for CAT 37 µg (leaf samples) and 17 µg (root samples). The gels were incubated in POX staining solution consisting of: 45 mL 0.1 M Na-phosphate buffer pH 6.4, 5 mg α-chloronaphthol, 5 mL of methanol, and 50 µL 30% H_2O_2. For specific CAT staining, the gels were incubated for 5 minutes in 0.003% H_2O_2 solution. After incubation, the gels were washed with distilled water and incubated for 10 minutes in the dark in a solution containing 1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$. Gels were scanned and processed in the TotalLab program. All results were processed in the GraphPad Prism 9 ordinary one-way Anova program.

RESULTS AND DISCUSSION

The highest protein concentration in the leaves was measured in the L150, (19.33 mg/g FW), and it was 20% higher than in LC ($p<0.001$). However, in L50 there was a decrease in the concentration of soluble proteins by 18% compared to LC ($p<0.01$). The concentration of soluble proteins was higher in L150 by 47% compared to L50 ($p<0.0001$) (Figure 2A). Wang *et al*. (2022) examined the effect of different concentrations of NaCl on two varieties of maize, labeled *B46* and *NC236*, for 7 days. The authors showed that the content of soluble proteins in leaf samples gradually increased with increasing NaCl concentration. Based on the obtained results, the authors concluded that, compared to *NC236*, *B46* could improve the ability of osmotic regulation of seedlings by increasing the content of soluble proteins, which was suitable for maintaining the normal morphological structure and metabolic processes of seedlings under conditions of osmotic stress.

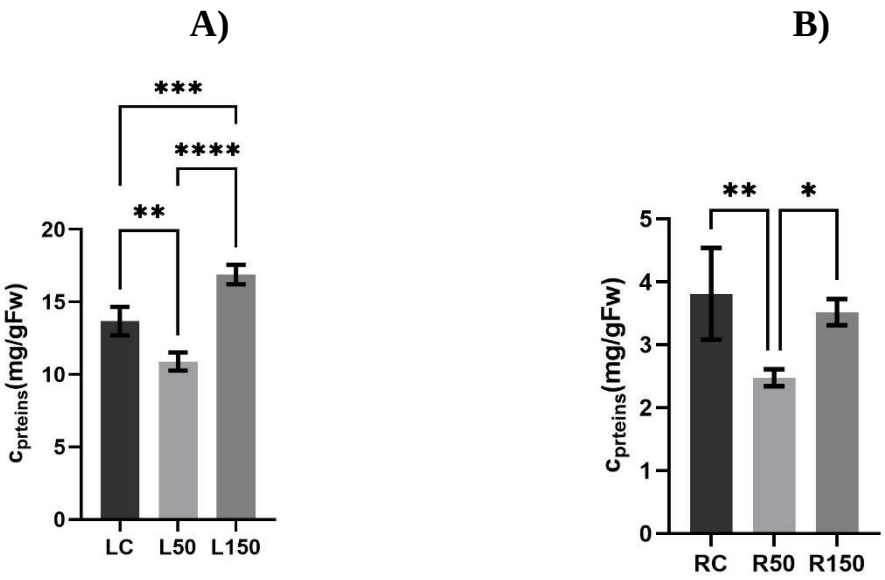

Figure 2. Protein concentrations in maize leaves (A); and roots (B). The results are expressed as mean value ± standard deviation. Statistical significances are marked with asterisk: *p<0.05, **p<0.01; ***p<0.001, ****p<0.0001

In the R50 sample, the concentration of soluble proteins decreased by 20% compared to the highest concentration measured in RC, (6.07 mg/g FW) ($p<0.01$). Also, in the R150 sample, a slight decrease in the concentration of soluble proteins compared to RC was measured. However, an increase in the concentration of soluble proteins was measured in R150, of around 20% compared to R50 ($p<0.05$) (Figure 2B).

In maize leaves, treatment with NaCl led to an increase in GSH concentration: in the L50 sample, by 3.7 ($p<0.0001$) fold, and in the L150 sample by 5.1 ($p<0.0001$) fold compared to LC (Figure 3A). GSH concentration in L150 increased by 36.6% compared to L50 ($p<0.001$). Wang *et al*. (2022) showed that the GSH content in the leaves of *B46* maize cultivar decreased with increasing NaCl concentration compared to the control sample. The GSH content of *NC236* cultivar also decreased with increasing NaCl concentration, which is opposite to our results. Our results showed that the concentration of GSH increased during the treatments, and the highest content of GSH was measured in the samples treated with the lower NaCl concentration. Upon studying the influence of NaCl on the GSH content in the leaves of two wheat varieties (*Triticum aestivum* L.), the results showed a linear increase in GSH with increasing salinity levels in the first variety marked as *Arta*, approximately 2 and 4 fold, respectively, for NaCl concentrations of 75 mM and 150 mM, respectively, compared to the control sample. However, in the second variety marked as *Darab2*, the GSH content remained unchanged in the treated samples.

