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DETERMINATION OF PRODUCTIVITY AND CHLORINE CONCENTRATION IN SOME BEAN CULTIVATION, FROM THE REGION OF MOLDOVA, UNDER SALT STRESS

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Abstract

Surfaces affected by excess salinity arrived at 200 million ha., at the level of the whole planet and to about 400.000 ha in Romania; around 7% of the world's total the surface area is affected by salinity. Salinization is a frequent process and is a major constraint for food production because it limits yield of the crops. Cl^- ion is dominant in saline substrates; it is essential as a microelement for all higher plants, but the high concentration of this ion leads to toxicity to the salt-sensitive plants; the stress caused by salinity is also a stress of dehydration. High salinity causes an internal water shortage, and plants that are affected by saline stress can sometimes develop xeromorphic structures. Therefore, in present study was pursued the influence of saline solutions of 100 mM and 200 mM NaCl on productivity of 7 local populations of common beans, collected from areas with saline soils, from North-East of Romania and grown under greenhouse conditions, in 2018. Thus, they were analyzed after 15 and 30 days of saline stress: the largest/ lowest amount of chlorine, the number of pods/plant and the weight of the grains/plant, elements that define productivity. As a result, the concentration of 100 mM and 200 mM NaCl negatively influenced the fruit weight against the control variant, to all the genotypes studied, and the Trușești 2, Blăgești, Săveni and Moșna genotypes recorded the highest number of grains/plants for the 100 mM variant and 200 mM.

Key words: *bean, correlations, NaCl, productivity.*

INTRODUCTION

Salinity is one of the major obstacles for increasing production in cropping areas throughout the world. The deleterious effects of salinity on plant growth are associated with: low osmotic potential of soil solution, nutritional imbalance, specific ion effect (salt stress) or a combination of these factors (Ashraf, 1994; Marschner, 1995). All of these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Gorham et al., 1985; Levitt, 1980; Munns, 2002) and at the molecular level (Mansour, 2000; Tester & Davenport, 2003; Winicov, 1998). Crop plants will not grow in high concentrations of salt: only halophytes grow in concentrations of sodium chloride higher than about 400 mM (Marschner, 1996). Species resistant to excess chlorine are: beans, potatoes, tomatoes etc. (Șumălan, 2004; Covașă, 2016).

The bean (*Phaseolus vulgaris* L.) is an important source of dietary protein in many developing countries but is considered as sensitive species to salinity compared to other vegetables (Bouzid & Chaabane, 2012). For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production of the plants as an indicator of salt stress tolerance.

Chlorine is an element present in all plants. The role of chlorine in plant metabolism is little known. Haas (1945) supported the influence of chlorine on enzymatic systems. It appears that $C1^-$ activates cytochrome oxidase.

It is accumulated in larger amounts in marine algae, ferns and halophilic plants. Higher plants take it from the ground through the root system and from the gaseous atmosphere through the stomachs of the leaves.

Nutritional insufficiency, causes leaf chlorosis, wrinkling of leaf edges and inhibition of root growth, deregulation of plant metabolism.

Such a insufficiency is also manifested by the wilting of the plants, stopping the growth, the easy breaking of the roots, which are generally small. The leaves have chlorotic spots, which later become yellowish.

The concentration of chlorine in the plant determines the yield and quality of production, for two reasons. First, chlorine is a mineral nutrient, and its deficiency induces metabolic problems that interfere with plant growth. However, due to the low requirements of most cultures, chlorine deficiency it does not appear hard in the field. Second, excess chlorine, an occurrence with salinity, results in serious physiological dysfunctions that affect both the quality and the yield of plants.

Most quality deficiencies are based on physiological dysfunctions that occur under toxic conditions (Zorb et al., 2018).

An important constraint on plants growing on saline soils is the toxicity of ions, especially those of Na^+ and Cl^-.

The ions can cause toxicity in different ways, as Bowen (1966) has shown, because they act as antimetabolites by binding or precipitating different metabolites, catalyzing the rapid breakdown of essential elements by combining with cell membranes and affecting thus permeability, but also embellish the essential elements, but cannot replace their functions (Marschner, 2002).

The experiment is part of a broader research, and the determination of chlor content is only one stage of this research.

We have tested the resistance of this species to a high degree of salinity, as there is currently an expansion of saline soils with high salt concentrations. Another reason for testing of these genotypes is that more and more, in agriculture, the use of seawater is increasingly desirable, for two reasons: the water crisis on the horizon, but also the nutritional value that seawater could provide by irrigating the crop plants.

As novelty, in the current study, new genotypes were found of bean, from Nord-East of Romania, tolerant to this factor. For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production of the plants as an indicator of salt stress tolerance.

