THE INFLUENCE OF SALINE STRESS ON THE WATER REGIME IN BITTER CUCUMBERS (*MOMORDICA CHARANTIA*)

Ștefănica OSTACI[1], Cristina SLABU[1], Alina Elena MARTA[1], Mihaela COVAȘĂ, Iulia MINIAȚĂ[1], Carmen Doina JITĂREANU[1]

e-mail: stefanaostaci@gmail.com

Abstract

Bitter cucumber (*Momordica charantia*) is a tropical and subtropical plant widely distributed in China, Malaysia, India and tropical Africa, with a long history of use in traditional medicine. Soil salinity is a major abiotic stress worldwide that affects plant morphology and physiology leading to reduced growth, reduced production yield or in some cases plant death. Due to the reduction of water accessibility, plants try for its optimal absorption, increasing the concentration of vacuolar juice as a method of combating the hypertonicity of the external environment. To determine the adaptation capacity to saline stress, which is increasingly common, two varieties (Brâncusi and Rodeo) and three experimental lines (Line 1, Line 3 and Line 4) of bitter cucumber were treated with saline solutions of different concentrations (100 mM NaCl and 200 mM NaCl), and following the treatments determinations of free water and bound water content were performed. Research has shown that with the onset of salt stress, the amount of free water in bitter cucumber leaves decreased and the amount of bound water increased in most of the studied plants, which highlights the triggering of a pronounced reaction to this type of abiotic stress. The statistical analysis shows significant differences between the control and the treated variants both in the case of free and bound water.

Key words: *Momordica charantia*, salinity, water regime

Bitter cucumber (*Momordica charantia*) commonly known as bitter melon or bitter gourd is a tropical and subtropical plant. It is widely distributed in China, Malaysia, India and tropical Africa with a long history of use as a hypoglycemic agent (Agarwal S., Shaheen R., 2007; Blum A., *et al*, 2012; Grover J., Yadav S., 2004).

Soil salinity is a major abiotic stress worldwide that affects plant morphology and physiology leading to reduced growth, reduced production yield or in some cases their death (Balal *et al*, 2011). The accumulation of salts in the soil is harmful to plants, because it increases the concentration of the soil solution and disrupts the root absorption process, having a toxic effect on them (Jităreanu D.C., 2007). Due to the reduction of water accessibility, plants try for its optimal absorption, increasing the concentration of vacuolar juice as a method of combating the hypertonicity of the external environment (Farooq M., *et al*, 2015).

Research has shown that plants with better resistance to abiotic factors are characterized by cuticular transpiration and a lower rate of leaf dehydration (Petcu E., *et al*, 2007).

Water is the mineral component of all living organisms, predominant over other substances. The water content is variable depending on the species, organ and tissue. Variations in the water content are also found in the different organs and tissues of the same plant (Ievinsh G., 2023; Maggio A., *et al*, 2002).

The regulation of water absorption in plant cells is achieved by processes of simple, facilitated diffusion, osmosis and active transport, which occur at the level of plasma membranes. Less than 1% of the total amount of absorbed water is used in photosynthesis and growth processes, the rest being eliminated through transpiration processes (Lobet G., *et al*, 2014; Brendel O., 2021).

MATERIAL AND METHOD

Bitter cucumber leaves (*Momordica charantia*) belonging to two Romanian varieties: Brâncusi and Rodeo and three experimental lines: Line 1, Line 3 and Line 4 were used to carry out the work. The seedlings were obtained in the greenhouse of the Agricultural Research Institute and Environment (ICAM) belonging to the "Ion Ionescu de la Brad" University of Life Sciences Iași. They were planted in 12-liter pots of

[1] Iasi University of Life Sciences, Romania

vegetation, the cultivation being carried out in the greenhouses of the vegetable growing discipline within the didactic resort "Vasile Adamachi Farm".

The plants were treated with saline solutions of different concentrations: 0 mM NaCl, 100 mM NaCl, and 200 mM NaCl. 3 treatments were carried out 10 days apart, in different phenophases according to the BBCH scale: 201 which corresponds to the appearance of the first lateral shoot, 501-502 which corresponds to the appearance of the first flowers and 701 which corresponds to the appearance of the first fruit.