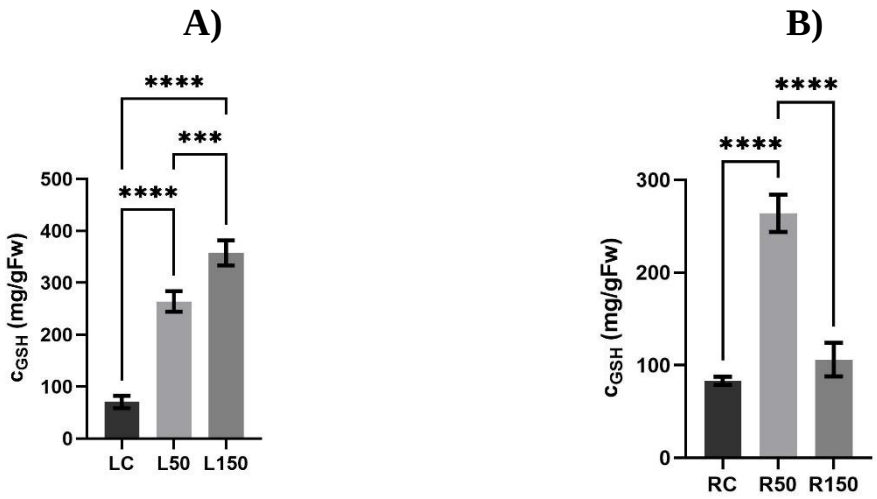

Figure 3. GSH concentrations in maize leaves (A) and roots (B). The results are expressed as mean value ± standard deviation. Statistical significances are marked with asterisk: ***p<0.001; ****p<0.0001

Our results show that in the R50 sample there was a statistically significant increase in GSH concentration, by 3.2 fold compared to RC ($p<0.0001$), which was also the highest measured concentration (264.04 mg/g FW). By increasing the NaCl concentration, an increase in GSH concentration in R150 was recorded, by 27% compared to RC. However, in the R150 a statistically significant decrease in GSH concentration, by 60% compared to R50 ($p<0.0001$) was measured (Figure 3B). In an experiment conducted by AbdElgawad *et al*. (2016), maize plants were treated with 75 mM or 150 mM NaCl per day. Three weeks after the start of the treatment, the maize roots were taken for measurement of the GSH content. The results showed an increase in the level of GSH in response to high salinity that was specific to the roots (AbdElgawad *et al*., 2016). The content of GSH in the treated samples was very similar and more than 50% higher than in the control sample. Similarly, our results showed a significant increase in GSH content in the roots, but in our case the content increased slightly at 150 mM NaCl. This could be attributed to the increased demand and metabolism of sulfur under stress for the biosynthesis of antioxidants such as GSH (Gill *et al*., 2013). Sensitivity to stress caused by increased NaCl concentrations could be a consequence of reduced antioxidant capacity. Other authors have also shown that GSH content is significantly elevated in tolerant varieties such as *Arab* compared to sensitive varieties such as *Darab2* (Esfandiari and Gohari, 2017).

The highest POX activity was measured in the L150 sample (18.61 $\mu mol/mg_{proteins}$*min), and it was 38% higher compared to the control, LC ($p<0.0001$). In contrast, 50 mM concentration of NaCl led to a statistically significant decrease in POX activity in the L50 sample, (39%) compared to LC ($p<0.0001$). POX activity in the L150 sample was 56% higher compared to L50 ($p<0.0001$) (Figure 4A).

