THE INFLUENCE OF SALINE STRESS ON THE CONCENTRATION OF TOTAL POLYPHENOLS IN BITTER CUCUMBERS (*MOMORDICA CHARANTIA*)

Ștefănica OSTACI [1]*, Cristina SLABU [1], Alina Elena MARTA [1], Mihaela COVAȘĂ [1] and Carmenica Doina JITĂREANU [1]

e-mail: stefanaostaci@gmail.com

Abstract

Momordica charantia is a climbing plant from the *Cucurbitaceae* family known and widely used for its many medicinal properties including the fight against diabetes. Salinity is the main abiotic stress factor affecting every aspect of plant physiology and biochemistry significantly affecting growth, development and production yield. Polyphenols are natural phytokinins synthesized in plants as secondary metabolites with the role of signaling, plant defense, mediating auxin transport, antioxidant activity and free radical scavenging. To determine salinity resistance, 5 bitter cucumber genotypes were studied that were treated with different salt concentrations. The highest amount of total polyphenols was observed in the case of Line 4 where the value recorded at the treatment with saline solution 200 mM NaCl determined an increase by 125.9% compared to the untreated control. This value correlated negatively with the number of lateral shoots, which highlights a poor adaptation of the genotype to salt stress. Compared to this genotype, Line 3 showed a reduced increase in the content of polyphenols in the variants subjected to saline stress, but also an increase in the number of lateral shoots, observing a positive correlation in the two factors. This response of the genotype highlights a good adaptation to salt stress.

Key words: *Momordica charantia*, polyphenols, spectrophotometry, salinity

Momordica charantia is a climbing plant in the *Cucurbitaceae* family known and widely used in areas such as China, India, Malaysia and the tropical part of Africa (Ahmad *et al*, 2016). The popular name of bitter cucumber or bitter gourd comes from the particularly bitter taste that characterizes all the organs of the plant. It has a long and rich history of use in traditional medicine to treat conditions such as eczema, pneumonia, rheumatism, hypertension, psoriasis, cancer and diabetes (Alam *et al*, 2015). The bitter taste of the plant is given by momordicin, a bioactive compound with a strong hypoglycemic effect called vegetable insulin (Chanda and Banerjee, 2019). Analyzes have shown that this plant has the highest nutritional value of all species of the cucurbitaceae family, being an excellent source of fiber, vitamins, minerals, carbohydrates and proteins. Bitter cucumber is also used due to the high content of minerals such as: Cu, Ca, Fe, Mg and Zn. The fruit is rich in nutrients such as vitamins A and C, tocopherols, thiamin, riboflavin, niacin and folic acid (Yuwai *et al*, 1991, Singla *et al*, 2023). In food, the aerial parts of the plant are consumed, especially the fruits, leaves and young shoots.

Salinity is the main abiotic stress factor affecting every aspect of plant physiology and biochemistry significantly affecting growth, development and production yield (Munns, 1993). Saline stress affects almost half of the irrigated agricultural lands causing significant economic losses and negatively affecting food security (Zhu 2001, Ondrasek *et al*, 2022). A soil is considered to be saline when it exhibits an electrical conductivity (EC) of the soil solution greater than 4 dS m^{-1} (equivalent to 40 mM NaCl). This concentration of salts generates an osmotic pressure of about 0.2 MPa and significantly reduces the yields of most crops. High salinity compromises carbon fixation, leading to over-reduction of light-harvesting complexes that cause the production of reactive oxygen species (ROS) such as: hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), ozone (O_3), singlet oxygen ($^1O_2$), superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-), perhydroxyl radicals ($HO_2^\bullet$), organic alkoxy ($RO^\bullet$) and organic peroxyl radicals (Misra and Gupta, 2005; Hameed *et al*, 2015).

