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THE ROLE OF ANTIOXIDANT ENZYMES IN SALINITY STRESS TOLERANCE OF BITTER CUCUMBER (*MOMORDICA CHARANTIA L.*)

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Abstract

Momordica charantia, a tropical and subtropical species belonging to the family Cucurbitaceae, is valued both for its nutritional importance and its medicinal properties. Salinity stress represents a major factor limiting agricultural productivity by affecting processes such as photosynthesis, plant growth, and development. Catalase and peroxidase are the principal antioxidant enzymes involved in the degradation of excess accumulated hydrogen peroxide. The aim of the present study was to assess the salinity stress tolerance of five bitter cucumber genotypes (Brâncuși, Rodeo, Line 3, Line 4, and Line 1) by determining their enzymatic activity. The results showed that genotype Line 3 exhibited the highest enzymatic activity (over 50%) under salinity stress conditions (100 mM and 200 mM NaCl), indicating superior antioxidant capacity and increased tolerance to saline conditions. These findings were statistically validated using Pearson's correlation analysis.

Key words*: salinity stress, catalase, peroxidase, Momordica charantia.*

INTRODUCTION

Momordica charantia is a climbing plant species belonging to the genus Momordica, family Cucurbitaceae, native to regions of Asia and Africa (Wang et al., 2017). Bitter cucumber has a long history of use in traditional medicine and is particularly consumed for its antidiabetic properties, which have earned it the designation of "plant insulin" (Chanda et al., 2019). In addition to its medicinal role, this species also possesses significant nutritional value. It is rich in vitamins, especially A, C, E, and the B-complex group, and provides essential minerals such as potassium, zinc, and iron. Furthermore, it contains valuable bioactive compounds, including polyphenols, flavonoids, and carotenoids (Kumar & Bhowmik, 2010; Gayathry & John, 2022; Sheikhalipour et al., 2023).

In the context of rapid global population growth, projected to exceed 9 billion by 2050, and the increasing demand for food security, abiotic stress represents a major constraint on agricultural production, significantly affecting crop growth, development, and yield (Oshunsanya et al., 2019; Moulick et al., 2024; Kopecká et al., 2023).

Salinity is one of the principal abiotic factors threatening the future of agricultural crops. This type of stress is characterized by elevated concentrations of mineral salts accumulated in water and soil, which disrupt essential physiological and biochemical processes in plants, thereby limiting productivity and ecosystem sustainability (Meloni et al., 2004; Ahmad & Prasad, 2013; Schubert, 2011).

High salinity induces osmotic stress, reducing the plant's ability to absorb water and mineral nutrients. Mark Tester Munns described this as the first phase of salinity effects, which is immediately perceived by plants (Munns, 1993). The second phase of salt stress occurs with the excessive accumulation of sodium and chloride ions in plant organs. Ionic stress is accompanied by oxidative stress, defined by the excessive accumulation of reactive oxygen species (ROS), such as the superoxide radical (O_2^-), hydroxyl radical (OH•), and hydrogen peroxide (H_2O_2). This elevated accumulation is referred to as an "oxidative burst" (Sharma et al., 2012). ROS alter molecular and cellular components by oxidizing proteins, carbohydrates, lipids, enzymes, and even DNA, ultimately leading to

programmed cell death (Huang et al., 2019; Kesawat et al., 2023).

Plants respond to oxidative stress by increasing the levels of enzymatic and non-enzymatic antioxidants, which act synergistically to neutralize reactive oxygen species and protect cells against oxidative damage (Zheng et al., 2025). The primary enzymatic mechanisms for H_2O_2 scavenging in plants involve catalase and peroxidase. However, their modes of action differ: catalase directly converts H_2O_2 into H_2O and $½O_2$ and is the main enzyme involved in detoxification (Ann et al., 2020). When catalase balance is altered in plants, compensatory mechanisms such as peroxidase activation are triggered. Peroxidase activity requires the regeneration system of ascorbate and glutathione. The first reaction of this cycle, catalyzed by ascorbate peroxidase (APX), is:

H_2O_2 + Ascorbate → H_2O + Monodehydroascorbate (MDA).