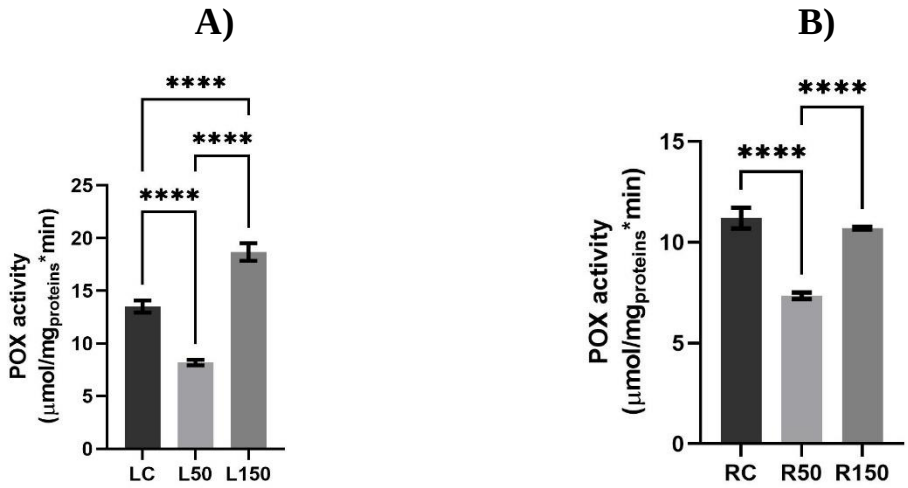

Figure 4. POX activities in maize leaves (A) and roots (B). The results are expressed as mean value ± standard deviation. Statistical significance is marked with asterisk: ****p<0.0001

In maize roots, the highest POX activity was measured in the control, RC (11.20 μmol/$mg_{proteins}$*min). The obtained results showed that in the R50 sample there was a statistically significant decrease in POX activity, by 34% compared to RC ($p<0.0001$), while the treatment with 150 mM NaCl caused a slight decrease. On the other hand, POX activity was about 31% higher in the R150 sample compared to the R50 sample ($p<0.0001$) (Figure 4B). Wang *et al*. (2017) investigated the effect of different concentrations of NaCl on leaf samples on six maize cultivars. The plants were treated with seven concentrations of NaCl (0, 45, 95, 145, 195, 245, 295 mM) and the treatment lasted for 20 days. The results showed that POX activity in leaves remained unchanged at lower NaCl concentrations (0-145 mM), then increased as the NaCl concentration further increased from 145 mM to 195 mM, and finally declined as the NaCl concentration increased from 195 to 295 mM. In relation to the results of Wang *et al*. (2017), our results showed that the lower concentration of NaCl in the maize leaf led to a decrease in POX activity (Figure 4A). The results of Wang *et al*. (2017) study showed that NaCl treatments at 195 mM and above caused a significant decrease in plant biomass, and the threshold of tolerance to increased salt concentration in the six cultivars varied greatly, which indicates the existence of large variation in tolerance to increased salt concentrations among those six maize cultivars. This is generally in agreement with previous studies (Carrasco-Ríos and Pinto 2014; AbdElgawad *et al*. 2016). Maize is considered to be a crop of medium sensitivity to increased salt concentrations. AbdElgawad *et al*. (2016) showed that treatment with 150 mM NaCl reduced leaf biomass by 20%. This reduction in growth may be due to ion toxicity, injury caused by oxidative stress, low osmotic potential, and limitation of cell wall flexibility. Menezes-Benavente *et al*. (2004) indicated that salt concentration >250 mM damages maize plants and can slow down growth. The results of this study suggest that the activity of antioxidant enzymes was higher in cultivars tolerant to increased NaCl concentrations than in sensitive cultivars under conditions of very high salt concentrations (245 mM NaCl or more) (Menezes-Benavente *et al*., 2004). This level of stress caused severe plant damage. A higher activity of the antioxidant enzyme may indicate a greater ability to suppress ROS and protect the integrity and functions of the cell membrane.

On native gel, after specific staining, two POX isoforms were detected in the maize leaves (labeled as lPOX1 and lPOX2). Both isoforms were present in both control and treated sample (Figure 5). The isoenzyme profile of maize roots showed the presence of five POX isoforms (marked as rPOX1-5). Isoforms rPOX1-POX4 were present in all three samples, while isoform rPOX5 was observed only in the control sample (Figure 5).

