

## EFFECT OF SALT STRESS ON THE GROWTH AND PHOTOSYNTHESIS RATE OF BEAN PLANTS (PHASEOLUS VULGARIS L.)

### ЕФЕКТ НА СОЛЕВИЯ СТРЕС ВЪРХУ РАСТЕЖА И ФОТОСИНТЕТИЧНАТА АКТИВНОСТ ПРИ ФАСУЛА (PHASEOLUS VULGARIS L.)

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#### ABSTRACT

The effect of salt stress on some physiological parameters in young bean plants (cv. Lody) was studied under controlled conditions in a climatic room. The plants were grown in pots as hydroponic cultures in half-strength Hoagland nutrient solution. The plants were treated for 7 days with 50 and 100 mM NaCl and Na<sub>2</sub>SO<sub>4</sub>, starting at the appearance of the first trifoliolate leaf unfolded. The salts were added to the nutrient solution.

It was established that the applied doses of both salt types caused stress on the young bean plants, which found expression in the suppression of growth, photosynthesis activity and the plastid pigment content. The amount of proline in the tissues of the salt-treated plants was increased, while the cell water potential was reduced.

Key words: bean, salt stress, growth, leaf-gas exchange and pigments, content of proline, water potential.

#### РЕЗЮМЕ

Беше изследван ефектът от засоляването върху някои физиологични параметри в млади растения фасул (сорт Лоди). Опитът беше проведен в климатичен бокс при контролирани условия. Растенията бяха отглеждани като хидропонни култури в ½ хранителен разтвор на Хогланд. След появата на първия троен лист растенията бяха третирани за 7 дневен период с 50 и 100 mM NaCl и Na<sub>2</sub>SO<sub>4</sub>.

Беше отчетено, че двата вида соли предизвикват стрес в младите растения фасул, който се проявяваше в потискане на растежа, фотосинтетичната активност и съдържанието на пластидните пигменти. Съдържанието на пролин в тъканите на засолените растения беше повишено, докато водният потенциал на клетките беше силно намалено.

Ключови думи: фасул, солеви стрес, растеж, листен газообмен и пигменти, съдържание на пролин, воден потенциал.

## INTRODUCTION

At present about 20% of the world's cultivated land and approximately half of all irrigated land is affected by salinity [16]. Therefore, salinity is one of the most significant abiotic factors limiting crop productivity [11, 3]. This is attributed to the fact that  $\text{Na}^+$  competes with  $\text{K}^+$  for binding sites essential for cellular function [15] and the latter implication of these two macronutrients in salinity is thought to be the one of the factors responsible for the reduction of the biomass and yield components of plants. High concentration of salt in the root zone (rhizosphere) reduces soil water potential and the availability of water [8]. As a result of this, reduction of the water content leading to dehydration at cellular level and osmotic stress is observed. The increased amount of  $\text{Na}^+$  and  $\text{Cl}^-$  in the environment affects the uptake of many indispensable nutrients through competitive interactions and by affecting the ion selectivity of membranes. For example, external  $\text{Na}^+$  negatively impacts intracellular  $\text{K}^+$  influx, attenuating the acquisition of this essential nutrient by cells.

The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular  $\text{CO}_2$  concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids [14].

The most studied compound under salinity stress is proline. The amount of proline usually increases under salinity [7].

In most cases, salinity problems are linked to an excess of  $\text{NaCl}$  in the irrigation water, but sometimes other salts, like  $\text{Na}_2\text{SO}_4$ , are present. There are few studies on the effect of  $\text{Na}_2\text{SO}_4$  on plant growth.

The objective of the present study was to compare the physiological reactions of young bean plants to two salt types -  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$ . It was assumed that the two salt types could have different effect with respect to plant growth and leaf gas-exchange, as well as the toxicity in beans.

For our experiment we chose bean (*Phaseolus vulgaris* L.) that is an important crop for the agricultural production, limited by the quality of irrigation water. Previous researches have suggested that beans is a salt-sensitive plant.