Glutathione peroxidase (GPX) also detoxifies H_2O_2 to H_2O but directly uses glutathione as a reducing agent (Sofo et al., 2015).

The aim of the present study was to identify *Momordica charantia* genotypes resistant to salinity stress by investigating their enzymatic profiles.

MATERIALS AND METHODS

Materials

Leaves of *Momordica charantia*, KH_2PO_4, Na_2HPO_4, NaOH, $C_7H_8O_2$ (guaiacol), and H_2O_2 were used in the present study.

Experimental Design

Five genotypes of *Momordica charantia* were used, including two Romanian cultivars (Rodeo and Brâncuși) and three experimental lines (Line 1, Line 3, and Line 4). The experiment was conducted in the greenhouse of the Research Institute for Agriculture and Environment, affiliated with the "Ion Ionescu de la Brad" Iași University of Life Sciences, as well as in the vegetable-growing tunnels of the Horticulture discipline located at the Vasile Adamache Experimental Farm.

Seeds of uniform size and appearance were sown in greenhouse trays. After seedling establishment, the plants were transplanted into the tunnel system. The plants were divided into the following variants: control (no salt treatment), V1 (plants treated with 100 mM NaCl), and V2 (plants treated with 200 mM NaCl).

A total of three saline treatments were applied at the following BBCH phenological stages: 201 BBCH (corresponding to the appearance of the first lateral shoot), 501 BBCH (appearance of the first flower), and 701 BBCH (appearance of the first fruit). Leaves used for enzymatic analyses were harvested seven days after the application of the final saline treatment.

Catalase Activity

Catalase enzymatic activity was determined using the protocol described by Hugo Aebi (1984), with minor modifications. A 30% hydrogen peroxide solution was diluted in phosphate buffer (pH = 7.0) until the absorbance reached 0.55.

A quantity of 0.2 g of fresh plant material was homogenized in a mortar with 3 mL of buffer solution and subsequently centrifuged for 15 minutes at 10,000 rpm.

The reference sample consisted of 2.9 mL of buffer solution mixed with 0.1 mL of supernatant obtained from the plant extract. The reaction mixture for analysis contained 2.9 mL of hydrogen peroxide solution (prepared in buffer) and 0.1 mL of supernatant.

Ten absorbance readings were recorded at intervals of 15 seconds.

One unit (U) is defined as the amount of enzyme required to degrade 1 μmol of hydrogen peroxide per minute. Catalase activity was determined by monitoring the decrease in absorbance at 240 nm over time. By plotting absorbance (A) versus time (min), the reaction rate (slope) was calculated and subsequently used in the equation for determining specific activity:

$U/g\ FW = (slope \times V_r) / (\varepsilon \times m)$

where:

- slope - reaction rate,
- V_r - total reaction volume,
- ε - molar extinction coefficient of hydrogen peroxide,
- m - mass of fresh plant material used for analysis.

Peroxidase Activity

Peroxidase activity was determined spectrophotometrically by monitoring the oxidation of guaiacol in the presence of hydrogen peroxide, measuring the increase in absorbance at 470 nm, and calculating the reaction rate to determine the

specific activity. The protocol and calculation method used for peroxidase determination were identical to those described for catalase.

RESULTS AND DISCUSSIONS

High salinity significantly disrupts photosynthesis and respiration processes, leading to the formation of the superoxide radical ($O_2^{\bullet -}$). Superoxide dismutase catalyzes its dismutation into hydrogen peroxide (H_2O_2), which is subsequently decomposed, primarily by catalase, into H_2O and O_2 (Hasanuzzaman et al., 2021). According to the scientific literature, tolerant genotypes exhibit elevated enzymatic activity even under normal environmental conditions, enabling them to respond rapidly when salinity triggers oxidative stress (Xiao and Zhou, 2023; Ghiasy & Majid, 2025).

Among the studied *Momordica charantia* genotypes, the highest catalase activity under control conditions was recorded in genotype Line 3 (9.82 U/g FW), suggesting a potentially high tolerance of this experimental line to stress. In contrast, the lowest catalase activity was observed in genotype Line 4 (1.41 U/g FW).