Seven days after the application of each treatment, determinations of free water and bound water were made. To carry out these determinations, leaves were harvested from the middle of the main shoot of the plants and inserted with the petiole into test tubes with water, then they were fixed with parafilm.

The analyzes were carried out in the laboratory of the Plant Physiology discipline within the Faculty of Agriculture. For the analysis of water loss at the foliar level, the free water was determined by weighing at 24 hours the leaves kept at room temperature. Before weighing, the petiole was removed and the surface of the section was covered with petroleum jelly to occlude the conducting vessels. The first weighing represents the initial weight of the turgid leaf (G0), and the last weighing (G24) gives by difference the amount of water lost by the leaves in the respective time interval. The water lost after 24 hours, as a percentage of the initial weight of the plant material, is considered free water (Jităreanu C.D., Marta A.E., 2020).

To determine the bound water, the plant material was placed in ampoules of known weights and placed in the oven at a temperature of 105 C° for 4 hours. After weighing, the vials were placed back into the oven and weighed until their weight remained constant. The amount of water lost through oven drying is considered bound water. This was determined by the difference between the initial weight and the dry matter (Jităreanu C.D., *et al*, 2011). Dry matter was calculated according to the formula:

$$b*100/a$$

a= initial weight

b=final weight.

RESULTS AND DISCUSSIONS

In the body of the plant, water is found in two states, liquid and gaseous. Liquid water is the main component of all cells, being present in maximum quantity in vacuoles and minimum in cell organelles. This is in the form of free water and bound water (Toma D.L., Jităreanu C.D., 2007).

Free water

Free water is considered to be the amount of water lost through sweating and dehydration. It is retained with weak forces in the plant body and circulates easily intra and intercellularly.

Since the reaction to excess salts manifests itself, in a first phase, as osmotic stress, in order to identify the varieties and lines of bitter cucumbers tolerant to salinity, it is necessary to assess the capacity to retain water at the leaf level, expressed by a speed of dehydration of the leaves less.

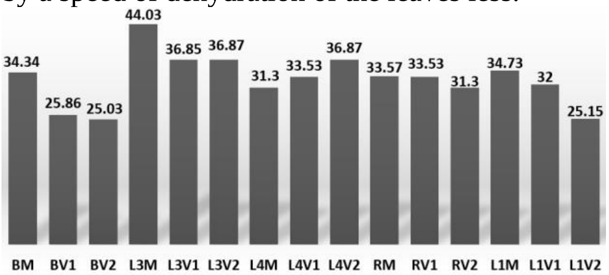

Figure 1 **Effect of salt stress on free water content after the first treatment**

After determining the amount of free water, it was possible to observe a tendency to decrease free water in plants treated with saline solutions, except for Line 4 where an opposite effect was highlighted, the lowest value of free water being observed in the control variant (31, 3%) and the highest value in the version treated with 200 mM NaCl (36.87%) (*figure 1*). This implies a poor adaptation of plants to salt stress. The most pronounced difference was observed in Line 3 where the control presented the highest value of free water (44.03%) in contrast to the variant treated with 100 mM NaCl (36.85%) and the variant treated with 200 mM NaCl (32 ,5%).

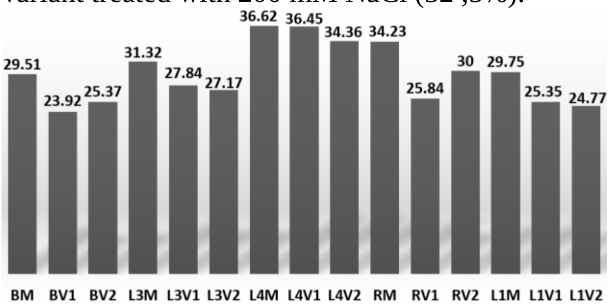

Figure 2 **Effect of salt stress on free water content after treatment two**

After the second treatment, the same phenomenon of decrease in free water content was observed in plates treated with saline solution. According to the graph (*figure 2*), pronounced differences between the control and the treated variants were observed in the Brâncusi variety, Line 3 and Line 1. In the case of Line 4, the highest value of free water was recorded in the control variant (36.32%) and the lowest amount in the variant treated with 200 mM saline solution, which may imply a slight adaptation to the saline conditions to which the plants were subjected.