## MATERIALS AND METHODS

The experiments were carried out in a climatic room in the Department of Plant Physiology and Biochemistry

at the Agricultural University of Plovdiv, Bulgaria. The conditions, maintained during the experiments, were the following: light duration – 14 hours, light intensity (PAR)  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ , temperature –  $22 \pm 2 \text{ }^\circ\text{C}$  and relative air humidity –  $60 \pm 5\%$ . The tested plants (beans cv. Lady) were grown in pots (4 plants/pot) filled by half-strength Hoagland nutrient solution. At the appearance of the first trifoliolate leaf, an experimental design with five treatments was arranged:

- control – plants, supplied by  $\frac{1}{2}$  of Hoagland solution;
- plants, supplied by  $\frac{1}{2}$  of Hoagland solution enriched with 50 mM  $\text{NaCl}$ ;
- plants, supplied by  $\frac{1}{2}$  of Hoagland solution enriched with 50 mM  $\text{Na}_2\text{SO}_4$ ;
- plants, supplied by  $\frac{1}{2}$  of Hoagland solution enriched with 100 mM  $\text{NaCl}$ ;
- plants, supplied by  $\frac{1}{2}$  of Hoagland solution enriched with 100 mM  $\text{Na}_2\text{SO}_4$ ;

The treatment of plants with salts lasted was for 7 days. The plants were harvested and the fresh and dry weight of roots, shoots, and leaves, and the leaf area (electronic area meter – NEO-3, TU-Sofia) were measured. The leaf gas exchange elements were determined by means of a portable infrared gas analyzer LCA-4 (Analytical Development Company Ltd., Hddesdon, England) equipped with a PLCB-4 chamber. The water potential ( $\Psi$ ) in leaves was measured with a pressure chamber (ELE-International, England). The photosynthetic pigments content in the leaves was determined spectrophotometrically [9]. Proline was extracted in 3% sulphosalicylic acid and was determined calorimetrically according to the method of Bates et al. (1973) [1].

## STATISTICAL ANALYSIS

Statistical analysis was performed using a one way ANOVA (for  $P < 0.05$ ). Based on the ANOVA results, a Tukey test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments. In the tables, different letters (a, b, c) express significant differences, with a representing the highest value.

## RESULTS AND DISCUSSION

The seven-day salt-treatment of bean plants caused symptoms of phytotoxicity – the leaves became brown, yellow and dry. The root tips turned brown and a considerable inhibition of the root system growth was observed (Figure 1).

**Table 1. Biometrical parameters of control and salt-treated plants**

Young bean plants are subjected to salt stress by adding 50 and 100 mM of NaCl and Na<sub>2</sub>SO<sub>4</sub> to the Hoagland nutrient solution. FM<sub>pl</sub> – fresh mass of plants (g), DM<sub>pl</sub> - dry mass of plants, DM<sub>r</sub> – dry mass of roots, DM<sub>st</sub> - dry mass of stems DM<sub>l</sub> – dry mass of leaves and LA – leaf area (cm<sup>2</sup>).

**Таблица 1. Биометрични параметри на контролните и засолени растения.**

Младите растения фасул бяха подложени на засоляване с добавяне на 50 и 100 mM of NaCl и Na<sub>2</sub>SO<sub>4</sub> към хранителния разтвор на Хогланд. FM<sub>pl</sub> – свежа маса на растенията (g), DM<sub>pl</sub> – суха маса на растенията, DM<sub>r</sub> – суха маса на кореният, DM<sub>st</sub> - суха маса на стъблата DM<sub>l</sub> – суха маса на листата и LA – листна площ (cm<sup>2</sup>).