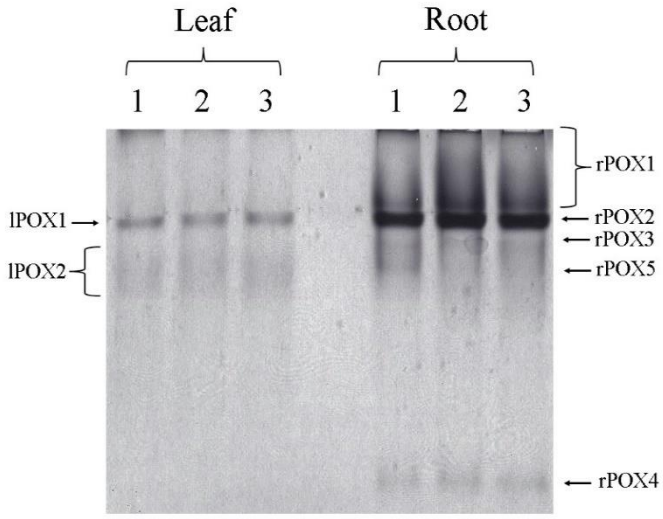

Figure 5. Native gel with separated POX isoforms in maize leaves and roots (Sample labels: 1-control; 2-50 mM NaCl; 3-150 mM NaCl. POX-labels for individual peroxidase isoforms in maize leaf and root)

POX activity in wheat shoots under NaCl stress (0, 50, 100, 150, 200 mM) was significantly higher in the NaCl-resistant genotype compared to the sensitive genotype at all concentrations and duration of stress. POX activity increased with increasing NaCl concentration (Singh *et al*., 2015). The isozyme profile of POX showed significant variation with only two isoforms in the NaCl-resistant genotype, but three isoforms in the NaCl-sensitive genotype (Singh *et al*., 2015). Our results show a lower POX activity in the leaf at 50 mM NaCl than in the control sample, but in L150 the POX activity was the highest, while in the root the highest activity was measured in the control sample. In our maize leaf samples, two lPOX isoforms were detected, while in the roots, four rPOX isoforms were present in all three samples (0, 50, 150 mM), and inhibition of rPOX5 isoform occurred during treatment. Transient increases in POX activity and isozyme polymorphism have been reported to play a role in the antioxidant defense mechanism by detoxifying ROS (Singh *et al*., 2015). Koo *et al*. (2007) showed, by native electrophoresis, the presence of three POX isoforms in control and in samples treated with NaCl (100, 200, 300 mM) in rice shoots. POX1 was strongly stimulated by salt, and POX2 and POX3 isoforms also showed increased expression under salt stress.

The highest CAT activity was measured in the L50 sample (6.227 μmol/$mg_{proteins}$*min). A statistically significant increase in CAT activity occurs in L50, by 91% compared to LC ($p<0.01$). However, in L150 there was a statistically significant decrease in CAT activity compared to LC, by 38%. CAT activity in L150 was 68% lower than in L50 with a statistically significant difference ($p<0.001$) (Figure 6).

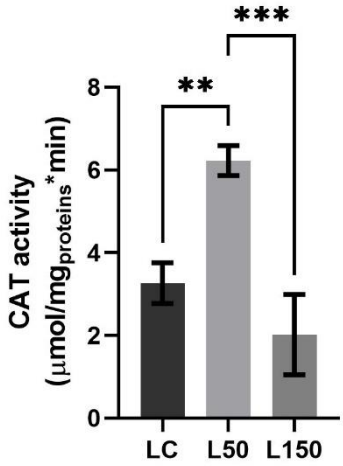

Figure 6. CAT activity in maize leaves. Statistical significances are marked with asterisks: **p<0.01; ***p<0.001. The results are expressed as mean value ± standard deviation (A)

In the NaCl-tolerant maize variety (*SC 129*), CAT activity increased sharply compared to the control sample (Azooz *et al*., 2009). In another NaCl-resistant maize variety (*SC 13*), the CAT activity gradually increased with increasing NaCl concentration up to 200 mM NaCl, while at the highest concentration (250 mM NaCl) the CAT activity decreased but was still higher than the activity in the control sample. On the other hand, all concentrations of NaCl caused a very significant reduction of CAT activity in the sensitive maize variety (Azooz *et al*., 2009). One CAT isoform was detected in the leaves of control and treated plants (data not shown).