Polyphenols are naturally occurring phytokinins synthesized in plants as small, secondary metabolites that play numerous

[1] Department of Plant Science, "Ion Ionescu de la Brad" Iasi University of Life Sciences, 700490 Iasi, Romania

biochemical and molecular roles in plants, such as signaling molecules, plant defense, mediating auxin transport, antioxidant activity, and scavenging free radicals (Frisvold, 2022; Tohidi *et al*, 2017). Among the non-enzymatic antioxidants, phenols and flavonoids contribute significantly to the scavenging of free radicals in plants to tolerate salt stress by accumulating in various tissues. Polyphenols are present in free and bound forms in plant materials (Kiani *et al*, 2021).

MATERIAL AND METHOD

The plant material used for the analyzes in this work was represented by the leaves and lateral shoots of bitter cucumber. To obtain them, seeds of equal size, of bitter cucumbers belonging to two varieties (Brâncusi variety and Rodeo variety) and three experimental lines (Line 1, Line 3 and Line 4) were sown in cells in the greenhouse of the Agricultural Research Institute and Environment (ICAM) belonging to the "Ion Ionescu de la Brad" University of Life Sciences, Iași, Romania. To achieve germination, the seeds were kept at an average temperature of 28 °C. After germination, the seedlings were moved to 12-liter pots of vegetation in the University greenhouses. To determine the effect of salinity on the 5 genotypes, the plants were treated with saline solutions of concentrations 0 mM NaCl – Control (M), 100 mM NaCl – V1 and 200 mM NaCl – V2. In total, three treatments were applied at intervals of approximately 10 days. The first treatment was applied in phenophase 201 BBCH (representing the appearance of the first lateral shoot), the second treatment was applied in phenophase 501 BBCH (representing the appearance of the first flower) and the last treatment was applied in phenophase 701 BBCH (being equivalent to the appearance of the first fruit). The plants were treated with an amount of 300 ml of saline solution per treatment. The plant growth procedure was similar to that presented in our previous works on bitter cucumber (Ostaci *et al*, 2024; Ostaci *et al,* 2023, Ostaci *et al*, 2024). The leaves used for the analysis of the total content of polyphenols were harvested 7 days after the application of the last treatment. The analysis of the number of lateral shoots was carried out in the field on the 7th day after the last treatment.

The determination of polyphenols was carried out using the Folin Ciocalteu reagent. 0.1 g of plant sample was used which was mortared with 1 ml of distilled water. From the resulting sample, 0.1 ml was used, over which 0.5 ml of Folin Ciocalteau reagent and 2 ml of distilled water were added. After 5 minutes, an amount of 0.75 ml of 20% $NaCO_3$ and 2.95 ml of distilled water was added. The obtained mixture is left to rest for one hour at room temperature, after which it is read by the UV-Vis method at 675 nm.

Three repetitions were performed for each determination.

Statistical analysis was performed by applying Two Way Anova, Tukey and Pearson correlation matrix tests. Statistical analyzes were performed using Origin Pro 2022 software.

RESULTS AND DISCUSSIONS

Polyphenolic compounds are considered a group of molecules with similar biological activity, especially in physiological and biochemical studies, where they are used as a marker of biological activity (Šamec *et al*, 2021).

