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DETERMINATION OF CHLORINE CONCENTRATION AND PRODUCTIVITY IN SOME BEAN GENOTYPES, FROM NORTH-EAST OF ROMANIA, UNDER SALT STRESS

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Abstract

From the environmental stress factors, salinity is one of the most important causes which affects the security of mankind. Impact of soils with excessive salinity on the productivity of different cultures is sometimes disastrous, which determines the identification and the creation of new genotypes of plants tolerant to osmotic stress conditions. From the viewpoint of stress concept, chlorine (Cl^-) is viewed as being biologically aggressive osmolite, based on its small ionic diameter and its strong tendencies to attract water (high hydration capacity). High concentrations of these ions in apoplast lead to imbalances in hydric and ionic relationships. For these reasons, stress caused by salinity is also a dehydration stress and ionic stress. In present study, it was pursued the influence of saline solutions of 100 mM and 200 mM NaCl on Cl^- concentration and productivity, to 7 local populations of common beans (Phaseolus vulgaris L.) collected from areas with saline soils, in North-East of Romania and grown under greenhouse conditions, at the pots, analyzing the largest/lowest amount of chlorine in the leaves and the correlations between the chlorine content and the average number of pods/plants, the average number of grains/pods, the average weight of the grains/pods and the average yield/plant, indicators that define productivity.

Key words: *correlations, NaCl, Phaseolus vulgaris L., productivity.*

INTRODUCTION

Excess salinity in the soil affects total growth and yield and the impact extent depends on the degree of salinity (Flowers and Yeo, 1995; Munns, 2002). Crop plants will not grow in high concentrations of salt: only halophytes grow in concentrations of sodium chloride higher than about 400 mM. The physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress are reviewed at the cellular, organ, and whole-plant level. Salinity is considered one of the major factors affecting the agricultural productivity worldwide. In the arid and semiarid regions, soil salinization may be caused by poor irrigation water which contains considerable amounts of salts, salt accumulation in the soil surface layer due to over-irrigation, proximity to the sea and/or the capillarity rise of salts from underground water, into the root zone, due to the excessive evaporation (Gama et al., 2007). Salinity reduces the ability of plants to utilize water, causes a reduction in the growth and yield, and changes in the plant metabolic processes (Munns, 1993, 2002).

The chloride 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 in most plants (Marchner, 1986). The influence of the chloride ion on plant growth depends on the plant variety (Tottingham, 1919). The dependence of modern agriculture on irrigation and chemical fertilization causes more concern about the toxicity of chloride than about Cl^- deficiency (Marschner, 1986).

Generally, most non woody crops tolerate excessive levels of Cl^-, whereas many woody plants species and beans are susceptible to Cl^- toxicity (Maas, 1986).

Chloride toxicity in plants is often hard to diagnose, for two reasons: it is difficult to separate the effects of chloride from those of any accompanying cations, commonly sodium;

and it is difficult to distinguish between the specific toxic effects of ions and the cellular dehydration caused by their excessive external concentrations (Bar et al., 1997).

Chlorine is involved in photosynthesis to remove harmful oxidants from photochemical systems and stimulate electron transport. It has a role in regulating the osmotic potential by maintaining the hydric cell level, ensuring the opening of the stomata. On the other hand, it stimulates enzymatic activity and is antitranspirant (Davidescu, 1988). In the chloroplast, the Cl^- concentration remains relatively constant regardless of whether the plant growth medium is characterized by deficienty or excess.

Excess of chlorine negatively affects plant growth, by degradation of carbohydrate metabolism. Species resistant to excess chlorine are: beans, potatoes, tomatoes etc. (Șumălan, 2004). Chlorine deficiency reduces foliar growth, followed by wasting, chlorosis, brunification, and ultimately necrosis; at the same time, the fruit decreases in number and size, because beans are a sensitive plant from this point of view (Toma et Jităreanu, 2007).

Phaseolus vulgaris L. is a salt - sensitive species. For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production as an indicator of salt stress tolerance.