Carrasco-Ríos and Pinto (2014) investigated the effect of elevated NaCl concentrations (50 mM and 100 mM) in the leaves of two maize cultivars (*Lluteño* and *Jubilee*). The NaCl treatment lasted for 15 days. CAT activity increased in *Lluteño* across treatments and was 50% higher than activity in *Jubilee* in all treatments. In our samples, the highest CAT activity was measured after treatment with the lower NaCl concentration and was 90% higher than in the control sample (Figure 6). Maize has three genes for catalase isoforms: *Cat-1*, *Cat-2* (peroxisome and cytosol) and *Cat-3* (mitochondria) coded by genes located on different chromosomes and regulated independently (Polidoros and Scandalios, 1999). We detected one CAT isoform in control and treated leaf samples, whereby we do not know the expression of which gene led to its synthesis. Lopez-Huertas *et al*. (2000) showed that H_2O_2 induces peroxisome gene biogenesis in plant and animal cells. The authors suggested that numerous stressful situations that cause H_2O_2 production could be alleviated by the proliferation of peroxisomes that were generated to restore the cellular redox balance.

The highest APX activity was measured in L150 (144.39 μmol/$mg_{proteins}$*min), which was higher than the APX activity measured in the LC sample by 4.6 fold (p<0.0001) and higher then APX activity measured in L50 by 64% (p<0.001). Also, APX activity was 2.4 fold higher in the L50 sample than in LC (p<0.0001) (Figure 7).

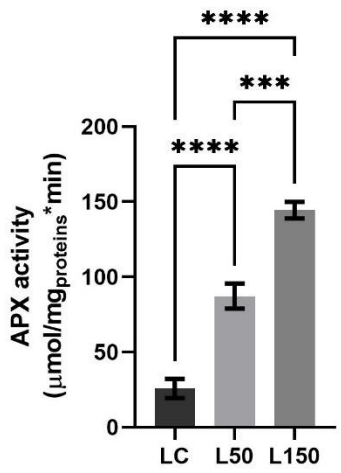

Figure 7. APX activity in maize leaves. Statistical significances are marked with asterisk: ***p<0.001 ****p<0.0001. The results are expressed as mean value ± standard deviation

APX activity was measured in the leaves of three maize varieties after exposure to different concentrations of NaCl (0, 50, 100, 150, 200, 250 mM) for 15 days (Azooz *et al*., 2009). In the variety *SC129*, APX activity was not significantly changed with increasing NaCl concentration in the soil, while in variety *SC13*, APX activity was significantly increased with increasing NaCl concentration up to 200 mM, and then decreased at 250 mM NaCl, where the reduction percentage was 28.7% in relation to the control. However, in *SC155*, APX activity increased with increasing NaCl levels (Azooz *et al*., 2009). Our results showed, similarly, that APX activity increased with increasing NaCl concentration. Stepien and Klobus (2005) examined the effect of NaCl (50, 100 or 150 mM) on two wheat cultivars, *Kobra* (winter variety) and *Opatka* (summer variety), and two maize cultivars, *Limko* and *Iman*, and concluded that APX activity in control plants was higher in both maize cultivars compared to wheat cultivars. There was an evident increase in APX activity in plants growing under conditions of increased NaCl concentration. The first changes were observed after the 3rd day of treatment with NaCl. The absolute increase in enzyme activity was similar for wheat and maize samples exposed to increased NaCl concentrations. The results obtained in the course of the experiments showed that the response to salinity in varieties of the same species was similar. Consequently, for the purpose of this paper, we chose a single variety of wheat (*Kobra*) and a single variety of maize (*Limko*) for comparison. By the 8th day of the experiment, APX activity was increased in wheat leaves of the *Kobra* variety by 45% after treatment with 50 mM NaCl, 61% after treatment with 100 mM NaCl and 72% after treatment with 150 mM NaCl, while in the maize leaves of *Limko* variety there was an increase in APX of 10, 25 and 35%, respectively. No significant difference was observed between the two varieties of maize and between the two varieties of wheat grown under conditions of increased NaCl concentration.

Lukić *et al*. (2021) examined the influence of flooding (after 6, 24, 72 and 144 h) on antioxidant parameters in the leaves of the maize hybrid *ZP 555*, which was also used in our research. This study showed a significant increase in protein concentration, which reduced injury caused by flood stress. Our results show an increase in leaf protein concentration with higher NaCl concentration. It has been observed that the improvement of protein synthesis is an advantage of flood resistant genotypes. Flooding stress caused an increase in CAT activity

after 144 h by 74%, while in our case the highest CAT activity was at the lower concentration. Also, POX activity increased by 27% after 6 h and by 21% after 72 h compared to the control. Finally, the highest activity was measured at the end of the treatment, and it was 34% higher compared to the control, while in our case the highest POX activity was at the lower concentration. Plant isoenzyme profiles were not affected by flooding, which is consistent with our results for the leaves. Three POX isoforms were detected after exposure to flooding stress, while in our research two lPOX isoforms were detected.

