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ESTIMATION OF CHLOROPHYLL CONTENT AND DETERMINATION OF CHLOROPHYLL FLUORESCENCE IN BITTER CUCUMBER (*MOMORDICA CHARANTIA* L.) LEAVES UNDER SALINE STRESS CONDITIONS

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Abstract

It has been scientifically proven that one of the physiological processes most affected by salt stress is photosynthesis. In this context, it is important to identify the main mechanisms involved in increasing plant tolerance to salt stress. The photosynthetic intensity is directly related to the productive capacity of plant species, thus increasing the tolerance of plants to saline stress is a priority. The chlorophyll content and chlorophyll fluorescence were studied in two varieties (Rodeo and Brâncuși) and three lines (Line 1, Line 3 and Line 4) of bitter cucumber (Momordica charantia), subjected to salt stress. Following the determinations, a higher concentration of chlorophyll was estimated in the control plants of the Brâncuși variety and Line 4, in contrast to the Rodeo variety, Line 3 and Line 1. In the case of this variety and the two lines, the plants treated with the highest concentration of saline solution prepared by using sodium chloride (NaCl) (200 mM) shows a higher concentration of total chlorophyll content. During the treatments, the fluorescence was higher in the treated plants compared to the control plants.

Key words: *bitter cucumber, salt stress, chlorophyll, fluorescence.*

INTRODUCTION

Bitter cucumber (*Momordica charantia* L.) belongs to the: Class Dycotyledonnae, Order Cucurbitales, Cucurbitaceae Family, *Momordica* genus. Bitter cucumber also known as bitter gourd is a tropical and subtropical plant grown in India, Asia, South America, Caraibbes, South Africa (Agarwal & Shaheen, 2007; Şesan, 2020).

The bitter cucumber was cultivated for the first time in Romania in 1990 when some seeds were supplied from Nepal. It is cultivated and researched in Romania within the Vegetable Development Research Station Buzau where the two varieties and the three lines studied in this paper were obtained (Şesan, 2020).

The plant is well known for its many medicinal properties, including anti-inflammatory; anti-carcinogenic; antitumor; antimicrobial; antibacterial; antifungal; antiviral; and hypoglycemic. The fight against diabetes being the main reason for eating bitter cucumbers (Martin et al., 2004). The fresh juice of *M. charantia* could control blood sugar and insulin levels as well as improve the human digestive system (Busuioc et al., 2020).

In addition to its polyphenol-rich content, this plant contains certain phytocompounds with a significant hypoglycaemic role, such as: charantin, momordicin, momordenol, zeatin, zeinoxanthin amino acids, polypeptides, sometimes being referred to as insulin plant (Ştefan et al., 2022).

Salt stress is one of the main factors that question the future of plant crops (Săulescu et al., 2010). Environmental stress, including salinity, affects almost every aspect of plant physiology, biochemistry and significantly diminishes the yield (Heidari, 2011).

About 20% of the world cultivated lands and about half of all irrigated lands are affected by saline stress (Zhu, 2001).

The high concentrations of salt in the soil, especially sodium chloride, cause osmotic stress by lowering the potential of water in cells and ion stress due to inhibition of metabolic processes.

In response to saline stress, plants accumulate toxic ions in vacuoles. Plant cells must also undergo osmotic adjustment, which is accomplished in many ways, including the production of organic osmolytes such as glycine betaine, proline, some sugars, and polyamines, most of which are synthesized in the chloroplast (Di Martino et al., 2003; Hameed et al., 2021).
One of the main physiological processes affected by excessive soil salinity is photosynthesis. Photosynthesis is the most important physiological process by which green plants synthesize organic substances from CO_2, H_2O and mineral salts in the presence of sunlight and assimilatory pigments, yielding O_2 to the environment (Toma & Jităreanu, 2007).
Reduced photosynthesis due to excessive salinity in the environment is not only attributed to stomatal closure, which leads to reduced intercellular CO_2 concentration, but also to non-stomatal factors such as overproduction of reactive oxygen species (ROS) and depletion of potassium (K^+) within plant cells due to natrium (Na^+) accumulation (Stepien & Kłbus, 2006; Hameed et al., 2021).
The present work aims to test the varieties and lines of bitter cucumber to salt stress by following the content of assimilatory pigments and chlorophyll fluorescence.