MATERIALS AND METHODS

The experience was done under greenhouse conditions 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, Moşna, Săveni, Trușești 2) collected from saline soils from the North-Eastern region of Romania, 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

Analysis of the Cl^- content of bean leaves subjected to saline stress, over a period of 30 days reflects the fact that in the case of the control variant, values ranged from 0.15 to 0.48 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 Moșna genotype (6.60 mg/100 mg DW) and the minimum value at the Blăgeşti 2 (5.25 mg/100 mg DW).

The values are significantly higher than the control variant and for the 200 mM NaCl stress, genotypes with a maximum of 12.10 mg/100 mg DW to the local population Blăgeşti 1, which denotes the resistance of this genotype to saline stress (Table 1).

Table 1. 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	0.23	6.35	12.10
Blăgești 2	0.48	5.25	5.45
Blăgești 3	0.22	6.40	10.10
Blăgești 4	0.29	6.60	7.15
Moșna	0.34	6.95	9.75
Săveni	0.15	5.40	7.95
Trușești 2	0.19	5.50	5.75

As a result of the T-test, it is noted that there are insignificant statistical differences between the control variant and the variant subjected to 100 mM NaCl; between the control variant and the one with a NaCl concentration of 200 mM statistical differences are very significant, and statistically significant values were recorded between variants treated with the two saline solutions (Table 2).

Table 2. 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	Signification
Control -100 mM NaCl (I)	-22.5725	2.474255	**Ns**
Control - 200 mM NaCl (II)	-8.58179	0.000006	***
100 mM NaCl - 200 mM NaCl (III)	-2.87781	0.014069	*

-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.

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 pods/plant, average number of grains/pods, average weight of grains/pods and average production/plant.
Analyzing the average number of pods/plants in saline-stressed bean plants over a period of 30 days, it is noted that, in comparison to the control variant, for all genotypes treated with 100 mM NaCl and 200 mM NaCl solutions the average number of pods/plant was low.
From table 3 it can be observed that the highest genotype of the analyzed character is the number of pods/plant is Blăgești 4, which records a number of 25.33 pods/plant in the control variant, 24 pods/plant for the variant 100 mM NaCl and 23.66 for the 200 mM NaCl variant. The lowest number of pods/plants was recorded in the Moșna: 3.33 pods/plant for the control variant, 1.66 pods/plant for the 100 mM NaCl (Table 3) .

Table 3. Effect of saline stress on the average number of pods/plant after 30 days exposure to saline stress

Population	**Control**	**100 mM NaCl**	**200 mM NaCl**
Blăgești 1	15.33	11.33	8.00
Blăgești 2	25.00	18.66	12.00
Blăgești 3	7.00	4.00	3.00
Blăgești 4	25.33	24.00	23.66
Moșna	3.33	1.66	0.00
Săveni	3.33	4.00	1.00
Trușești 2	15.33	9.00	6.00

The correlation between Cl^- content and average number of pods/plants 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).

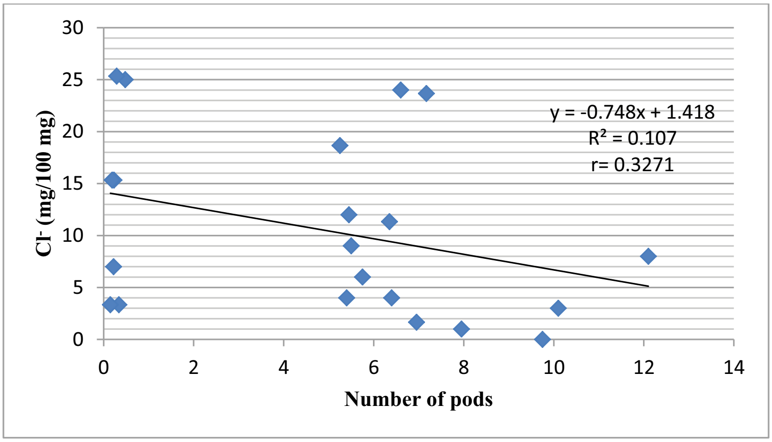

Figure 1. Correlation between Cl^- (mg/100 mg DW) and average number of pods/plant and after 30 days exposure to saline stress

Regarding the determination of the average number of beans/pods it is noted that compared to the control variant, all local populations treated with 100 mM NaCl and 200 mM NaCl solutions determined a decrease of the analyzed parameter.

