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The Influence of Salt Stress on the Content of Vitamin C in the Leaves of some Varieties and Lines of Bitter Cucumber (*Momordica charantia*)

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RESEARCH ARTICLE

Abstract
Bitter cucumber is a tropical and subtropical plant with a long history of medicinal use. Soil salinity is a major abiotic stress that affects the morphology and physiology of plants leading to reduced growth, production yield or in some cases their death. Salt stress increases the accumulation of reactive oxygen species in plants. They developed adaptation mechanisms by increasing the level of antioxidants, including vitamin C. The aim was to estimate the amount of vitamin C in the studied plants to determine the ability to adapt to salinity and improve nutritional properties. To carry out the experiment, two varieties and three experimental lines of bitter cucumber subjected to saline stress through treatments with saline solutions of different concentrations were used. The estimation of vitamin C content was carried out by UV-Vis spectrophotometry using the calibration curve method with standard L-ascorbic acid and $KMnO_4$. After performing the analysis of variance on the varieties and lines of bitter cucumber, subjected to salt stress by applying the two treatments, it was found that the accumulation of vitamin C was insignificantly influenced by the factor *a*-the number of the treatment, but it was significantly influenced by the factor *b*-the concentration saline solution.

Keywords: *Momordica charantia*, salinity, stress, vitamin C

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INTRODUCTION

Momordica charantia L., commonly known as bitter melon, is an annual, tropical plant with pharmaceutical properties of the Cucurbitaceae family and cultivated in various areas of the world for its edible fruits, leaves and young shoots. It is traditionally used in India, China, Africa and South America for its antidiabetic, antioxidant, contraceptive and antibacterial properties (Alisofi et al., 2020). Nutritional analyses have shown that this plant has the highest nutritional value among cucurbits, being a good source of carbohydrates, proteins, fibres, vitamins and minerals. Green fruits and leaves contain vitamin C, A and P, thiamin, riboflavin, niacin and minerals (Bortolotti et al., 2019). Soil salinization is one of the most important causes of land degradation and desertification, posing an important threat to land management, agricultural activities, water quality and sustainable development in arid and semi-arid areas (Wang et al., 2019). Salinity is an abiotic stress, widely encountered globally. It causes a severe limitation of germination, growth and production yield of plants, due to their sensitivity to high concentrations of salts in soil or irrigation water (Stepien & Johnson, 2009). 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, 2007). Salinity causes numerous physiological, morphological, metabolic reactions and can induce osmotic and oxidative stress by increasing the formation of reactive oxygen species (ROS), including free radicals, hydrogen peroxide and singlet oxygen (Garg & Manchanda, 2009). ROS damage lipids in the composition of cell membranes and other macromolecules such as proteins, DNA and photosynthetic pigments, thus leading to the inactivation of antioxidant enzymes (Alisofi et al., 2020). Plants with a high level of antioxidants, either constitutive or induced, showed greater resistance to this oxidative damage. The toxic effects of ROS are counteracted by both the enzymatic and non-enzymatic antioxidant systems (Smirnoff, 2007). The non-enzymatic antioxidant system mainly includes low molecular weight and water-soluble compounds such as ascorbate (Asc) and glutathione as well as fat-soluble α-tocopherol. Ascorbic acid (vitamin C) is a multifunctional metabolite with strong reducing properties that allows the neutralization of ROS and the reduction of oxidized molecules in cooperation with glutathione in the Foyer-Halliwell-Asada cycle, which is known as the major antioxidant defense pathway (Bilska et al., 2019). In humans, vitamin C participates in various physiological processes such as iron absorption, collagen synthesis, immune stimulation and epigenetic regulation. For this reason, increasing the vitamin C content of crops could have beneficial effects on human health (Paciolla et al., 2019, Bologa et al., 2017). This paper aims to identify some varieties and variants of bitter cucumber (*Momordica charantia*) with a high content of vitamin C and analyse the effects of salt stress on its amount.

MATERIALS AND METHODS

Obtaining plant material

The plant material used in this work is represented by two Romanian cultivars of bitter cucumber (Brâncuși and Rodeo) and three experimental lines (Line 1, Line 3 and Line 4). The seedlings were obtained in the greenhouse of the Research Institute for Agriculture and the Environment (ICAM) belonging to the "Ion Ionescu de la Brad" University of Life Sciences in Iași. The seedlings were moved to the greenhouses of the vegetable growing discipline located on the territory of the Didactic Station Ferma Vasile Adamachi. They were planted in 12 litter pots. The pots were filled with universal soil for flowers "Florasol" mixed with peat "Kekkila" DSM 2 W. The mixture was made in the proportion of 65% Florasol - 35% Kekkila. The plants were treated with saline solutions of different concentrations: 0 mM NaCl, 100 mM NaCl, 200 mM NaCl. Plant material samples were collected 7 days after the application of each treatment. These were frozen to facilitate the extraction of vitamin C, according to studies by Nojavan et al (2008).