MATERIALS AND METHODS

The experience was carried out in the greenhouse of the Research Institute for Agriculture and the Environment (ICAM) located on the territory of the Ferma Vasile Adamachi didactic resort, which belongs to the Ion Ionescu University of Life Sciences from Brad in Iași and in the solariums of the Vegetable Cultivation discipline located on the territory of the didactic resort Vasile Adamachi in 2022.
Two varieties (Brâncuși and Rodeo) and three lines (Line 1, Line 3 and Line 4) of *Momordica charantia* (bitter cucumber) obtained at the Buzău Vegetable Research and Development Station (SCDL) were used for the experiment. To obtain the plants used, the seeds were sterilized with sodium hypochlorite (NaOCl), then went through the imbibition process for 24. The seedlings were obtained in the greenhouse under controlled conditions of temperature and humidity. Seedlings were moved to the greenhouse when they had between two and three leaves. They were planted in 12 liter pots filled with a mixture of Florasol flower soil and Kekkila peat.
The bifactorial experiment was performed in randomized blocks with 3 replications. The two cultivars and three lines of bitter cucumber were subjected to salt stress for a period of 40 days.
Plants were treated with saline solutions of different concentrations (M = untreated control plants; V1 = 100 mM saline; V2 = 200 mM saline). The concentrations applied in the current study were chosen to be approximately the average of the maximum and minimum concentrations found in the literature for bitter cucumber. The plants were subjected to a number of four treatments and the determinations were made 7 days after the application of each treatment.
In the present study, the aim was to determine total chlorophyll content and fluorescence.
Total chlorophyll pigment content was estimated using the Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter, which uses a non-destructive method for evaluating chlorophyll content. The method is based on the determination of the chlorophyll concentration index. The chlorophyll concentration index is defined as the difference in optical density at two wavelengths: 650 nm and 940 nm (Bologa, 2016). 10 determinations were made on the leaves from the base of the plant to the middle and from the tip, in total obtaining a number of 30 determinations per plant.
Fluorescence is the characteristic of a solution of pigments to show a different color depending on how it is viewed. If it is seen transparently, in direct light, it has a green color. If seen in reflection, it is ruby-red. Thanks to this property, chlorophyll transforms the wavelength of light radiation, from a short wavelength to a long wavelength (Jităreanu, 2007).
Fluorometer OS30p+ is an instrument that measures fluorescence parameters in the visible spectrum by observing the wavelength intensity distribution of the emission spectrum during excitation with a certain light stimulus. The measurements were aimed at determining the values of the Fv/Fm ratio in which it represents the maximum quantum yield of photosystem II (PS II), the indicator of the maximum efficiency of excitation energy transfer.

The determinations were made on leaves from the base of the plant, from its middle and from the tip. 10 determinations were made from each area, in total 30 determinations for each plant. To make a correlation between the two studied parameters, the Pearson Test was used as a method of correlation.

RESULTS AND DISCUSSIONS

Compared to the control variants watered exclusively with water, the plants exposed for 40 days for saline stress have recorded higher SPAD values, which denotes that the variants of bitter cucumber are included in the biphasic model proposed by Munns. Due to their resistance to saline stress factors, throughout the treatments the plants remained in the first phase of this model (Munns, 1993).

Seven days after the application of the first treatment, in the case of the treated plants, a higher SPAD value is observed compared to the control plants. According to Figure 1, this phenomenon appears in the case of the two varieties: Brâncuși and Rodeo, but it is also observed in Lines 1 and 3. The only variant where the phenomenon is not visible is Line 4, where in the case of control plants, the average of SPAD value shows 42.616. In the case of plants treated with 100 mM saline solution, the SPAD value is 35.888 and in the case of plants treated with 200 mM saline solution, the average SPAD value is 36.722.