In another study, hybrid *ZP 555* plants were treated with different concentrations of nicosulfuron (150 μg/mL and 250 μg/mL) for four days (Kuvelja *et al*., 2021). Authors showed a decrease in protein concentration in all samples except for the root sample treated with 150 μg/mL, compared to control. In addition, the specific activity of POX in the leaves was higher in the treated samples compared to the control sample. In roots, POX activity increases at a higher concentration of herbicide, while at a lower concentration it was lower than in the control sample. The increase in the specific activity of POX in the leaves, in this study, may indicate the resistance of this hybrid. Our results show a reduction in POX activity in treated roots. The specific activity of APX in the leaves increased at a lower herbicide concentration (Kuvelja *et al*., 2021), while it decreased at a higher concentration, which was opposite to our results. Based on the above mentioned, it can be concluded that the antioxidant response of a maize to different stress conditions depends on its development stage, the duration of stress and the intensity of stress, and that individual antioxidant parameters can have different patterns of activation/inactivation for different type and level of stress.

Table 1 summarizes our results: changes in the measured antioxidant parameters for different concentrations of NaCl in the leaf and root of hybrid ZP 555.

Table 1. Activation (↑) and inhibition (↓) of antioxidant parameters during treatment with NaCl (50 and 150 mM) in leaves and roots of maize hybrid ZP 555 compared to control

	Leaf		**Root**	
Parameters	50 mM	150 mM	50 mM	150 mM
Proteins	↓ 18%	↑ 20%	↓ 20%	↓ 5%
GSH	↑ 3.7 fold	↑ 5.1 fold	↑ 3.2 fold	↑ 27%
POX	↓ 39%	↑ 38%	↓ 34%	↓ 4%
CAT	↑ 91%	↓ 38%	-	-
APX	↑ 2.4 fold	↑ 4.6 fold	-	-

The most significant changes in leaves of maize hybrid 555 compared to the control were recorded in GSH concentration (increase for both NaCl concentrations), for CAT activity for a lower concentration and increase in APX activity for both NaCl concentrations. In the root, the GSH concentration increased the most for both NaCl concentrations, and a significant decrease in POX activity was measured at the lower salt concentration (Table 1).

CONCLUSION

The increased concentration of NaCl (50 and 150 mM) caused different responses of antioxidant parameters in leaves and roots of maize hybrid ZP 555. According to the obtained results, we could conclude that the ZP 555 hybrid shows medium resistance to increased NaCl concentrations.

REFERENCES

AbdElgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H., & Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Frontiers in Plant Science*, *7*, 276. https://doi.org/10.3389/fpls.2016.00276

Aebi, H. (1974). Catalae. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis 2* (pp. 673-684). New York, London: Academic press. https://doi.org/10.1016/B978-0-12-091302-2.X5001-4

Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G., & Sharma, S. (2010). Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, *30*(3), 161-175. https://doi.org/10.3109/07388550903524243

de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, *56*(1), 87-94. https://doi.org/10.1016/j.envexpbot.2005.01.008

Azooz, M. M., Ismail, A. M., & Elhamd, M. A. (2009). Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of maize cultivars grown under salinity stress. *International Journal of Agriculture & Biology*, *11*(1), 21-26.

Carrasco-Ríos, L., & Pinto, M. (2014). Effect of salt stress on antioxidant enzymes and lipid peroxidation in leaves in two contrasting corn,'Lluteno'and'Jubilee'. *Chilean Journal of Agricultural Research*, *74*(1), 89-95. http://dx.doi.org/10.4067/S0718-58392014000100014

Esfandiari, E., & Gohari, G. (2017). Response of ROS-scavenging systems to salinity stress in two different wheat (*Triticum aestivum* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *45*(1), 287-291. https://doi.org/10.15835/nbha45110682

Eyer, P., Worek, F., Kiderlen, D., Sinko, G., Stuglin, A., Simeon-Rudolf, V., & Reiner, E. (2003). Molar absorption coefficients for the reduced Ellman reagent: reassessment. *Analytical biochemistry*, *312*(2), 224-227. https://doi.org/10.1016/S0003-2697(02)00506-7

Foyer, C. H., Theodoulou, F. L., & Delrot, S. (2001). The functions of inter-and intracellular glutathione transport systems in plants. *Trends in Plant Science*, *6*(10), 486-492. https://doi.org/10.1016/S1360-1385(01)02086-6