Thus, the Blăgeşti 4 genotype, which had a number of 6.03 number of grains/pod in the control variant, 5.61 number of grains/pod for the 100 mM variant and 4.06 grains/pod for the 200 mM NaCl variant, was particularly highlighted.

The lowest value of this analyzed parameter was recorded in the Mosna genotype: 1.72 number of grains/pod for the control variant, 1.25 number of grains/pod for the 100 mM NaCl variant and number of pod/plant for the 200 mM NaCl treated variant (Table 4).

Table 4.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
Blăgeşti 1	3.56	3.16	2.72
Blăgeşti 2	4.42	3.19	2.72
Blăgeşti 3	2.72	2.26	2.16
Blăgeşti 4	6.03	5.61	4.06
Moşna	1.72	1.25	0.00
Săveni	3.31	3.00	1.33
Truşeşti 2	4.20	3.54	2.40

The correlation between Cl^- content and the average number of grains/pod after 30 days of exposure to saline stress has shown an acceptable degree of association, indicating that the chlorine concentration recorded at the leaf level negatively influenced the average number of grains/pod, from this point of view the excess of chlorine having a toxic effect (Figure 2).

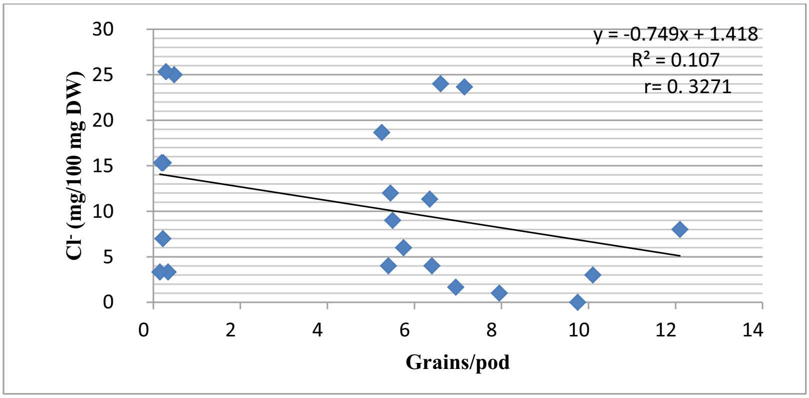

Figure 2. Correlation between Cl^- (mg/100 mg DW) content and average number of grains/pod after 30 days exposure to saline stress

Determinations by the influence of saline stress on weight grains/pod after 30 days of saline stress, in the case of the 200 mM NaCl variant, four genotypes recorded lower values, except the Blăgeşti 3 genotype at which the average weight of the grains/pod was the same, as in the 100 mM NaCl treated variant. And this time the Moşna genotype was noted for lack of productivity (Table 5).

Table 5. 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**
Blăgeşti 1	0.82	0.56	0.37
Blăgeşti 2	1.10	0.96	0.73
Blăgeşti 3	0.56	0.32	0.32
Blăgeşti 4	1.85	1.70	1.30
Moşna	0.39	0.31	0.00
Săveni	0.63	0.54	0.33
Truşeşti 2	1.36	1.22	0.74

The correlation between Cl^- content and average weight of grains/pod after 30 days of exposure to saline stress also indicates an acceptable degree of association (Figure 3).

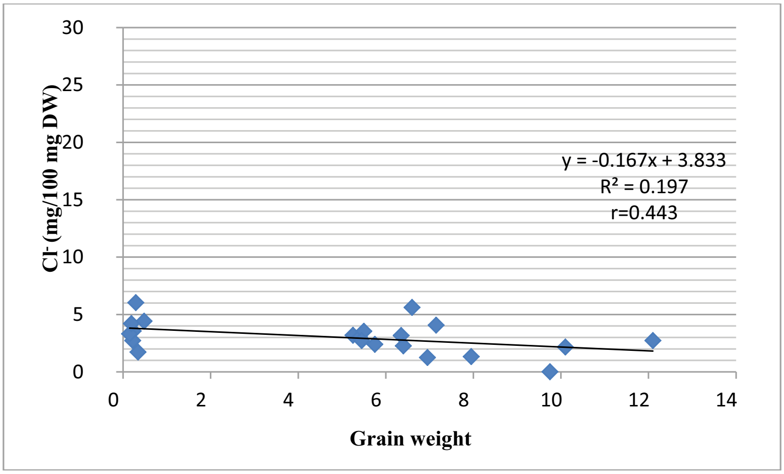

Figure 3. Correlation between Cl^- content and average weight of grains/pod after 30 days exposure to saline stress