Parameters	Control	50 mM NaCl	50 mM Na <sub>2</sub> SO <sub>4</sub>	100 mM NaCl	100 mM Na <sub>2</sub> SO <sub>4</sub>
FM <sub>pl</sub>	10.24 a	8.92 b	8.55 b	5.42 c	4.90 c
DM <sub>pl</sub>	1.29 a	1.04 b	1.03 b	0.59 c	0.55 c
DM <sub>pl</sub> /FM <sub>pl</sub>	0.13 a	0.12 a	0.12 a	0.11 b	0.10 b
DM <sub>r</sub>	0.24 a	0.23 a	0.25 a	0.09 b	0.14 b
DM <sub>st</sub>	0.30 a	0.20 b	0.21 b	0.13 c	0.15 c
DM <sub>l</sub>	0.75 a	0.61 b	0.58 b	0.31 c	0.28 c
DM <sub>r</sub> /DM <sub>sh</sub>	0.30 a	0.28 b	0.32 a	0.31 a	0.33 a
LA	258.95 a	184.45 b	165.15 b	93.65 c	79.73 c

Mean values are the average of three replicates. Within the same column, values flanked by the same letters are not significantly different for  $P = 0.05$  following one-way ANOVA test. Data in parenthesis are expressed as percent of control value.

Comparing the plants on the picture, it is evident that the toxicity in the chloride treatment is expressed more clearly in older leaves, while in the case of the sulphate treatment, a similar effect was observed with respect to younger leaves and the plants' tops (Figure 2).

The results regarding the changes of the basic biometrical parameters of young bean plants indicate that the applied salt treatment causes a considerable inhibition of their initial development. The data on the accumulation and the distribution of the fresh and dry biomass in the organs of the young bean plants are shown in Table 1. They change in accordance with the salt type and its concentration. In case of salt treatment with 50 mM NaCl and Na<sub>2</sub>SO<sub>4</sub>, the inhibition of the fresh and dry biomass was within the limits of 17 – 23%.

In both types of salt treatments the higher dose caused a stronger inhibition effect. This tendency was observed with respect to all parameters and was averagely at 50% below the control level (FM<sub>pl</sub> and DM<sub>pl</sub>). The addition of Na<sub>2</sub>SO<sub>4</sub> to the nutrient environment caused a stronger inhibition effect. This tendency was more clearly expressed with respect to the plants' dry biomass.

The dry biomass changes in the different plant organs had similar values. Once more the dose of 100 mM Na<sub>2</sub>SO<sub>4</sub> caused stronger inhibition than 100 mM NaCl. The applied salt treatment caused considerable changes in the ratios between the dry biomass in the roots and in the above-surface organs (DM<sub>r</sub>/DM<sub>sh</sub>), and these values

were at about 30-40% above the control.

A strong inhibition effect was also observed with respect to the leaf area. The reduction of this index at the dose of 100 mM was 64-67% below the control, for the chloride and sulphate treatments, respectively.

Salinity type (NaCl and Na<sub>2</sub>SO<sub>4</sub>) would be expected to have an impact on plant growth, where a direct ion toxicity or nutritional imbalance occur [6].

Salt stress in beans caused significant differences in gas exchange parameters. In our experiment there was an evident decrease of P<sub>N</sub> in the leaves of bean plants subjected to salinity (Table 2). Increasing salinity level progressively decreased P<sub>N</sub>.

On the 7<sup>th</sup> day of treatment P<sub>N</sub> was reduced to 65% (50 mM NaCl) and 56% (50 mM Na<sub>2</sub>SO<sub>4</sub>). High dose salinity (100mM) decreased P<sub>N</sub> to 40% and 20% respectively.

The stronger inhibition of this parameter at the higher salinity dose (100 mM) at the end of the seven-day period could be a result of the stomata closure, as well as of the biochemical activity.

Similar results have been reported by Stepien, 2006 [14]. This tendency, with respect to the water use efficiency, observed in the leaves suggests that the non-stomata factors, in addition to the stomata ones, affected photosynthesis.