Moderate salinity (100 mM NaCl) induced a significant increase in catalase activity in all studied genotypes, with the most pronounced difference compared with the untreated control observed in Line 3 (28% increase relative to the control). The smallest increase was recorded in Line 4, where no statistically significant difference was observed. The 200 mM NaCl treatment elicited a differential response among the bitter cucumber genotypes. Increases in catalase activity relative to the control variant were recorded in Line 1 (14%) and Line 3 (58%). In contrast, the Brâncuși, Rodeo, and Line 4 genotypes exhibited decreases in enzymatic activity compared with both the V1 treatment and the untreated control (Figure 1). Under severe salinity stress conditions, catalase activity declines in sensitive plants due to excessive ROS accumulation, direct enzymatic inhibition caused by ionic toxicity, and accelerated protein degradation. This response indicates that the plant's antioxidant defense capacity has been exceeded (Sofo et al., 2015).

In contrast to catalase, the *Momordica charantia* genotypes exhibited a stronger peroxidase activity, which may be explained by their prolonged exposure to abiotic stress conditions. Under control conditions, the highest enzymatic activity was recorded in Line 3 (13.37 U/g FW), followed by Line 1 (9.34 U/g FW). The remaining studied genotypes showed lower peroxidase activity, highlighting increased sensitivity.

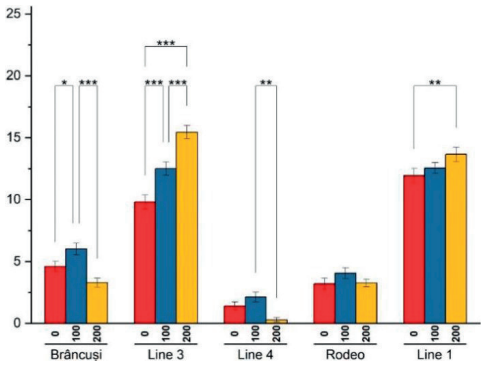

Figure 1. Effect of salinity stress on catalase activity expressed as U/g FW in the studied bitter cucumber genotypes, seven days after the application of the final saline treatment. Error bars represent the standard deviation, and asterisks indicate statistical significance according to Tukey's post hoc test as follows: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$; n = 3

Treatment with 100 mM NaCl led to an intensification of enzymatic activity in all studied genotypes, with a maximum observed in Line 3 (23.47 U/g FW), representing a 76% increase compared to the control. A significant increase in peroxidase activity reflects the plant's rapid response to salinity-induced stress conditions (Wu et al., 2022).

Application of the 200 mM NaCl treatment further enhanced enzymatic activity compared to both the untreated control and the 100 mM NaCl treatment. Relative to the control, the highest enzymatic activity was recorded in Line 1 (98% increase), followed by Line 3 (92%). Regarding the variation between stress treatments, highly statistically significant differences were observed only in experimental Lines 1 and 3, further emphasizing their rapid response to unfavorable environmental conditions (Figure 2).

Significant increases in peroxidase activity under salinity stress have also been reported by Neto and colleagues (2005). The authors investigated enzymatic activity in both salt-tolerant and salt-sensitive maize cultivars and

observed an accelerated increase in peroxidase activity in salt-tolerant cultivars following the onset of salinity stress (Neto et al., 2006).

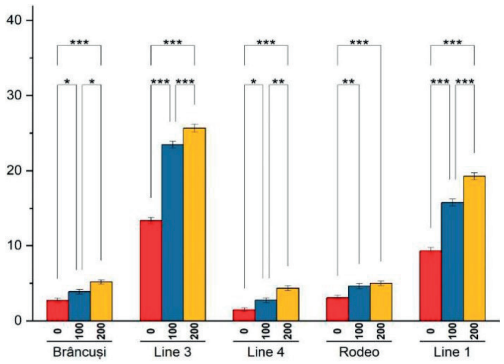

Figure 2. Effect of salinity stress on peroxidase activity expressed as U/g FW in the studied bitter cucumber genotypes, seven days after the application of the final saline treatment. Error bars represent the standard deviation, and asterisks indicate statistical significance according to Tukey's post hoc test as follows: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$; n = 3

In Figure 3, the Pearson correlation coefficient was calculated for catalase and peroxidase analyses performed on the leaves of the bitter cucumber genotypes. For the Pearson correlation analysis, specific abbreviations were used as follows: B, R, L1, L3, and L4 represent the studied genotypes Brâncuși, Rodeo, Line 1, Line 3, and Line 4, respectively. These were combined with the notations M, V1, and V2, corresponding to the control, 100 mM NaCl treatment, and 200 mM NaCl treatment. The abbreviations C and P refer to catalase and peroxidase, respectively.

