ABSTRACT

Keywords: *Momordica charantia*, salinity, photosynthesis, water regime, secondary metabolites, mineral nutrition.

Salt stress affects almost every aspect of plant physiology and biochemistry, leading to significant decreases in agricultural production. It is an important abiotic factor, affecting almost 50% of irrigated agricultural land. It is estimated that irrigated agriculture will increase by 20% in the coming years, which will most likely lead to the expansion of arable areas affected by salinity, representing a serious challenge for food security.

Most species of economic and agricultural interest do not show increased resistance to salt stress, which can cause strong physiological imbalances, considerable decreases in crop yield and in extreme cases complete drying of plants. In order to avoid these situations, the identification of salinity-resistant genotypes that can be used in future breeding programs is aimed at.

Momordica charantia is a climbing, tropical and subtropical plant commonly found in many areas of Asia such as China and India, but also in South America such as Brazil, Guatemala and Argentina.

Among the species belonging to the Cucurbitaceae family, the bitter cucumber is one of the most important, due to its very high nutritional value, but especially due to the over 60 active substances that can be used in the treatment of a very large number of diseases, starting from skin problems such as eczema and up to cancer and diabetes.

The doctoral thesis entitled **"Research on the physiological reaction of bitter cucumber (*Momordica charatia*) subjected to salt stress"** records and analyzes the physiological & biochemical characteristics of some bitter cucumber genotypes in the context of salt stress, obtained within the Buzău Vegetable Research and Development Station.

The research was conducted during 2021-2024 and aimed to improve bitter cucumber production in the context of salt stress, through a better understanding of the defense mechanisms and resistance to salinity of the studied genotypes. The following objectives were set for the preparation of this thesis:

- Establishing the influence of salt stress on the studied bitter cucumber genotypes;
- Specifying the influence of salt stress on biomass production, through biometric analyses;
- Determining the influence of salinity on the intensity of the photosynthesis process through physiological and biochemical analyses;
- Analysis of the response of some metabolites involved in the fight against salt stress;
- Study of mineral nutrition and the antagonism of some elements involved in the stress response of plants;
- Reusing plant material in the circular economy.

The research was carried out both in the greenhouse belonging to the Research Institute for Agriculture and Environment and in the solariums of the "Ion Ionescu de la Brad" University for Life Sciences in Iași.

This doctoral thesis is structured in two parts and includes seven chapters to which are added the introduction, bibliography and annexes. The thesis presents a number of 20 tables, 85 figures, a scheme and over 250 bibliographical sources.

Part I of the work includes the introduction and the current state of research, according to the specialized literature, on the bitter cucumber culture and the influence of salt stress on the main physiological and biochemical processes summarized in a chapter (I).

Chapter I is made up of four main subchapters that synthesize information about salt stress and its spread in the context of continuous population growth; the reaction of plants to salinity depending on the level of resistance to this stress factor and bitter cucumber from the point of view of nutritional and medicinal importance but also the classification of the species depending on its resistance to stress conditions. At the same time, this chapter presents literature studies regarding *Momordica charantia* and both the physiological and biochemical reaction in the context of high amounts of sodium chloride in the soil.

The second part of the thesis is represented by the own research and consists of six main chapters (II-VII).

Chapter II includes the description of the experimental conditions and the way of organizing the two experiments carried out in this thesis. During the research, a bifactorial experiment consisting of randomized blocks with three plants was designed. Factor A of the experiment was represented by the plant material used and factor B assumed the saline treatments applied.

The plant material was composed of five bitter cucumber genotypes, two of which were Romanian varieties (Rodeo and Brâncuși) and three experimental lines (Line 1, Line 3 & Line 4). These genotypes were each divided into three variants as follows: M – Untreated control, V1 - Variant treated with 100 mM NaCl and V2 – Variant treated with 200 mM NaCl. Treatments were applied in the 201 BBCH, 501 BBCH and 701 BBCH phenophases.

The secondary experiment involved the use of the same plant material, which was divided as follows: M – Untreated control, V1 - Variant treated with 50 mM NaCl and V2 – Variant treated with 50 mM NaCl and 0.5% Algevit biostimulator. The plants were watered with the corresponding solutions according to needs.

Chapter III presents the purpose, objectives, particularities of each genotype studied and also the research methodology within the present thesis. In order to achieve the proposed objectives, physiological, biochemical and physical analyses were carried out.

Physiological analyses are represented by biometric determinations such as: plant height and number of flowers per plant but also the average number of fruits. Determinations were made on the water regime through stomatal conductance. The same category also includes analyses on the content of photosynthetic pigments, carried out both by studying the total content of photosynthetic pigments and by analyzing chlorophyll a and chlorophyll b.