Salinity strongly decreased g<sub>s</sub> (Table 2) and the declined g<sub>s</sub> resulted in reduced transpiration rate (E). Stomata closure is known to be an effective mechanism



Figure 1



Figure 1

for economical water utilisation under salt stress and for limitation of the harmful salt ions uptake [5]. Unfortunately, the decrease in  $g_s$  caused a simultaneous decrease in  $P_N$ .

After 7 days of salt treatment, E had fallen by 13% and 23% for those plants grown at 50 mM and by 50% and 70% for those grown at 100 mM NaCl and  $Na_2SO_4$ , respectively. The water use efficiency ( $P_N/E$ ) was modified as a result of the reported changes in  $P_N$  and E (Table 2). In the leaves of beans, subjected to 100

mM NaCl and  $Na_2SO_4$ , the  $P_N/E$  was reduced to 50% compared to that in the control plants.

Water status is highly sensitive to salinity and therefore is dominant in determining the plant responses to stress [14]. The results in the Table 2 showed that water potential ( $\Psi$ ) decreased considerably in the 100 mM sodium chloride- and sulphate-treated plants, because salinity increased cellular water loss. However, there is substantial evidence that glycophytic as well as halophytic species adjust to high salt concentrations by

**Table 2. Effect of salinity on the parameters of leaf-gas exchange and water status in bean plants.**

Young bean plants are subjected to salt stress by adding 50 and 100 mM of NaCl and Na<sub>2</sub>SO<sub>4</sub> to the Hoagland nutrient solution. P<sub>N</sub> – Net photosynthetic rate [ $\mu\text{mol}(\text{CO}_2)\text{ m}^{-2}\text{ s}^{-1}$ ]; E – Transpiration rate [ $\text{mmol}(\text{H}_2\text{O})\text{ m}^{-2}\text{ s}^{-1}$ ]; g<sub>s</sub> – stomata conductance [ $\text{mol m}^{-2}\text{ s}^{-1}$ ]; P<sub>N</sub>/E ratio – water use efficiency;  $\Psi$  – Water potential [-MPa] and P – proline content (mg g<sup>-1</sup> FM).

**Таблица 2. Ефект на засоляването върху параметрите на листния газов обмен и водния статус във фасулевите растения.**

Младите растения фасул бяха подложени на засоляване с добавяне на 50 и 100 mM of NaCl и Na<sub>2</sub>SO<sub>4</sub> към хранителния разтвор. P<sub>N</sub> – Скорост на фотосинтезата [ $\mu\text{mol}(\text{CO}_2)\text{ m}^{-2}\text{ s}^{-1}$ ]; E – Скорост на транспирацията [ $\text{mmol}(\text{H}_2\text{O})\text{ m}^{-2}\text{ s}^{-1}$ ]; g<sub>s</sub> – Устична проводимост [ $\text{mol m}^{-2}\text{ s}^{-1}$ ]; P<sub>N</sub>/E ratio –Ефективност на използваната вода;  $\Psi$  – Воден потенциал [-MPa] и P – Съдържание на пролин (mg g<sup>-1</sup> FM).

Parameters	Control	50 mM NaCl	50 mM Na <sub>2</sub> SO <sub>4</sub>	100 mM NaCl	100 mM Na <sub>2</sub> SO <sub>4</sub>
P <sub>N</sub>	7.93	6.16	4.19	1.95	0.77
E	1.80	1.57	1.40	0.88	0.33
g <sub>s</sub>	0.07	0.036	0.03	0.02	0.01
P <sub>N</sub> /E	4.40	4.36	2.95	2.30	2.29
$\Psi$	1.66	2.96	3.93	5.13	5.80
Proline	10.1	27.5	35.2	42.5	43.5

**Table 3. Content and ratios between photosynthetic pigments of control and salt-treated bean plants.**

Young bean plants are subjected to salt stress by adding 50 and 100 mM of NaCl and Na<sub>2</sub>SO<sub>4</sub> to the Hoagland nutrient solution. Chl. a – chlorophyll a; Chl. b – chlorophyll b; Car – carotenoids

**Таблица 3. Съдържание и съотношения между фотосинтетичните пигменти. В контролните и подложените на солеви стрес растения.**

Младите растения фасул бяха подложени на засоляване с добавяне на 50 и 100 mM of NaCl и Na<sub>2</sub>SO<sub>4</sub> към хранителния разтвор на Хогланд. Chl. a – Хлорофил а; Chl. b – хлорофил b; Car –каротиноиди.