MATERIALS AND METHODS

Soil salinity is one of the best known stress factors of plants that can lead to crop yield reduction. Therefore, it is important to identify new tolerance varieties of plants that can grow on saline soils. The experience was done under greenhouse conditions, to "Vasile Adamachi" farm in Iasi, and the research took place in the Plant Physiology Laboratory, USAMV Iași. The biological material was represented by seven local bean populations (Blăgeşti 1, Blăgeşti 2, Blăgeşti 3, Blăgeşti 4, Trușeşti 2, Moşna and Săveni) collected from saline soils from the region of Moldova, known as the region of Moldova (Iași, Vaslui and Botoșani).

The bifactorial experience was set up in 12 liter seed pots, in randomized blocks with three repetitions. They were exposed to saline stress for a period of 30 days, being constantly watered with concentrations of 100 mM NaCl and 200 mM NaCl.

The concentration of chlorine was determined by potentiometric titration with silver ions, using the Analytical Chloride Titrator, as described by Slabu et al. (2009), and expressed as mg/100 g DW. The results were statistically interpreted using the Microsoft Excel - Data Analysis application, determining the correlation coefficient and magnitude of the effect in the linear correlation r (Pearson).

RESULTS AND DISCUSSIONS

Chlorine is involved in the photosynthesis process to remove harmful oxidants to photochemical systems and stimulate the transport of electrons. It also has the role of regulating the osmotic potential by maintaining the hydric cell level, ensuring the opening of stomates (Davidescu, 1988). The excess of this microelement influences negatively the growth of plants by the degradation of carbohydrate metabolism (Ciobanu & Şumălan, 2009). The chlorine ion is present in abundance almost everywhere in the world. It is required as a micronutrient for optimal plant growth, at a rate of only 0.3-1 mg/g dry matter (DW) in most plants (Marschner, 1986). The influnce of the chloride ion on plant growth depends on the plant variety (Tottingham, 1919). As an example, high salt - tolerant barley cultivars cultured with 150 mmol/liter of NaCl produced 42-86% of the yields of the same cultivars grown under no saline conditions (Sopandie et al., 1993).

Analysis of the Cl^- content of bean leaves subjected to saline stress, over a period of 15 days reflects the fact that in the case of the control variant, values ranged from 0.97-3.37 mg/100 mg DW. For the variant treated with 100 mM NaCl, the values were higher compared to the control variant, so the maximum was recorded at the Săveni genotype (10.20 mg/100 mg DW) and the minimum value at the Blăgești 3 (3.90 mg/100 DW). The values are significantly higher than the control variant and for the 200 mM NaCl stress, genotypes with a maximum of 15.26 mg/100 mg DW is Săveni, followed by genotypes: Blăgești 4 (12.95 mg/100 mg DW), Moșna (7.89 mg/100 mg DW) and Trușești 2 (7.09 mg/100 mg DW) which denotes the resistance of these genotype to saline stres (Table 1).

Table 1. The content of Cl^- (mg/100 mg DW) of bean leaves under salinity stress for a period of 15 day

Population	Control	100 mM NaCl	200 mM NaCl
Blăgești 1	0.97	5.59	4.88
Blăgești 2	1.24	4.52	3.48
Blăgești 3	1.77	3.90	4.88
Blăgești 4	3.37	8.69	12.95
Moșna	2.32	6.65	7.89
Săveni	0.97	10.20	15.26
Trușești 2	1.33	5.23	7.09

Following analysis to variance in the seven saline stressed bean genotypes for 15 days, it was found that Cl^- accumulation was insignificantly influenced by genotype and very significantly by the applied NaCl concentration (Table 2).

Table 2. Variance analysis for bean genotypes subjected to NaCl stress for a period of 15 days with respect to Cl^- of the leaf content

Source of variation	SP	GL	MS	F	P-value	F crit	Influence
Genotype	97.4073	6	16.23.455	3.438095	0.032568	2.99612	NS
Concentration	151.8529	2	75.92643	16.07943	0.000402	3.8853	***
Error	56.66353	12	4.721961				
The total	305.9237	20					

*Anova Two-Factor: NS- statistic differences insignificant (p≥0.05); *Significant statistical differences (p≤0.05); ** Significantly significant statistical differences (p≤0.01); *** Very significant statistical differences (p≤0.001), F> F crit reject the null hypothesis.*

As a result of the T-test, it is noted that between the control variant and the variant subjected to a 100 mM saline stress, there are very significant statistical differences. There are statistically significant differences between the blank control and the 200 mM concentration. Significant distinct statistical differences are manifested between saline-treated variants (Table 3).