According to the spectrophotometric analysis of the total content of polyphenols (Figure 1), significant differences were noted between the genotypes studied under normal and saline stress conditions. The general trend was to increase the total amount of polyphenols in plants subjected to abiotic stress. In the control plants, the lowest amount of total polyphenols was recorded in the case of the Brâncusi variety (229.19 mg/L) and the highest amount was observed in the Line 3 genotype (421.11 mg/L). A high amount of polyphenols naturally existing in plants shows their good resistance to saline stress (Zagoskina *et al*, 2023). In the case of plants stressed with 100 mM saline solution, the lowest amount of polyphenols was recorded in the Brâncusi genotype (351.19 mg/L) and the highest amount was observed in Line 4 (524.83 mg/L). The plants subjected to the treatment with 200 Mm NaCl showed the lowest amount of total polyphenols in the case of the Brâncusi genotype (392.05 mg/L) and the highest amount in the Linia 4 genotype (780.55 mg/L). According to specialized literature, when saline stress occurs, the biosynthetic pathways of several phenolic compounds are stimulated, thus causing an increase in the amount of total polyphenols (Zagoskina *et al*, 2023). The highest increase in the total amount of polyphenols was recorded in the case of Line 4 where the treatment with 100 mM saline caused an increase of them by 51.89% compared to the control, and the treatment with 200 mM NaCl caused an increase of 125.9%. This increase in the total amount of polyphenols can highlight the effort made by the plants to resist the new conditions to which they were subjected. The low amount of polyphenols recorded in control plants of this genotype is an important indicator of their sensitivity to salt stress. Compared to Line 4, the smallest increases in phenolic compounds were recorded in the case of Line 3 where the treatment with 100 mM NaCl caused an increase of 11.11% compared to the control and the treatment with 200 mM NaCl caused a 17.76% increase. This reduced growth associated with a high amount of total

polyphenols in the control plants indicates a good resistance of the genotype to salt stress and the absence of the installation of oxidative stress at a toxic level.

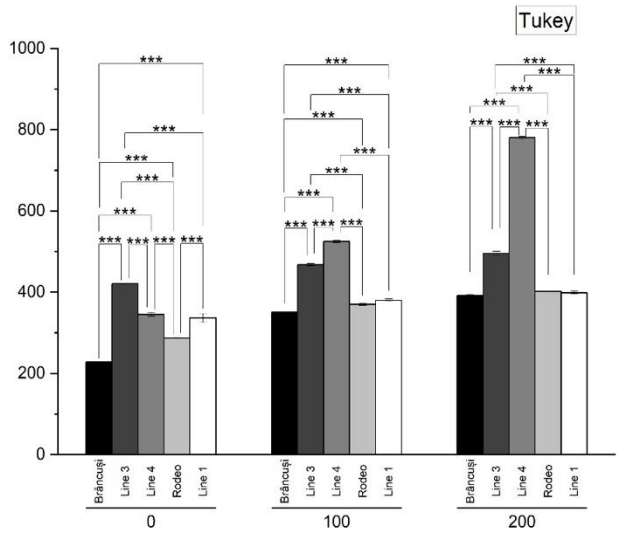

Figure 1 **Total polyphenol content of bitter cucumber leaves after the third saline treatment, measured in ml/L. The bars represent the standard deviation and the asterisks on the graph are placed using the Tukey test. *** - p < 0.001.**

Growth is the process of stable and irreversible increase in the weight and volume of plant cells, tissues and organs, due to the continuous increase in the amount of dry matter (Jităreanu, 2007). Salt stress caused a decrease in the number of lateral shoots in all the bitter cucumber genotypes studied in this work except Line 3 where an increase was observed compared to the untreated control. In the case of the control plants, the lowest number of lateral shoots was observed in the Line 4 genotype (5 shoots) and the highest number of shoots was recorded in Line 1 (7 shoots). The plants treated with 100 mM NaCl showed the lowest number of lateral shoots in the Line 4 genotype (4 shoots) and the highest number compared to the untreated control in Line 1 (6.3 shoots). Treatment with 200 mM saline revealed the lowest number of lateral shoots in Line 4 (3 shoots) and the highest number compared to the control in Line 3 (6.3 shoots). Polyphenols, in addition to the essential antioxidant role they have in plants, are indirectly responsible for energy transfer, growth regulation, the intensity of photosynthesis and morphogenesis (Duda-Chodak and Tarko, 2023). High amounts of polyphenols can negatively affect plant growth and development through their interaction with growth hormones. Thus, a large amount of polyphenols indicates a strong abiotic stress affecting the plant, which causes the use of a significant amount of energy and the reduction of essential processes. This effect could be noted from the analyzes performed. The lowest value of lateral shoots and the highest value of total polyphenol content were recorded in Line 4.