Gill, S. S., Anjum, N. A., Hasanuzzaman, M., Gill, R., Trivedi, D. K., Ahmad, I., Pereira, E., & Tuteja, N. (2013). Glutathione and glutathione reductase: A boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry*, *70*, 204-212. https://doi.org/10.1016/j.plaphy.2013.05.032

Gray, J. S., & Montgomery, R. (2003). Purification and characterization of a peroxidase from corn steep water. *Journal of Agricultural and Food Chemistry*, *51*(6), 1592-1601. https://doi.org/10.1021/jf025883n

Hussain, S., Rao, M. J., Anjum, M. A., Ejaz, S., Zakir, I., Ali, M. A., Ahmad, N., & Ahmad, S. (2019). Oxidative stress and antioxidant defense in plants under drought conditions. In M. Hasanuzzaman, K. Hakeem, K. Nahar & H. Alharby (Eds.), *Plant abiotic stress tolerance* (pp. 207-219). Springer: Cham. https://doi.org/10.1007/978-3-030-06118-0_

Koo, J. S., Choo, Y. S., & Lee, C. B. (2007). Changes in ROS-Scavenging Enzyme Activity in Rice (*Oryza sativa* L.) Exposed to High Salinity. *Journal of Ecology and Environment*, *30*(4), 307-314. https://doi.org/10.5141/JEFB.2007.30.4.307

Kukavica, B. M., Veljovicć-Jovanovicć, S. D., Menckhoff, L., & Lüthje, S. (2012). Cell wall-bound cationic and anionic class III isoperoxidases of pea root: biochemical characterization and function in root growth. *Journal of Experimental Botany*, *63*(12), 4631-4645. https://doi.org/10.1093/jxb/ers139

Kuvelja, A., Davidović-Plavšić, B., Lukić, D., Gajić, N., Žabić, M., Škondrić, S., & Kukavica, B. (2021). Impact of nicosulfuron on biochemical markers of oxidative stress in maize leaves and roots. *Biljni lekar*, *49*(2), 201-217. https://doi.org/10.5937/biljlek2102201k

Lopez-Huertas, E., Charlton, W. L., Johnson, B., Graham, I. A., & Baker, A. (2000). Stress induces peroxisome biogenesis genes. *The EMBO Journal*, *19*(24), 6770-6777. https://doi.org/10.1093/emboj/19.24.6770

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265-275. PMID: 14907713

Lukić, N., Trifković, T., Kojić, D., & Kukavica, B. (2021). Modulations of the antioxidants defence system in two maize hybrids during flooding stress. *Journal of Plant Research*, *134*(2), 237-248. https://doi.org/10.1007/s10265-021-01264-w

Menezes-Benavente, L., Kernodle, S. P., Margis-Pinheiro, M., & Scandalios, J. G. (2004). Salt-induced antioxidant metabolism defenses in maize (*Zea mays* L.) seedlings. *Redox Report*, *9*(1), 29-36. https://doi.org/10.1179/135100004225003888

Miyake, C., & Asada, K. (1992). Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant and Cell Physiology*, *33*(5), 541-553. https://doi.org/10.1093/oxfordjournals.pcp.a078288

Pandey, V. P., Awasthi, M., Singh, S., Tiwari, S., & Dwivedi, U. N. (2017). A comprehensive review on function and application of plant peroxidases. *Biochemistry & Analytical Biochemistry*, *6*(1), 308. DOI: 10.4172/2161-1009.1000308

Polidoros, A. N., & Scandalios, J. G. (1999). Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). *Physiologia Plantarum*, *106*(1), 112-120. https://doi.org/10.1034/j.1399-3054.1999.106116.x

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, *2012*, 217037. https://doi.org/10.1155/2012/217037

Singh, A., Bhushan, B., Gaikwad, K., Yadav, O. P., Kumar, S., & Rai, R. D. (2015). Induced defence responses of contrasting bread wheat genotypes under differential salt stress imposition. *Indian Journal of Biochemistry and Biophysics*, *52*(1), 75-85. PMID: 26040114

Stepien, P., & Klobus, G. (2005). Antioxidant defense in the leaves of C3 and C4 plants under salinity stress. *Physiologia Plantarum*, *125*(1), 31-40. https://doi.org/10.1111/j.1399-3054.2005.00534.x