Table 3. Statistical differences between control (I) and variants treated with saline solutions: 100 mM (II) and 200 mM (III) in terms of Cl^- content, expressed as mg/100 mg DW

Comparative variants	t-stat	P two-tail	Significance
I-II	-5.51981	0.001487	***
I-III	-3.9656	0.007404	*
II-III	-1.91005	0.01047	**

*Test Paired Two Sample for Means: Ns-statistically insignificant differences (p≥0.05) between variants; *Significant statistical differences (p≤0.05) between variants; **Significant distinct statistical differences (p≤0.01) between variants; ***Very significant statistical differences (p≤0.001) between variants.*

Sensitivity to high Cl^- concentrations varies widely between plant species and cultivars. Generally, most nonwoody crops tolerate excessive levels of Cl^-, where as many woody plant species and beans are susceptible to Cl^- toxicity (Mass, 1986). The critical toxicity concentration is about 4-7 and 15-50 mg/g for Cl^- sensitive and Cl^- tolerant plant species. Critical toxicity concentration to bean is 1.0 mmol Cl^-/liter (maximum Cl^- concentration in saturated soil extracts without loss in yield) to an EC of 1.0 (dS/m) (Jing et al., 1992).

After 30 days, after application of saline treatments, in the case of the control variant, the Cl^- content of the leaves shows values ranging from 1.30-2.66 mg.u./100 mg s.u. For the variant treated with 100 mM saline, the values are higher compared to the control variant; the maximum was recorded to genotype Blăgești 1 (11.75 mg/100 mg DW) and the lowest value for Blăgești 4 genotype (3.19 mg/100 mg s.u.). In contrast, the values are significantly higher than the control variant for four populations subjected to a 200 mM saline stress; In this case, the maximum value reaches 14. 44 mg/100 mg s.c. to the Blăgeşti 2 genotype, and the minimum value is 5.56 mg/100 mg s.u. at Blăgești 4 (Table 4).

Table 4. The content of Cl^- (mg/100 mg DW) of bean leaves under salinity stress for a period of 30 days

Population	Control	100 mM NaCl	200 mM NaCl
Blăgești 1	2.43	11.75	18.67
Blăgești 2	1.30	12.21	14.44
Blăgești 3	2.64	5.45	11.73
Blăgești 4	2.53	3.19	4.56
Moșna	2.30	7.74	7.55
Săveni	2.66	3.75	6.81
Trușești 2	2.13	5.02	5.64

Following variance analysis, to bean genotypes subjected to saline stress for 30 days, it is noted that Cl^- accumulation was insignificantly influenced by genotype and significantly distinct from the applied NaCl concentration (Table 5).

Table 5. Analysis of variance to bean genotypes subjected to stress with NaCl over a period of 30 days, with respect to the Cl^- content of leaf

Source of variation	SP	GL	MS	F	P-value	F crit	Influence
Genotype	140.7583	6	23.45972	2.685111	0.068602	2.99612	NS
Concentration	207.6784	2	103.8392	11.88504	0.001426	3.8853	**
Erorr	104.8436	12	8.736966				
Total	453.2803	20					

Anova Two-Factor: NS- statistic differences insignificant ($p \geq 0.05$); *Significant statistical differences ($p \leq 0.05$); **Significantly significant statistical differences ($p \leq 0,01$); ***Very significant statistical differences ($p \leq 0,001$), F> F crit reject the null hypothesis.

This time, following the T-test, it is noted that between the control variant and the variant subjected to a 100 mM saline stress, there are very significant statistical differences. There are statistically significant differences between the control and the 200 mM variant. Significant distinct statistical differences are manifested between saline-treated variants (Table 6).

Table 6. Statistical differences between control (I) and variants treated with saline solutions: 100 mM (II) and 200 mM (III) in terms of Cl^- content, expressed as mg/100 mg DW

Compared variants	t-stat	P two-tail	Significance
I-II	-3.91308	0.007864	**
I-III	-2.97548	0.024781	*
II-III	-0.50368	0.0632443	**

Paired Two Sample for Means: Ns- statistically insignificant differences ($p \geq 0.05$) between variants; *Significant statistical differences ($p \leq 0.05$) between variants; **Significant distinct statistical differences ($p \leq 0.01$) between variants; ***Very significant statistical differences ($p \leq 0.001$) between variants.

Assessing the influence of saline stress on plant production is a very important aspect in determining how salinity affects productivity, and that is why the fructification process has been studied on the basis of determinations of different indices: average number of grains/ plant, and average weight of grains/pods.
Analyzing the average number of grains/plants and average weight of grains/pods, after treatment with 30 day saline solution, it was observed that, in comparison to the control variant, for all genotypes treated with 100 mM NaCl and 200 mM NaCl solutions, these parameters, decreased considerable**.** From Tables 7 and 8, it can be noticed that four genotypes recorded a constant number of grains and a constant grain weight, to all three analyzed variants (Trușești 2, Blăgești 4, Săveni and Moşna) and three of the genotypes had high values only at the control variant (Blăgești 1, Blăgeşti 2 and Blăgeşti 3); to the 100 mM treated variants, they had very low production, and to the variant treated with 200 mM was inexistent.
The results obtained are in full agreement with those presented in the literature, according to which high concentrations of NaCl have a negative influence on the production of cultivated plants (Giannakoula & Ilias, 2013; Covaşă, 2016).