Biochemical analyses included the study of: proline, malondialdehyde, vitamin C, aromatic amino acids, total polyphenol content and nitrates, and physical analyses were represented by the Maximum Quantum Yield of PS II, the Maximum Fluorescence Yield in the absence of photosynthetic light (Fm), the Minimum Fluorescence Yield in the absence of photosynthetic light (F0) and the determination of mineral elements such as potassium, calcium, phosphorus and sulfur through X-ray fluorescence analysis.

Chapter IV presents the results of the analyses carried out on leaves & fruits within the main experiment, being divided into five subchapters according to the analyses presented.

The first section refers to the biometric analyses and highlights a similar behavior of the genotypes for each analysis carried out. Thus, after applying the first treatment, the variants treated with saline solutions for each genotype showed a trend of accelerated growth observed through a higher height value. This effect was not observed in the case of the Line 4 genotype, which reacted negatively to salt stress by reducing growth.

The application of the second treatment determined a decrease in the values in the variants subjected to salt stress, and after the application of the last treatment a sharp decrease in the growth process could be observed compared to the untreated control. In the context of a long period of exposure to salt stress, Line 3 was highlighted by maintaining higher values in the variants subjected to stress compared to the control.

The number of flowers and fruits were analyzed after the application of the second & third treatments, respectively, & the behavior of the genotypes was identical to that observed in the previous analyses. The most significant decreases compared to the control were reported in the Line 4 genotype where the number of flowers per plant decreased from 17 in the untreated control to 7 in the case of treatment with 200 mM NaCl, after the application of the second treatment. Compared to this genotype, Line 3 presented an upward trend in the number of flowers in the stressed variants, increasing by 27% in the variant treated with 200 mM NaCl.

In the case of the number of fruits in the saline treatments, the highest value was observed in Line 3 (3.3 fruits per plant in the treatment with 100 mM NaCl and 4 in the one with 200 mM NaCl) and the lowest values were noted in Line 4 (2.3 fruits in the variant V1 and 1 fruit per plant in the variant V2).

The second section of this chapter refers to the analyses performed on the water regime and the results obtained. The analysis of stomatal conductance revealed an initial reaction of its increase in the variants subjected to salinity, and a decrease after a long period of exposure to salinity. After the application of the last treatment, the strongest decrease in stomatal conductance was observed in genotype Line 4 with a decrease from 10.3 mol/m^{2}s^2 in the case of the control, to 9.4 mol/m^{2}s^2 in the V2 variant. Line 3 maintained an upward trend in stomatal conductance with an increase from 8.23 mol/m^{2}s^2 in the case of the control, to 9.1 mol/m^{2}s^2 in the case of the V2 variant.

The third subsection deals with the photosynthesis process by analyzing photosynthetic pigments and chlorophyll fluorescence. Photosynthetic intensity was maximum in the variants subjected to salt stress after the application of the first treatment due to the high amounts of photosynthetic pigments and in an accelerated decrease with the application of the following treatments.

Analysis of the total pigment content after the last treatment highlighted reduced values compared to the control in all genotypes studied, except for Line 3, where the V2 variant presented an increase of 7% compared to the control. Contrary to this increase, Line 4 presented the lowest values, decreasing from 46.51 u.s (control) to 38.5 u.s (V2).

The analysis of chlorophyll *a* and chlorophyll *b* confirms the results observed in the case of the total content of photosynthetic pigments, observing a similar behavior of chlorophylls. After a long period of exposure to salt stress, the chlorophyll content decreased significantly compared to the control.

The strongest decrease was noted in Line 4. In this genotype, chlorophyll *a* was reduced by 38% in the variant treated with 100 mM saline solution & by 58.6% compared to the untreated control in the variant watered with 200 mM NaCl. Chlorophyll *b* presented a behavior similar to that of chlorophyll *a*. Line 3 recorded higher values than the control even after the application of the last treatment. This high accumulation of chlorophyll highlights not only the resistance of the genotype to salt stress conditions, but also the fact that the plants fit into the biphasic model proposed by Munns in 1993. Chlorophyll fluorescence revealed optimal values of the controls for all genotypes analyzed, and the salt treatments influenced the values of the ratios. The most pronounced differences from the control could be observed in Line 4 for each of the three analyses, highlighting the genotype as being strongly affected by oxidative stress. Compared to this, the smallest differences from the control were observed in Line 3 (4% in treatment V1 & 6.9% in treatment V2), demonstrating good resistance to salt stress.

The fourth section presents the biochemical analyses performed on bitter cucumber leaves and fruits. Malondialdehyde and total polyphenols increased considerably in the variants subjected to salt stress for each genotype studied. The strongest increase in the amount of malondialdehyde was reported in Line 4, where treatment with 100 mM saline solution produced a 17% increase compared to the untreated control, and treatment with 200 mM saline solution caused a 41% increase in MDA compared to the control. This high concentration attracted a high amount of total polyphenols, highlighting the presence of high amounts of reactive oxygen species.