Materials

L-Ascorbic acid (Aldrich), Oxalic acid (Merck), Potassium permanganate (Sigma-Aldrich), Bitter cucumber (*Momordica charantia*) leaves.

Preparation of ascorbic acid solution for calibration curve

Standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01 g of standard ascorbic acid in small amount of oxalic acid solution (0.5%) and then completed to 100 mL with the same solution to obtain a concentration of 100 mg/L according to the protocol presented by Elgailani et al. (2017). A series of dilutions 1, 2, 3, 4, 5, 6, 8, 10 mg/L were prepared from the stock ascorbic acid solution.

Preparation of potassium permanganate solution

A solution of $KMnO_4$ of concentration of 100 mg/L was prepared by dissolving an accurately 0.01 g of $KMnO_4$ in a small amount sulphury acid solution (0.1 M), then transferred into a 100 mL volumetric flask and completed to the mark with distilled water.

Preparation of standard calibration curve of ascorbic acid

In a test tube, 10 mL of each standard solution (Ascorbic Acid) was put, then 10 mL of $KMnO_4$ (10 mg/L) was added. This solution was let to stand for 5 minutes. The absorbance of this standard solutions was read at 528 nm against blank. Blank sample was preparate by adding 10 mL distillate water and 10 mL $KMnO_4$ solution.

Preparation of leaf extracts for analysis by UV-VIS spectrophotometer

Five leaves per plant were collected from the middle area of the main shoot, then the average sample was made from which 1 g of the leaf material was used. This number of frozen leaves was added to a flask. A small amount of oxalic acid and 15 mL of distilled water were added to this. The mixture was brought to 90°C for 15 minutes. After 15 minutes, the mixture was left to cool at room temperature. A volume of 0.1 mL was pipetted from the extract and diluted to a final volume of 10 mL, after that 10 mL of $KMnO_4$ solution was added to the diluted extract solution. The absorbance was read at 528 nm.

UV–VIS absorption spectra were recorded with a Specord 210 Plus, Analytik Jena spectrophotometer on water solutions. Statistical analysis was performed with Anova and T tests.

RESULTS AND DISCUSSIONS

UV-Vis spectrophotometry is one of the most used methods of both qualitative and quantitative analysis. The method is suitable for the determination of substances that are colored or in the structure of which there are at least double bonds (Desai, 2019).

The UV-Vis spectra revealed that the maximum absorbance is at λ_{max}=528 nm Figure 1a. Calibration curves for the determination of ascorbic acid in the prepared leaves samples and ($KMnO_4$ solution) were constructed by plotting the absorbances as a function of the corresponding concentrations as shown in Figure 1b.

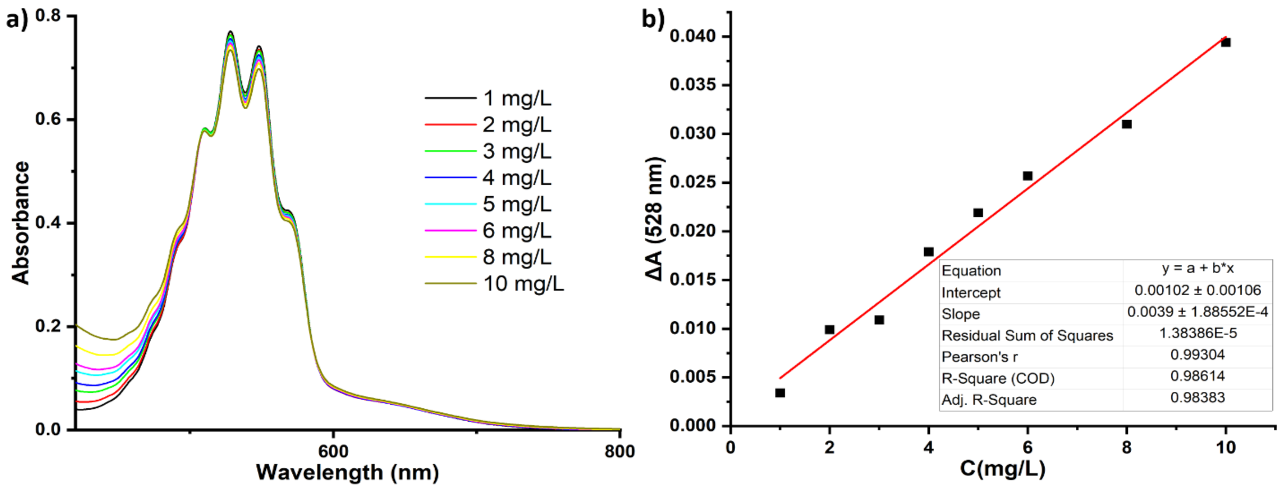

Figure 1. (a) Uv-vis spectra for the solutions used in the calibration curve, (b) calibration curve for *L*-ascorbic acid. $\Delta A=\Delta_{blank}-\Delta_{sample}$

Using the equation on the right, the values of the concentration of vitamin C related to 100 g of plant material (frozen) were estimated.