Concerning in the mean production per plant compared to the control variant, it is observed a decrease with the application of saline treatments to all seven studied.genotypes.
It was found that the highest production of the Blăgeşti 4 genotype, both for the control variant (46.86 g) and for the saline-treated variants (40.80 g and 30.75 g).
The smallest production was recorded in the Moşna genotype, from the 100 and 200 mM NaCl variants, namely 0.51 and 0 grains/plant (Table 6).

Table 6. Effect of saline stress on average production/plant after 30 days exposure to saline stress

Population	**Control**	**100 mM NaCl**	**200 mM NaCl**
Blăgeşti 1	12.57	6.34	2.92
Blăgeşti 2	27.50	17.91	8.76
Blăgeşti 3	3.92	1.28	1.28
Blăgeşti 4	46.86	40.80	30.75
Moşna	1.29	0.51	0.00
Săveni	2.09	2.16	0.33
Truşeşti 2	20.84	10.98	4.94

Regarding the correlation between Cl^- content and average production/plant after 30 days of exposure to saline stress also indicates an acceptable degree of association (Figure 4).

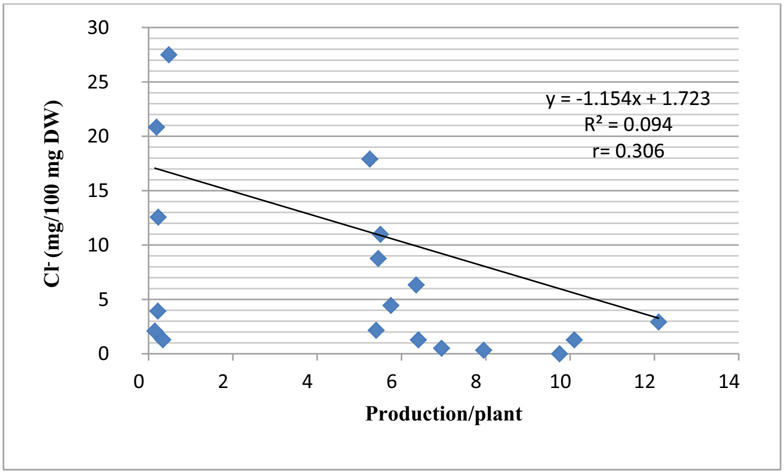

Figure 4. Correlation between Cl^- (mg/100 mg DW) content and average production/plant after 30 days exposure to saline stress

Excess chlorine accumulated in plants following NaCl treatments at 100 mM and 200 mM, respectively, has a negative influence on the average number of pods/plants, the average number of grains/pods, the average weight of the grains/pods and the average yield/plant, in which case chlorine becomes toxic to plants, inhibiting the fructification process. The differences in susceptibility to toxicity between the bean genotypes studied are correlated with the ability to reduce chlorine transport in stems and leaves.
This character is genetically determined and can be used to improve resistance to toxicity.
The results obtained are in full agreement with those presented in the literature, according to which the high concentrations of Cl^- negatively influence the production (Singh et al., 2012).

CONCLUSIONS

Cl^- content values were higher than control variant for genotypes subjected to a 200 mM NaCl. The maximum value reached was 12.1 mg/100 mg DW in the Blăgeşti 1, and the minimum value was 5.75 mg/100 mg DW at Truşeşti 2. The Blăgeşti 4 genotype recorded the highest production in all four indices analyzed, and the lowest values were registered on Moşna local population, with the specification that the variant treated with 200 mM NaCl did not have any production.

The fructification process decreased compared to the control variant of all treated genotypes with 100 mM NaCl and 200 mM NaCl. The correlation between the Cl^- content and the average number of pods/plant, the average number of grains/pods, the average weight of grains/pods and the average yield/plant in bean plants exposed to saline stress for a period of 30 days from exposure to saline stress was found to be acceptable in accordance with the rules established by Colton (1974).

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