Treatments	Content (mg / g FW) and ratios between photosynthetic pigments				
	Chl. a	Chl. b	Car	a/b	a+b / Car
Control	1.44	0.88	0.56	2.28	3.98
50 mM NaCl	1.39	0.64	0.52	2.18	3.88
50 mM Na <sub>2</sub> SO <sub>4</sub>	1.71	0.87	0.69	1.97	3.73
100 mM NaCl	1.08	0.64	0.53	1.76	3.24
100 mM Na <sub>2</sub> SO <sub>4</sub>	1.12	0.73	0.49	1.51	3.77

lowering tissue osmotic potentials with an increase of inorganic ions accumulation in tissues [2].

The water use efficiency was significantly modified as a result of the reported changes in the net photosynthetic rate and transpiration rate. In the bean plants grown at 50 mM sodium chloride and sulfate P<sub>N</sub> decreased to a lesser extent.

Proline accumulation is an important physiological index for plant response to salt stress [13], as well as to other types of stress. The relationship between proline accumulation in the shoots of bean plants and the two salinity types is shown in table 2. It is evident

that proline content increased parallel with the rise of the salinity levels.

In the case of higher salinity level (100 mM) the proline concentration was increased 3-3.5 times compared to the control. This result demonstrates that sulfate stress can cause higher accumulation of proline than the chloride type. This fact suggests that the induction of proline synthesis is related not only to changes in the water potential and to the salinity type – chloride and sulfate, but also resulted from metabolism interruption by high-stress intensity or from an adaptive response with special physiological function.

The increased levels of proline, under salt stress, have been reported in two wheat cultivars [7]. It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. The higher concentration of proline under salt stress is favorable to plants as proline participate to osmotic potential of leaf and thus to osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition.

The photosynthetic pigments are some of the most important internal factors, which in certain cases can limit the photosynthesis rate.

The data presented in Table 3 shows that the applied treatment tended to diminish the content of all pigments and to a certain extent changed their ratios. The decrease of Chl.a content at 100 mM NaCl and Na<sub>2</sub>SO<sub>4</sub> was considerably different from the controls and was 75% and 77%, respectively. The decreased content of both chlorophyll and carotenoids could probably be a result from the mineral deficiency. Total chlorophyll content in both salt-stressed plants declined with the salinity increase. It was established that Car decreased to a lesser extent than Chl.

Ion accumulation in leaves adversely affected Chl content [10]. The observed decrease of Chl content in the plants grown under saline conditions may be attributed to both of the increased degradation and the inhibited synthesis of that pigment [4]. Interestingly, Chl a was less sensitive or better protected against salt stress compared to Chl b.

## CONCLUSIONS

On the basis of the results obtained during the research, the following conclusion can be drawn:

With the increase of the salinity level the growth processes in the bean plants was suppressed, which is a result of the disturbed osmotic processes and the toxic effect of Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and Na<sup>+</sup>.

The leaf gas-exchange and the plastid pigments concentrations were considerably suppressed, and this tendency was more strongly expressed in the case of the sulphate treatment.

The water potential of the bean plants was reduced considerably, while the proline content was increased from 3 to 3.5 times in the case of the higher salinity level.

The isomolar concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> suppressed each to a different extent, the physiological processes in the plants. The weaker toxic effect in the plants treated with NaCl probably is a result, on the one

hand, of the weaker inhibition of the water potential and the stomata closure, and on the other hand, of the lower concentration of Na<sup>+</sup> in the tissues of the plants treated with chloride, compared to that in the plants treated with Na<sub>2</sub>SO<sub>4</sub>.

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