Štolfa, I., Pfeiffer, T. Ž., Špoljarić, D., Teklić, T., & Lončarić, Z. (2015). Heavy metal-induced oxidative stress in plants: Response of the antioxidative system. In D. Gupta, J. Palma & F. Corpas (Eds.), *Reactive oxygen species and oxidative damage in plants under stress* (pp. 127-163). Cham: Springer. https://doi.org/10.1007/978-3-319-20421-5_6

Veljović Jovanović, S., Kukavica, B., Vidović, M., Morina, F., & Menckhoff, L. (2018). Class III peroxidases: Functions, localization and redox regulation of isoenzymes. In D. Gupta, J. Palma & F. Corpas (Eds.), *Antioxidants and antioxidant enzymes in higher plants* (pp. 269-300). Cham: Springer. https://doi.org/10.1007/978-3-319-75088-0_13

Villalpando-Rodriguez, G. E., & Gibson, S. B. (2021). Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat. *Oxidative Medicine and Cellular Longevity*, *2021*, 9912436. https://doi.org/10.1155/2021/9912436

Wang, M., Gong, S., Fu, L., Hu, G., Li, G., Hu, S., & Yang, J. (2022). The involvement of antioxidant enzyme system, nitrogen metabolism and osmoregulatory substances in alleviating salt stress in inbred maize lines and hormone regulation mechanisms. *Plants*, *11*(12), 1547. https://doi.org/10.3390/plants11121547

Wang, Y., Jia, D., Guo, J., Zhang, X., Guo, C., & Yang, Z. (2017). Antioxidant metabolism variation associated with salt tolerance of six maize (*Zea mays* L.) cultivars. *Acta Ecologica Sinica*, *37*(6), 368-372. https://doi.org/10.1016/j.chnaes.2017.08.007

ANTIOKSIDATIVNI ODGOVOR BILJAKA KUKURUZA NA STRES IZAZVAN SOLJU

Biljana Pezić[1], Vanja Bjelić[1], Biljana Davidović-Plavšić[1*], Biljana Kukavica[1]

[1]*Univerzitet u Banjoj Luci, Prirodno-matematički fakultet, Mladena Stojanovića 2, 78000 Banja Luka, Republika Srpska, Bosna i Hercegovina*

***Autor za korespondenciju:** biljana.davidovic-plavsic@pmf.unibl.org

Sažetak

Cilj rada je bio da se ispita uticaj povećane koncentracije NaCl na koncentraciju proteina i antioksidativni sistem korijena i listova kukuruza. Biljke kukuruza (hibrid ZP 555) su gajene hidroponično i tretirane sa NaCl u koncentracijama od 50 i 150 mM tokom šest dana.

Od antioksidativnih parametara određeni su: koncentracija glutationa (GSH) i aktivnost antioksidativnih enzima: peroksidaze klase III (POX, EC 1.11.1.7), askorbat peroksidaza (APX, EC 1.11.1.11) i katalaza (CAT, EC 1.11.1.6). Nakon tretmana sa 50 mM NaCl, koncentracija proteina u listu i korijenu se smanjila, dok je nakon tretmana sa 150 mM NaCl izmjereno povećanje koncentracija proteina, ali samo u listu. Povećanje koncentracije GSH detektovano je za obje koncentracije NaCl u listu, a u korijenu samo za nižu koncentraciju, u poređenju sa kontrolom. Aktivnost POX je značajno porasla samo u listovima tretiranim sa 150 mM NaCl, dok je pri nižoj koncentraciji aktivnost POX smanjena, kako u listovima kukuruza tako i u korijenu. Pet izoformi rPOX detektovano je nativnom gel elektroforezom u kontroli, dok izoforma rPOX5 nije detektovana u tretiranim uzorcima. Dvije lPOX izoforme su detektovane u kontrolnim i tretiranim uzorcima lista. Nativna elektroforeza je pokazala prisustvo jedne CAT izoforme samo u listovima, i u kontrolnim i u tretiranim uzorcima. Najveća CAT aktivnost je izmjerena pri nižoj koncentraciji NaCl. Na osnovu dobijenih rezultata može se zaključiti da salinitet mijenja antioksidativni sistem u listovima i korijenu kukuruza. Pored toga, na osnovu mjerenih parametara može se zaključiti da hibrid ZP 555 ima umjerenu tolerantnost na ispitivane nivoe saliniteta.

Ključne riječi: salinitet, ZP 555, proteini, GSH, POX, APX, CAT

Received March 04, 2024
Accepted April 09, 2024