For an annual plant like bitter cucumber, there is a race against time to initiate flowers and form seeds, while the leaf area is still adequate to supply the necessary photosynthate, for this reason the plant fights in all ways including increasing the level of antioxidants (Munns, 2011). Ascorbic acid is a small, water- soluble antioxidant molecule which acts as a primary substrate in the cycling pathway of enzymatic detoxication of hydrogen peroxide (Abdelhamid et al., 2010).

The first treatment was applied in phenophase 51-52 according to the BBCH scale, which corresponds to the formation of the first flowers, Figure 2. Following the determinations, higher amounts of vitamin C were obtained in the variants treated with saline solutions compared to the untreated variant, with the exception of the Rodeo variety in which the amount of vitamin C was higher in the control plants (4.64 mg/100 g) compared to those treated with 100 mM saline (3.44 mg/100 g) and plants treated with 200 mM (4.46 mg/100 g).

The most pronounced difference in the amount of vitamin C was recorded in Line 3 where the values increased in direct proportion to the increase in the concentration of the saline solutions applied. The control presented the lowest value (2.56 mg/100 g) and the variant treated with 200 mM NaCl presented the highest value (5.62 mg/100 g). The same phenomenon was observed in the case of Lines 1 and 4, but the difference was less pronounced. In the case of the Brâncuși variety, the values recorded in the control variant were lower (4.77 mg/100 g) compared to the variants treated with saline solution, the highest value being obtained in the variant treated with 100 mM (5.49 mg/100 g). The results obtained regarding the content of vitamin C in the leaves of some varieties and lines of bitter cucumber subjected to saline treatments, showed that the synthesis of ascorbic acid was favored, in concentrations 1.04 to 2.19 times higher than the control variant.

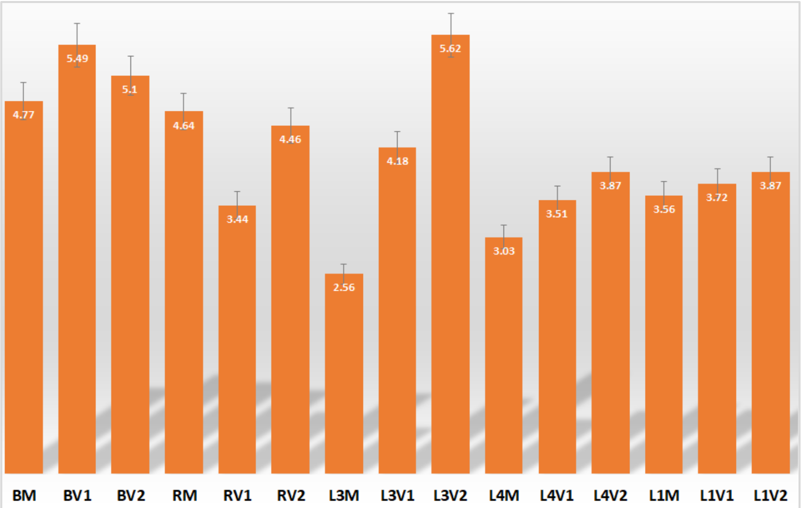

Figure 2. Amounts of vitamin C seven days after the application of the first treatment (mg/100 g)

The second treatment was applied in phenophase 71 of the BBCH scale, equivalent to the appearance of the first fruit (Figure 3).