Table 7. Effect of saline stress on the average number of grains/pod after 30 days of exposure to saline stress

Population	**Control**	**100 mM NaCl**	**200 mM NaCl**
Blăgești 1	17.40	11.40	0.00
Blăgești 2	11.60	0.00	0.00
Blăgești 3	7.00	2.40	0.00
Blăgești 4	6.20	4.20	2.40
Trușești 2	9.20	6.40	5.60
Săveni	6.40	5.00	1.60
Moșna	5.80	3.40	2.80

Table 8. Effect of saline stress on average weight of grains/pod after 30 days exposure to saline stress

Population	**Control**	**100 mM NaCl**	**200 mM NaCl**
Blăgești 1	0.92	0.59	0.00
Blăgești 2	1.21	0.00	0.00
Blăgești 3	0.51	0.12	0.00
Blăgești 4	0.48	0.28	0.12
Trușești 2	0.86	0.51	0.46
Săveni	0.90	0.64	0.32
Moșna	0.65	0.28	0.15

In nature, phenomena are in close association with the surrounding phenomena. That is why in practical applications we are interested in not only the presence and the meaning of the correlation, but also the extent to which it manifests itself; this grade is appreciated by statistical calculations. In this respect, the linear correlation coefficient Bravais-Pearson (Dragomirescu, 2003) was introduced to assess the correlation between two sizes. In this paper, in order to have statistical coverage, a correlation was made between two studied parameters, according to the rules established by Colton (1974).
The correlation between Cl^- content and average weight of grains/pod after 30 days of exposure to saline stress was found to be acceptable in accordance with the rules established by Colton (1974). Chlorine concentration at leaf level had a negative influence on the average number of grains/ pods, in this respect the excess of chlorine having a toxic effect (Figure 1).

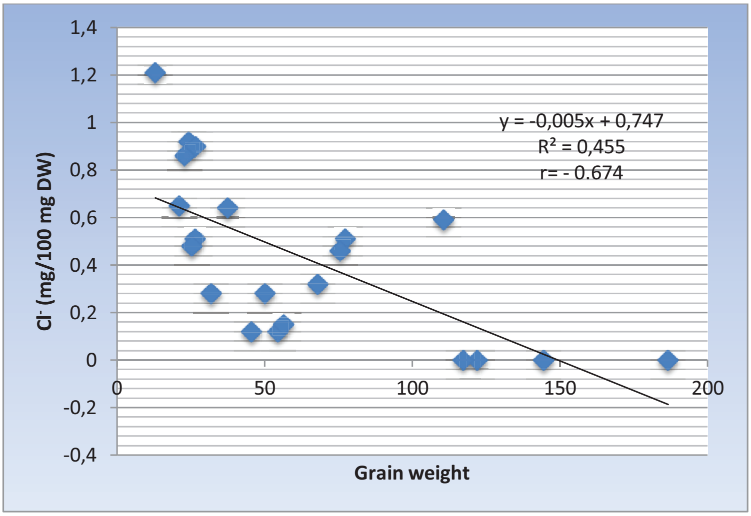

Figure 1. Correlation between Cl$^-$ (mg/100 mg DW) content and average number of grains/pod after 30 days exposure to saline stress

Figure 2. Aspects on how to determine the Cl$^-$ content of bean plants with the Analytical Chroride Titrator (original)

CONCLUSIONS

At the end of the treatment, the Cl$^-$ content values were greater than to the control variant for the 100 mM genotypes and 200 mM NaCl. At the last treatment applied, the maximum value achieved was 14.44 mg/100 mg DW at Blăgești 2, and the minimum value was 4.56 mg/100 mg DW at Blăgești 4.

From the observations on the fructification process we can conclude the following: the number of grains exposed to saline stress over a period of 30 days decreased compared to the control variant for all genotypes soaked to 100 mM and 200 Mm NaCl; to the last variant there were genotypes to which no production was obtained, because salinity had an effect of inhibiting growth, photosynthesis and subsequent production (Blăgeşti 1, Blăgeşti 2 and Blăgești 3).

The genotypes Trușești 2, Săveni, Moșna and Blăgeşti 4 recorded the highest number of grains/plants for the 100 mM and for the 200 mM variant.

The concentration of 100 mM and 200 mM NaCl negatively influenced the weight of the fruit against the control variant, the values oscillating from 0.12 grams to 0.59 grams of berries/pods.

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