The correlation matrix displays a color gradient ranging from light yellow, indicating a strong negative correlation, to violet, representing the absence of correlation, and up to black, indicating a strong positive correlation.

Analysis of the Pearson correlation matrix revealed strong positive relationships in all studied genotypes under the 100 mM NaCl treatment, demonstrating a favorable response of the genotypes to moderate abiotic stress conditions. In plants treated with 200 mM NaCl, strong negative correlations between the activities of the two studied enzymes were observed in the Brâncuși, Line 4, and Rodeo genotypes. This was associated with a decrease in catalase activity under severe stress, highlighting their sensitivity to harsh environmental conditions.

In contrast, Line 3 and Line 1 maintained strong positive correlations under severe salinity, emphasizing their enhanced tolerance to salt stress.

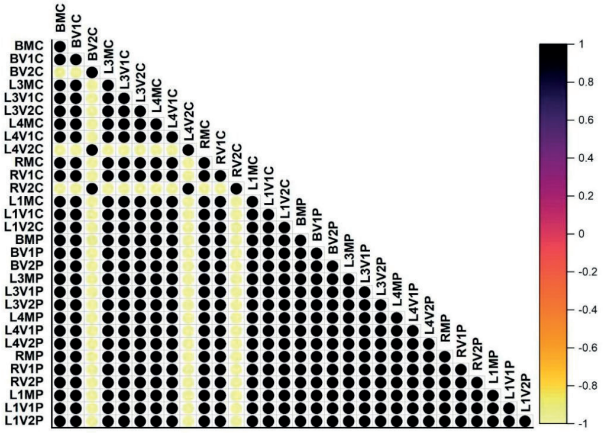

Figure 3. Pearson correlation results for catalase and peroxidase determinations following the application of the final saline treatment in the studied bitter cucumber genotypes

CONCLUSIONS

Hydrogen peroxide (H_2O_2) is one of the primary reactive oxygen species (ROS) produced in excess under high salinity conditions. To protect against cellular oxidative damage, plants enhance their enzymatic metabolism by increasing the levels of catalase and peroxidase.

Comparative analysis revealed that Lines 1 and 3 exhibited the highest catalase and peroxidase activities under salinity stress, indicating enhanced tolerance and the ability to maintain an efficient enzymatic response under severe oxidative stress.

These findings open new research perspectives, as the identified salt-tolerant genotypes can serve as a starting point for further studies on the mechanisms underlying salinity resistance.

REFERENCES

Aebi, H.B.T.-M. (1984). Catalase *in vitro*. *Oxygen Radicals in Biological Systems*, *105*, 121–126 .

Ahmad, P., & Prasad, A.M.N. V. (2013). Salt Stress in Plants. *Salt Stress in Plants,* 465–495

Ann, C., Karen, S., Jos, R., Kelly, O., Els, K., Tony, R., Nele, H., Nathalie, V., Suzy, V. S., Frank, V., B., Yves, G., J. C., & Jaco, V. (2020), The cellular redox state as a modulator in cadmium and copper responses in Arabidopsis thaliana seedlings, *Journal of Plant Physiology*, *168* (4), 309–316.

Chanda, R., Samadder, A., & Banerjee, J. (2019), Anti-diabetic Activity of Momordica Charantia or BitterMelon : A Review, *Acta Scientific Pharmaceutical Sciences*, *3*(5), 24–30.

Gayathry, K., S., & John, J. A. (2022), A comprehensive review on bitter gourd (Momordica charantia L.) as a gold mine of functional bioactive components for therapeutic foods, *Food Production, Processing and Nutrition*, *4* (1).