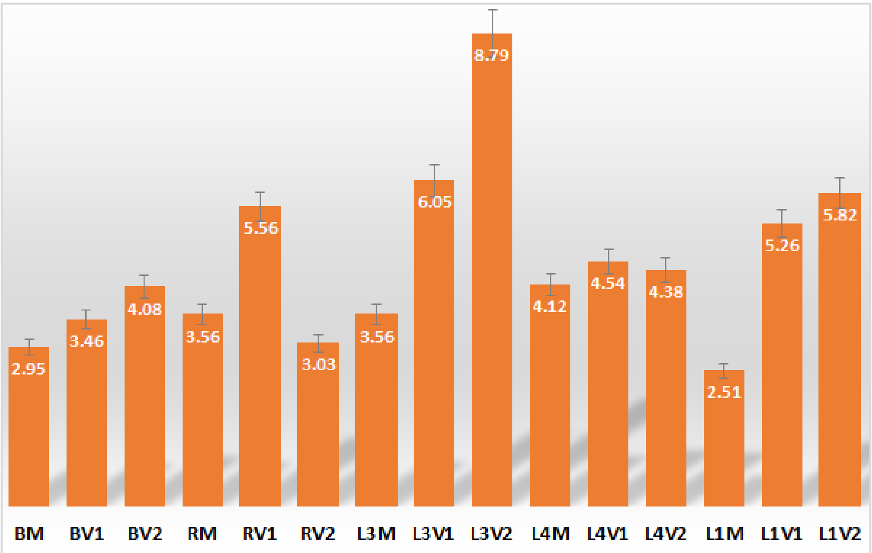

Figure 3. Amounts of vitamin C seven days after the application of treatment two (mg/100 g)

Following the determinations made with plant material harvested 7 days after the application, in the second treatment, higher values of vitamin C were observed in plants treated with NaCl compared to untreated plants. The most pronounced difference between the control and the treated variants was recorded, as in the case of the previous treatment at Line 3 where the control recorded an amount of 3.56 mg of vitamin C per 100 g of plant material, the variant treated with 100 mM NaCl recorded 6 .05 mg/100 g, and the variant treated with 200 mM NaCl registered a value of 8.79 mg of vitamin C. Line 1 and Line 4 retain the same character observed in the previous treatment, showing higher values of vitamin C in the treated variants with saline solutions. In the case of the Rodeo variety, the control showed a lower value (3.56 mg/100 g) than the plants treated with 100 mM NaCl (5.56 mg/100 g) and higher than the plants treated with 200 mM NaCl (3.03 mg /100 g). Saline stress favored ascorbic acid synthesis depending on its intensity and plant tolerance (Bologa et al., 2017). The obtained results showed the increase in the synthesis of ascorbic acid, in concentrations 1.10 to 2.46 times higher than the control variant. In the case of both treatments, the determined values of vitamin C were consistent with the values reported in the literature (Bakare et al. 2010).

Similar results were also recorded in the case of other antioxidants such as free proline, which showed higher values in varieties resistant to salt stress. Proline accumulation is one of the adaptations of plants to salinity (Misra & Gupta, 2005).

After performing the analysis of variance, it was found that the accumulation of vitamin C was insignificantly influenced by factor *a* - the number of treatments, but was significantly influenced by factor *b* - the concentration of the saline solution Table 1.

Table 1. Analysis of variance on leaf vitamin C content

Source of Variation	SS	df	MS	F	P-value	F crit
Treatment	1.14736	1	1.14736	0.795847	0.381196	4.259677
Concentration	10.0845	2	5.042249	3.49747	0.046456	3.402826
Interaction	2.261664	2	1.130832	0.784383	0.467754	3.402826
Error	34.60042	24	1.441684			
Total	48.09395	29				

Non-significant statistical differences ($p \geq 0.05$); significant statistical differences ($p \leq 0.05$); distinctly significant statistical differences ($p \leq 0.01$); highly significant statistical differences ($p \leq 0.001$).
Two-Factor Anova: F<F crit the null hypothesis is accepted; F >F crit the null hypothesis is rejected;
Null hypothesis: There are no statistically significant differences between the analyzed data groups.

The T-test was used to compare the amount of vitamin C obtained in the control with the amounts of vitamin C found in plants treated with 100 mM and 200 mM saline solutions. 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), Table 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 vitamin C content

Variants compared	T-stat	P one-tail
I-II	-2.604336536	0.01426728
I-III	-2.331391474	0.02232264

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 ($p \geq 0.05$) between variants; significant statistical differences ($p \leq 0.05$) between variants; distinctly significant statistical differences ($p \leq 0.01$) between variants; very significant statistical differences ($p \leq 0.001$) between variants.
Null hypothesis: There are no statistically significant differences between the analysed data groups.

CONCLUSIONS

After the first treatment with saline solutions, an amount of 1.04 - 2.19 times the content of vitamin C was recorded, compared to the control, the biggest differences being in Line 3. After the second saline treatment, they revealed a synthesis of ascorbic acid, in concentrations 1.10 to 2.46 times higher than the control variant. As in the case of the previous treatment, the most pronounced differences between the control and the treated variants were registered at Line 3. The statistical analysis revealed significant differences between the control variant and the variants treated with saline solutions. Salinity caused an increase in the content of ascorbic acid in the leaves of bitter cucumbers, thus improving their nutritional quality. Following the analyses carried out, Line 3 stood out as the most valuable from the point of view of adaptation to saline stress, as it can be grown on salty soils to obtain higher amounts of vitamin C.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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