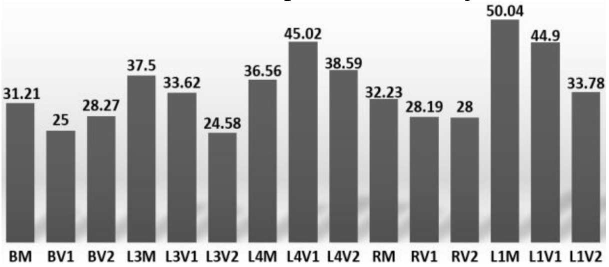

Figure 3 **Effect of salt stress on free water content after treatment 3**

After the application of the third treatment, the most pronounced differences between the control plants and those treated with saline solution were recorded in Line 1 where the control presented a free water value of 50.04% in contrast to the variant treated with 100 mM NaCl which recorded a value of 44.9%, and the variant treated with 200 mM with a value of 33.78%. In the case of Line 4, the highest value was recorded in the version treated with 100 mM saline solution (45.02%), and the lowest value in the control version (36.56%) (*figure 3*).

The T-test was used to compare the free water recorded in the control with the amounts of free water found in the 100 mM and 200 mM saline-treated variants. Following the application of the test, statistically significant differences were recorded both between the control (I) and the variant treated with 100 mM NaCl (II) and between the control and the variant treated with 200 mM NaCl (III). In the case of the two treatments, there are no significant differences, which may be due to the fact that they are close in terms of saline concentration (*tabel 1*).

Table 1

Statistical differences between the control variant (I) and the variants treated with saline concentrations of 100 mM (II) and 200 mM (III), regarding the free water content of the leaves

Variants compared	*t-stat*	*P one-tail*
I-II	2.848504699	0.00644394
I-III	3.636956011	0.00134681
II-III	1.565081015	0.06994084

t-Test Paired Two Sample for Means: P one-tail > α the null hypothesis is accepted; P one-tail ≤ α the null hypothesis is rejected;
Insignificant statistical differences (p≥0.05) between variants; significant statistical differences (p≤0.05) between variants; distinctly significant statistical differences (p≤0.01) between variants; very significant statistical differences (p≤0.001) between variants.

Bound water is retained in plants with high forces. This water is hardly lost by plants, because it consists of immobile molecules, lacking the property of diffusion. The bound water is removed by drying (Toma and Jităreanu, 2007).

In conditions of saline stress, when the vital activity of the plant decreases, there is an increase in the amount of bound water, thus ensuring survival in such conditions.

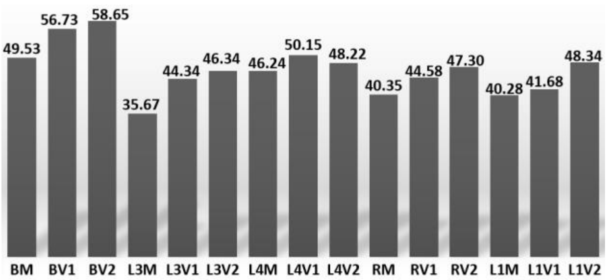

Figure 4 **The effect of salt stress on the bound water content after the application of the first treatment**

According to the determinations made after the first treatment, higher values of the bound water content were observed in the treated variants than in the control ones (*figure 4*). The most pronounced difference was observed in the Brâncusi variety where the bound water values increased directly proportional to the increase in NaCl concentration. The control showed the lowest value of bound water (49.53%) in contrast to the variant treated with 100 mM (56.73%) and the variant treated with 200 mM saline (68.65%).

Variants treated with 100 mM NaCl showed bound water values between 1.03-1.24 times higher than the control values. In the case of variants treated with 200 mM, the bound water values increased compared to the control by 1.04-1.29 times.