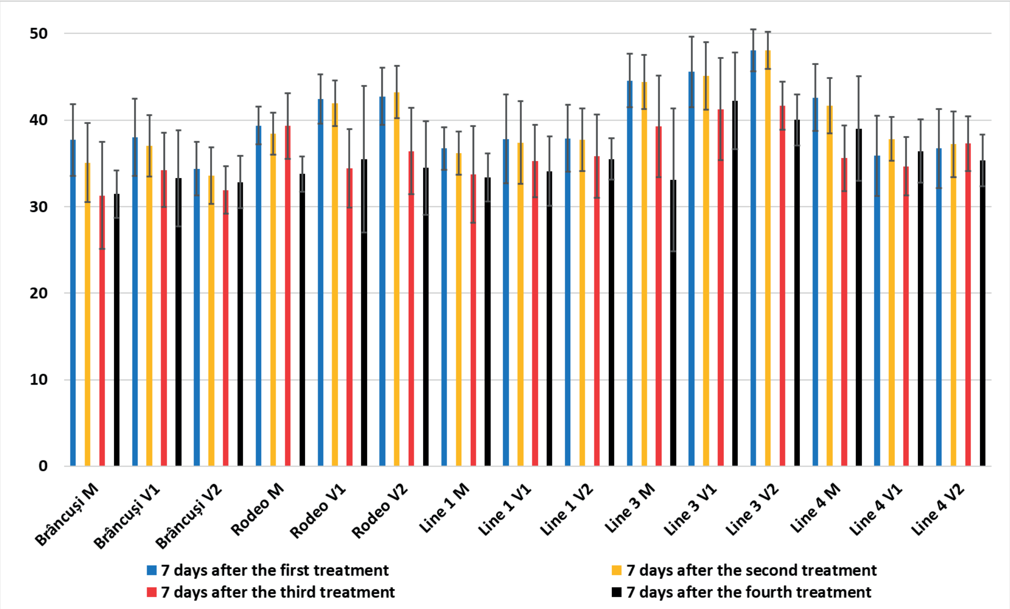

Figure 1. The estimated total chlorophyll content (SPAD values) of *Momordica charantia* L. leaves under saline conditions

After applying treatment number 2, the variants kept the same physiological reaction as in the case of the first treatment. In the case of the Rodeo variety, of Line 1 and Line 3, an increase in the SPAD value was observed, directly proportional to the increase in the concentration of the saline solution applied. In the case of the Brâncuși variety, the highest chlorophyll content is noted in the plants treated with a 100 mM saline solution where the average is 37.011. Control plants have a SPAD value of 35.083 and in the case of plants treated with 200 mM saline solution, the average SPAD value is 33.600. In the case of Line 4, the SPAD value decreases in direct proportion to the concentration of applied saline solutions, thus the highest SPAD value is recorded in control plants and the lowest in plants treated with the highest saline concentration.

Following the determinations made after the application of treatment number 3, the Brâncuși variety, Line 1 and Line 3 presented the same characteristics as in the case of the previous treatment. They show an increase in the SPAD value in the plants treated with the saline solutions compared to the control plants. The Rodeo variety shows a higher SPAD value in the case of the control slopes: 39.333 in contrast to the treated plants. In the case of the treatment with 100 mM, the average SPAD value is 34.422 SPAD units, and in the case of plants treated with 200 mM saline solution, the average SPAD value is 36.400. In the case of Line 4 a difference appears compared to the previous treatments. Plants treated with the highest concentration of saline have the SPAD value: 37.300, control plants have an average SPAD value of 35.616 and plants treated with 100 mM saline have an average photosynthetic pigment of 34.655. After applying treatment 4, it is observed that the Brâncuși variety, Line 1 and Line 3 remain constant. The Rodeo variety changes its pigment content in the control plants, which present the lowest concentration: 33.783. The plants treated with the 100 mM saline solution show the highest amount of chlorophyll: 39.911, and the plants treated with the 200 mM concentration solution show an average of chlorophyll of 34.466 SPAD units. Line 4 returns to the characteristics observed after the first two treatments where the content of chlorophyll decreases proportionally with the increase in the concentrations of saline solutions.