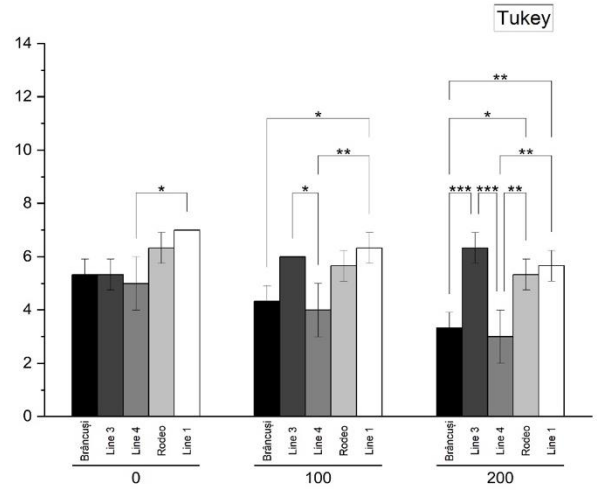

Figure 2 **The number of lateral shoots in the bitter cucumber genotypes studied after the application of the third saline treatment. Error bars represent the standard deviation and asterisks are placed using the Tukey test. *- p < 0.05, ** - p < 0.01, *** - p < 0.001.**

The correlation between the two analyzes was strongly negative (Figure 3) which may indicate a poor resistance of Line 4 to salt stress. Negative correlations were recorded in the case of all genotypes subjected to salt stress except for Line 3 where the variant treated with 200 mM NaCl recorded a positive mean correlation. This may represent the fact that this genotype is the least affected by salinity and therefore the small increase in the amount of polyphenols compared to the other genotypes has a positive role in the plant growth process.