Ghiasy, M., & Majid, O. (2025), Salt tolerance mechanisms in five Asteraceaespecies : seed germination and seedlinggrowth, cellulardamage, enzymatic and non - enzymatic antioxidants, *Acta Physiologiae Plantarum*, *47*(3), 1–14

Hasanuzzaman, M., Raihan, R.H., Awal, A., Masud, C., Rahman, K., Nowroz, F., Rahman, M., &

Masayuki, F. (2021), Regulation of Reactive Oxygen Species and Antioxidant Defense in Plants under Salinity. *International Journal of Molecular Sciences 22*(17), 9326

Huang, H. Ullah, F., Zhou, D.X., Yi, M., and Zhao, Y. (2019), Mechanisms of ROS regulation of plant development and stress responses, *Frontiers in Plant Science*, *10*, 1–10.

Kesawat, M.S., Satheesh, N., Kherawat, B.S., Kumar, A., Kim, H.U., Chung, S.M., & Kumar, M. (2023), Regulation of Reactive Oxygen Species during Salt Stress in Plants and Their Crosstalk with Other Signaling Molecules - Current Perspectives and Future Directions, *Plants*, *12*(4).

Kopecká, R., Kameniarová, M., Černý, M., Brzobohatý, B., and Novák, J. (2023), Abiotic Stress in Crop Production, *International Journal of Molecular Sciences*, *24*(7).

Kumar, K.P.S., & Bhowmik, D. (2010), Traditional medicinal uses and therapeutic benefits of Momordica charantia Linn, *International Journal of Pharmaceutical Sciences Review and Research*, *4*(3), 23–28.

Meloni, D.A., Gulotta, M.R., Martínez, C.A., & Oliva, M.A. (2004), The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in Prosopis alba, *Brazilian Journal of Plant Physiology*, *16*(1), 39–46.

Moulick, D., Choudhury, S., & Brestic, M. (2024), Editorial : The impact of abiotic stresses onagriculture : mitigation through climate smart strategies*, Frontiers in Plants Science, 15*, 1–3.

Munns, R. (1993), Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses, *Plant, Cell & Environment*, *16*(1), 15–24.

Neto, D.A., Prisco, T., En, J., Eduardo, C., & Abreu, B. De. (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.

Oshunsanya, S.O., Nwosu, N.J., & Li, Y. (2019), Abiotic Stress in Agricultural Crops Under Climatic Conditions BT - Sustainable Agriculture, Forest and Environmental Management, *Springer Singapore*, *1*, 71–100.

Schubert, S. (2011), Salt Resistance of Crop Plants: Physiological Characterization of a Multigenic Trait, *The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops*, 443–455.

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, *Hindawi Publishing CorporationJournal of Botany*.

Sheikhalipour, M., Gohari, G., Esmaielpour, B., Behnamian, M., Giglou, M.T., Milani, M.H., Bahrami, M.K., Kulak, M., Ioannou, A., Fotopoulos, V., & Vita, F. (2023), Effect of melatonin foliar sprays on morphophysiological attributes, fruit yield and quality of *Momordica charantia* L. under salinity stress, *Plant Physiology and Biochemistry*, *205*, 108194.

Sofo, A., Scopa, A., Nuzzaci, M. & Vitti, A. (2015), Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses, *International Journal of Molecular Sciences, 16*(6) 13561–13578.

Wang, S., Li, Z., Yang, G., Ho, C.T. & Li, S. (2017), Momordica charantia: A popular health-promoting vegetable with multifunctionality, *Food and Function*, *8*(5), 1749–1762.

Wu, L., Jiang, Q., Zhang, Y., Du, M., Ma, L. & Ma, Y. (2022), Peroxidase Activity in Tomato Leaf Cells under Salt Stress Based on Micro-Hyperspectral Imaging Technique, *Horticulturae*, *8*(9), 1–12.

Xiao, F. & Zhou, H. (2023), Plant salt response: Perception, signaling , and tolerance, *13*, 1–16.

Zheng, C. Chen, J., & Wang, X. (2025), Reactive Oxygen Species in Plants: Metabolism, Signaling and Oxidative Modifications*. Antioxidants*, *14*, 1–17.