In the specialized literature, I did not find similar studies to be able to compare the results.

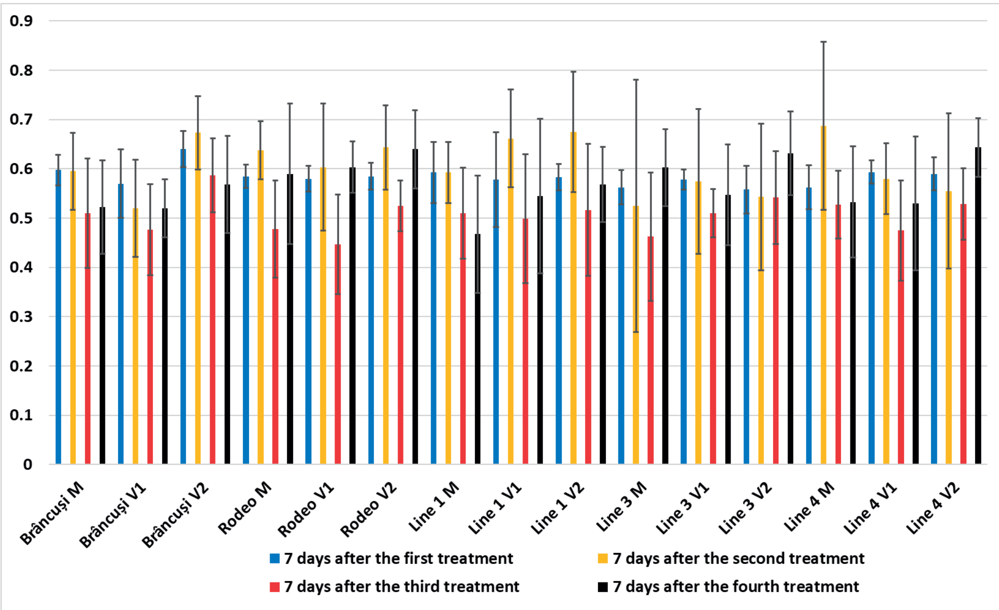

Figure 2. Chlorophyll fluorescence values (Fv/Fm) of *Momordica charantia* L. leaves under saline conditions

Exposure of bitter cucumber plants to saline treatments influenced their ability to capture light. These results correlate with the total content of photosynthetic pigments in leaves which is in the first phase of the biphasic model proposed by (Munns, 1993).

Determinations after the first treatment show a high level of fluorescence in plants treated with saline solutions compared to the control. Among all the variants taken in the fluorescence calculation, the highest fluorescence was recorded in the Brâncuși variety in the plants treated with 200 mM saline solution, where the average of the fluorescence determinations was 0.64 µmol. In the case of the Rodeo variety, no significant differences are observed between the control and the treated variants. In the case of Lines 3 and 4, the highest fluorescence values were recorded in plants treated with 100 mM saline solution. The lowest fluorescence value was recorded at Line 3 in the case of plants treated with 200 mM saline solution: 0.558