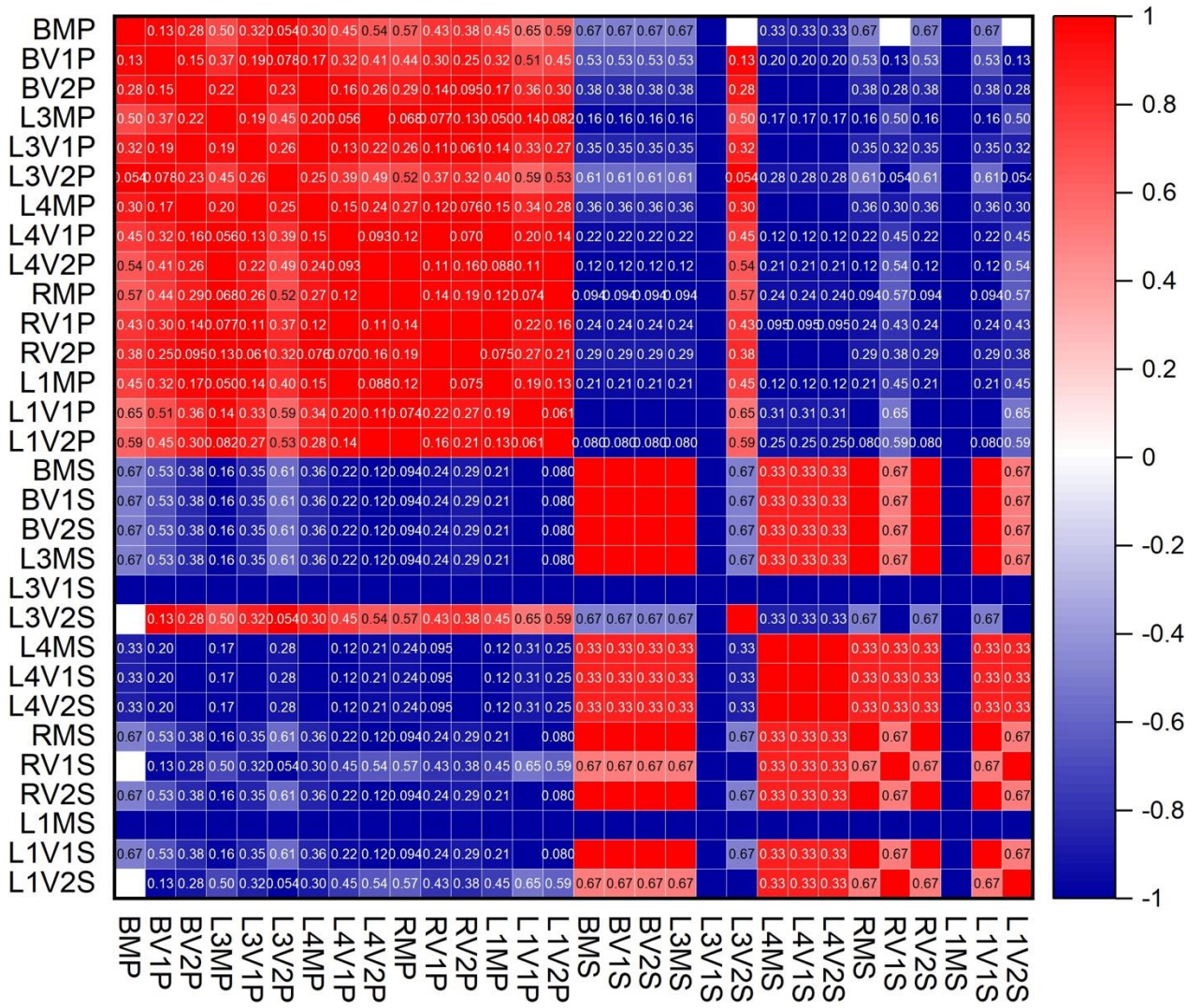

Figure 3 **Pearson correlation matrix between the total amount of polyphenols and the number of lateral shoots of the studied genotypes. The specific notations are composed of the first letter of each genotype (B, L3, L4, R, L1) followed by the treatment applied (M, V1, V2) and the analysis performed (P – polyphenols, S – shoots)**

CONCLUSIONS

Following the analyzes carried out, increases in the amounts of polyphenols were observed in all studied genotypes, the differences being significant both between treatments and between genotypes. The highest growth was observed in Line 4 where it was negatively correlated with the lowest number of lateral shoots. Compared to this genotype, the lowest increase compared to the untreated control was recorded in Line 3 which was positively correlated with an increase in the number of lateral shoots.

The statistical analyzes carried out had the role of validating the observations mentioned in this paper.

According to the analyzes carried out, we can recommend Line 3 as a salinity-resistant genotype, which makes it ideal for cultivation on saline soils.

REFERENCES

Ahmad N., Hasan N., Ahmad Z., Zishan M., Zohrameena S., 2016 - *Momordica Charantia: For Traditional Uses and Pharmacological Actions*. Journal of Drug Delivery and Therapeutics, 6(2):40–44.

Alam M.A., Uddin R., Subhan N., Rahman M., Jain P., and Reza H.M., 2015 - *Beneficial Role of Bitter Melon Supplementation in Obesity and Related Complications in Metabolic Syndrome*. Journal of Lipids, 2015:1–18.

Chanda R., Asim S., Janmajoy B., 2019 - *Anti-Diabetic Activity of Momordica Charantia or Bitter Melon : A Review*. Acta Scientific Pharmaceutical Sciences, 3(5):24–30.

Duda-Chodak A., and Tomasz T., 2023 - *Possible Side Effects of Polyphenols and Their Interactions with Medicines*. Molecules 28(6), 2436.

Frisvold M.H., 2022 - *Functional Foods and Nutraceuticals*.

Hameed A., Gulzar S., Aziz I., Hussain T., Gul B., & Khan M. A., 2015 - *Effects of salinity and ascorbic acid on growth, water status and antioxidant*

system in a perennial halophyte. AoB PLANTS, 7(1):1–11.