Considerable increases were noted, at the fruit level, in terms of proline & vitamin C. In both cases, the most pronounced increases compared to the control were noted in the Line 1 and Line 4 genotypes. In the analysis of proline, the maximum concentration was observed in Line 4, in which the treatment with 100 mM NaCl caused a 2.1-fold increase compared to the control, and the treatment with 200 mM NaCl increased the amount of proline by 4.68 times in green fruits. In the Line 1 genotype, vitamin C increased by 323.36%, reaching a value of 136.60 mg/100 g in plants treated with 200 mM NaCl. Compared to these differences, the lowest increases were reported in Line 3, highlighting a good reaction to salt stress of this genotype.

Regarding aromatic amino acids, they showed decreases in all genotypes analyzed, except Line 3. The most pronounced contrast between the plants in the control group and those treated with saline solutions was observed in Line 4, where the control presented an absorbance value of 0.6242; variant V1 had an absorbance of 0.5655, and variant V2 of 0.5177. In the context of previous results obtained in this research, it can be seen that Line 4 presents a reduced adaptation to salt stress conditions. Line 3 presented a slight tendency to increase the concentration of amino acids specific to stress-resistant genotypes.

The last subchapter aimed at the analysis of essential mineral elements for plants such as calcium, potassium and nitrogen. Each of the three analyses highlighted a strong tendency to decrease in the leaves of plants subjected to salt stress. This effect can be explained by both the occurrence of osmotic stress and ionic stress installed after long periods of exposure to salinity. The smallest differences were observed in Line 3 where the calcium ratio decreased by a maximum of 3.18% in plants treated with 100 mM NaCl and by 10.21% in plants treated with 200 mM NaCl. The same phenomenon was noted for both potassium & nitrate.

Chapter V highlighted the action of a commercial biostimulator, based on seaweed, on bitter cucumber genotypes, in the context of salt stress.

Thus, to evaluate the reaction of bitter cucumber to the application of the biostimulator, the following were analyzed: germination, proline, malondialdehyde, total polyphenols, calcium, potassium, phosphorus, sulfur and nitrogen.

The germination rate was strongly negatively influenced by soil salinity, with the most considerable differences being reported for the Brâncuși, Linia 4 & Rodeo genotypes where the decreases were approximately 40% compared to the untreated control. The application of the Algevit biostimulator improved both the germination rate and the time required for germination for Rodeo and Linia 4, thus demonstrating its positive role on the tested genotypes.

The strongest fluctuations in proline were recorded when applying saline treatments where the highest concentration was noted in Linia 4 (14.9 mg/L), and the lowest value was observed in Linia 3 (4.91 mg/L). A considerable decrease in the amount of proline observed when applying the Algevit 0.5% treatment highlighted a good response of the plants to the application of this biostimulator.

Malondialdehyde presented the highest values in the variants treated with saline solution. The strongest increase compared to the control was observed in the Brâncuși variety, followed by Line 4. Although the application of the biostimulator reduced lipid peroxidation, values close to those of the plants in the variant subjected to salt stress were observed in Brâncuși, Rodeo and Line 4, which highlights these genotypes as being strongly affected by oxidative stress.

Total polyphenols showed the highest concentrations in the saline treatment for all genotypes. The application of the Algevit biostimulator determined the reduction of polyphenol concentrations, with the closest values compared to the control being observed, according to the statistical analysis, in Line 3, Line 1 and Line 4.

Potassium, calcium, nitrogen, sulfur and phosphorus showed a very accelerated tendency to decrease in the plants in the variants subjected to salt stress. The largest fluctuations, compared to the untreated control, were recorded in Ca (59.7%) and S (55%). Variant V2 determined significantly higher ratio values compared to V1. The closest ratios to the untreated control were in the Line 3 genotype, where S/Si (6.89) was higher than the untreated control (5.34). Thus, a reduction in osmotic and ionic stress was highlighted upon application of the biostimulator.

Chapter VI presents an alternative method for using plant waste resulting from the analyses performed in this thesis. Thus, carbon dots were obtained that were tested for the purpose of purifying wastewater containing textile dyes.

The degradation experiments were designed to mimic contaminated wastewater. The degradation of the dyes took place under UV type A irradiation, this field being selected to reproduce natural conditions. The results showed that within 13 minutes both methylene blue and amaranth were degraded by over 50%.

This doctoral thesis concludes with a chapter dedicated to conclusions and recommendations for outlining future perspectives, followed by a bibliography that totals 250 titles from national and international literature.