μmol. In the case of Line 1, the highest fluorescence value is in the case of control plants.
After the second treatment, Brâncuși and Rodeo varieties remained constant. Line 3 showed the highest fluorescence value in plants treated with 100 mM saline where 0.575 μmol was recorded. In the case of Line 4, the fluorescence value decreases proportionally with the increase in the applied saline concentrations, and in the case of Line 1, the values increase directly proportionally with the increase in the concentration of saline solutions.
Following the determinations made after the application of treatment number 3, it is observed that the only variant that remained constant is the Brâncuși variety. The variety Rodeo, Line 1, Line 3 and Line 4 show the highest fluorescence value in the case of plants treated with the highest concentration of saline. Line 3 shows an increase in fluorescence values directly proportional to the increase in saline solutions applied.
After treatment 4, it was observed that the varieties Brâncuși and Rodeo but also Lines 1 and 4 show an increase in fluorescence values directly proportional to the increase in the concentration of the saline solutions applied. Line 3 shows a value of the control plants of 0.602 μmol compared to the value of the plants treated with 200 mM saline solution, where the value of 0.631 μmol was recorded, the lowest value being recorded in the plants treated with 100 mM concentration solution.
The Pearson test was determined to evaluate the impact of the treatment with saline solutions, on the degree of connection between the variables that define a series of physiological indicators involved in the response to salt stress, in the bitter cucumber (*Momordica charantia*).
In order to identify the behavior of the bitter cucumber to the abiotic stress factor applied during the successive treatments, determinations of the total chlorophyll content and fluorescence were performed for each treatment, which were then subjected to the Pearson test.
In the case of the 4 determinations made 7 days after the application of each treatment with saline solution, the Pearson test shows that increasing abiotic stress determined a different degree of association between the analyzed.
The Pearson test shows that the salt stress to which the bitter cucumber plants (*Momordica charantia*) were subjected, determined a different degree of association between the analyzed parameters. The values show that salt stress involves a specific behavior on the part of the plants depending on the phenophase.
In the Biologische Bundesanstalt Bundessortenamt and Chemische Industrie (BBCH) scale, 21 phenophase, according to the Pearson test, after the first applied treatment the correlation between the two variables taken into account was negative, falling between the values -0.5 and -0.75 (according to Table 1), values that assume a moderate to good correlation coefficient (Oancea, 2007).
In the BBCH 51 phenophase, which corresponds to the second treatment, the Pearson test indicates a correlation coefficient between the variables taken into account, between -0.25 and 0.50 (according to Table 1). The value corresponds to a correlation with acceptable degree of association (Oancea, 2007).
Treatment number three corresponds, depending on the bitter cucumber variant, to BBCH 61 or BBCH 62 phenophases. In these phenophases, the Pearson test indicates a correlation coefficient of the two variables between -0.25 and 0.25 (according to Table 1), values that correspond to a correlation weak or null (Oancea, 2007).

Table1. Pearson correlation coefficient between estimated chlorophyll content and chlorophyll fluorescence

BBCH Scale	21	51	61-62	71
Pearson corelation	-0.7173	-0.3069	-0.09857	-0.207368

The last treatment was applied in the BBCH 71 phenophase, which is equivalent to the appearance of the first fruit on the plant. The Pearson test indicates in this phenophase, values between -0.25 and 0.25 (according to Table 1). As with the determinations after the third treatment, the values show little or no correlation (Oancea, 2007).

Following the performance of the Pearson Test, a negative correlation is observed in the case of all the phenophases in which the determinations were made.

During the development of the plants and the phenophases of the BBCH scale, the correlation ratio between the studied parameters decreases. In the first studied phenophase, a good correlation is observed and in the last phenophase, a weak to zero correlation is noted.

CONCLUSIONS

The analysis of SPAD value revealed that the most affected variant of bitter cucumber (*Momordica charantia*) during the period of all four applied treatments was Line 4, which recorded the lowest values compared to the control plants.

The most resistant variant to salt stress during all treatments was Line 3, in which a greater amount of SPAD value of was observed in plants treated with the two salt concentrations compared to control plants.

The interpretation of the data obtained with the Fluorometer OS30p+ showed a good physiological reaction of the plants to salinity.

The most resistant variant of bitter cucumber was the Brâncuși variety where the plants treated with 200 mM saline solution showed a marked difference. Also, they showed a higher fluorescence compared to the control plants during the treatment period.

Following the performance of the Pearson Test, a negative correlation is observed in the case of all the phenophases in which the determinations were made.

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