J**ităreanu C.D., 2007** - *Fiziologia Plantelor*. Edit. "Ion Ionescu de la Brad", Iaşi, ISBN 978-973-7921-89-5.

Kiani R., Arzani A., Maibody S.A.M., 2021 - *Polyphenols, Flavonoids, and Antioxidant Activity Involved in Salt Tolerance in Wheat, Aegilops Cylindrica and Their Amphidiploids.* Frontiers in Plant Science, 12:1–13.

Misra N., Gupta A.K., 2005 - *Effect of Salt Stress on Proline Metabolism in Two High Yielding Genotypes of Green Gram.* Plant Science, 169(2):331–39.

Munns R. 1993 - *Physiological Processes Limiting Plant Growth in Saline Soils: Some Dogmas and Hypotheses*. Plant, Cell & Environment, 16(1):15–24.

Ondrasek G., Rathod S., Manohara K.K., Gireesh C., Anantha M.S., Sakhare A.S., Parmar B., Yadav B.K., Bandumula N., Raihan F., Zielińska-Chmielewska A., Meriño-Gergichevich C., Reyes-Díaz M., Khan A., Panfilova O., Seguel Fuentealba A., Romero S.M., Nabil B., Wan C.C., Shepherd J., Horvatinec J., 2022 - *Salt Stress in Plants and Mitigation Approaches*. Plants (Basel), 11(6):717.

Ostaci Ș., Slabu C., Marta A.E., Covașă M., Miniață, I., Jităreanu, C.D., 2023 - *The Influence of Salt Stress on the Content of Vitamin C in the Leaves of Some Varieties and Lines of Bitter Cucumber (Momordica Charantia).* Bulletin of University of Agricultural Sciences ond Veterinary Medicine Cluj-Napoca. Horticulture 80(2):65–70.

.**Ostaci Ș., Slabu C., Marta A.E., Covașă M., Miniață, I., Jităreanu C.D., 2024** - *Spectral Study of Some Metabolites Involved in the Adaptation Reaction of Bitter Cucumber (Momordica Charantia) to Saline Stress.* Horticulturae, 10, 309.

Ostaci Ș., Slabu C., Marta A.E., Jităreanu C.D., 2024 - *Evaluation of the Resistance of Bitter Cucumber (Momordica charantia) to Saline Stress through Physical, Biochemical, and Physiological Analysis*. Horticulturae, 10, 893.

Šamec D., Karalija E., Šola I., Vujčić Bok V., Salopek-Sondi B., 2021 - *The Role of Polyphenols in Abiotic Stress Response: The Influence of Molecular Structure.* Plants, 10(1):1–24.

Singla D, Sangha M.K., Singh M., Pathak M., Bala M., 2023 - *Variation of Mineral Composition in Different Fruit Parts of Bitter Gourd (Momordica charantia L.).* Biological Trace Element Research 201(10):4961–71.

Tohidi B., Rahimmalek M., Arzani A., 2017 - *Essential Oil Composition, Total Phenolic, Flavonoid Contents, and Antioxidant Activity of Thymus Species Collected from Different Regions of Iran.* Food Chemistry, 220:153–61.

Yuwai K.E., Rao K.S., Kaluwin, C., Jones G.P., and Rivett D.E., 1991 - *Chemical Composition of Momordica Charantia L. Fruits.* Journal of Agricultural and Food Chemistry, 39(10):1762–63.

Zagoskina N.V., Zubova M.Y., Nechaeva T.L., Kazantseva V.V., Goncharuk E.A., Katanskaya V.M., Baranova E.N., Aksenova M.A., 2023.- *Polyphenols in Plants: Structure, Biosynthesis, Abiotic Stress Regulation, and Practical Applications (Review).* International Journal of Molecular Sciences 24(18).

Zhu J.K., 2001 - *Plant Salt Tolerance*. Trends in Plant Science 6(2):66–71.