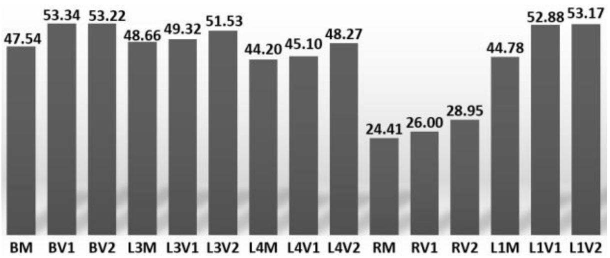

Figure 5 **Effect of salt stress on bound water content after application of treatment two**

After the second treatment, the same trend of increasing water values was noted, directly proportional to the increase in the concentration of the applied saline solution (*figure 5*). Pronounced differences were observed in the case of the Rodeo and Brancusi varieties, but also in the case of Line 1. In the variants treated with 100 mM saline solution, the bound water increased by 1.01-1.18 times, and in the case of the variants treated with 200 mM saline solution bound water increased over control by 1.05-1.08-fold. Smaller differences between the two treatments compared to the previous treatment could be noted, which may be due to the small difference between the two saline concentrations.

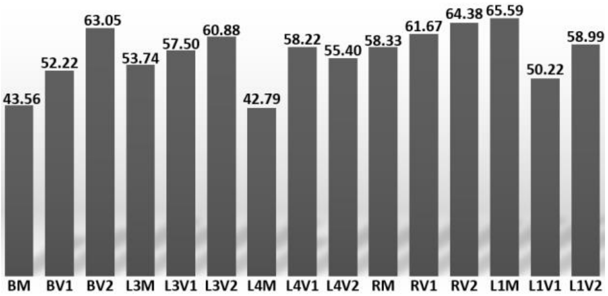

Figure 6 **Effect of salt stress on bound water content after application of treatment three**

Seven days after the application of the third treatment, a more pronounced difference in bound water was revealed between the control version and the variants treated with saline in the Brâncusi variety, where the control showed a value of 43.56% bound water and the version treated with 200 mM showed a value of 53.05% (*figure 6*). In the case of Line 1, the effect was opposite to the other varieties and lines, the control showing a higher bound water value (65.59%) than the variants treated with saline solutions. The lowest value was recorded for the variant treated with 100 mM (50.22%).

After the treatment, the most pronounced differences between the control and the treated variants were observed. The variety treated with 100 mM NaCl showed 3.76-15.43 times the amount of bound water and in the case of the variety treated with 200 mM saline, the amount of bound water was 6.5-19 times higher.

Following the application of the T test, statistically significant differences were observed both between the control and the two variants treated with saline solution and between the two concentrations of NaCl applied, which can highlight an adaptation of the bitter cucumber varieties and lines to saline stress (*tabel 2*).

Table 2

Statistical differences between the control variant (I) and the variants treated with saline concentrations of 100 mM (II) and 200 mM (III), regarding the bound water content of the leaves

Variants compared	*t-stat*	*P one-tail*
I-II	3.636956011	0.00134681
I-III	-2.27403464	0.01961622
II-III	-3.02802594	0.00451761

t-Test Paired Two Sample for Means: P one-tail > α the null hypothesis is accepted; P one-tail ≤ α the null hypothesis is rejected;
Insignificant statistical differences (p≥0.05) between variants; significant statistical differences (p≤0.05) between variants; distinctly significant statistical differences (p≤0.01) between variants; very significant statistical differences (p≤0.001) between variants.

CONCLUSIONS

Following the applied treatments, smaller amounts of free water lost by the leaves of the plants subjected to saline stress were observed, in contrast to the control, a phenomenon specific to water and saline stress. After analyzing the content of bound water lost by the leaves treated with saline solutions and the control, a pronounced difference was observed between the control and the treatments, with the stressed plants showing higher amounts of bound water.

Following the statistical analysis of the content of free water and bound water, statistically significant differences were recorded between control plants and those treated with saline solutions.

Among the varieties and lines tested, the Brâncusi variety and Line 3 presented the highest bound water content, which demonstrates their good adaptation to saline stress conditions.

Line 4 recorded the lowest amount of water related to the variants treated with saline solutions, which shows a low capacity to adapt to saline stress.

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