ОТГОВОР НА ФАСУЛЕВИ РАСТЕНИЯ (*PHASEOLUS VULGARIS L.*) КЪМ ЗАСОЛЯВАНЕ С ИЗООСМОТИЧНИ РАЗТВОРИ НА NaCl И Na_2SO_4

RESPONCE OF BEAN PLANTS (*PHASEOLUS VULGARIS L.*) TO ISO-OSMOTIC SOLUTIONS OF NaCl AND Na_2SO_4

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Резюме

Засоляването на почвата е един от основните екологични проблеми, които оказват влияние върху продуктивността на растенията навсякъде по света. Проучен беше ефектът на солевия стрес върху растежа, фотосинтезата, съдържанието на пролин и нивата на липидната пероксидация на растения от фасул (*Phaseolus vulgaris* L.). Експериментът беше проведен с млади фасулеви растения (сорт Чер Старозагорски) в климатичен бокс при контролирани условия. В настоящото проучване растенията бяха отгледани като хидропонни култури в съдове с 1/2 хранителен разтвор на Хогланд. След развитието на първия троен лист растенията бяха третирани с изоосмотични разтвори на NaCl (100 mM) и Na_2SO_4 (67 mM) в продължение на 7 дни.

Засоляването понижава съдържанието на хлорофил, като стойностите са по-ниски след засоляване с NaCl. Съдържанието на пролин и нивата на липидната пероксидация са повишени в тъканите на третираните растения, като изменението е сходно за двете соли. Получените от нас резултати показват, че приложените нива на засоляване предизвикват стрес в младите фасулеви растения. Понижението на устичната проводимост и фотосинтетичния капацитет след засоляване вероятно е свързано с инхибицията на растежа при третираните растения, отчетено в настоящото изследване.

Abstract

Soil salinity is one of the most important stress factors to limit crop productivity worldwide. The effect of salt stress în the growth, photosynthesis, proline content and lipid peroxidation level in bean plants (*Phaseolus vulgaris L.*) were studied. The experiment was caried out with young bean plants (*cv. Cher Starozagorski*) under controlled conditions in a climatic room. In the present study, plants were grown in pots as hydroponic cultures in half-strength *Hoagland* nutrient solution. The plants were treated for 7 days with iso-osmotic concentrations of NaCl (100 mM) and Na_2SO_4 (67mM) after the first trifoliate leaf unfolded.

Saline conditions decreased the chlorophyll content and values were lower after the NaCl treatment. The proline content and lipid peroxidation level increased in the tissues of the salt-treated plants and the changes were similar for both types of salinity. Our result suggests that the applied dose of both salt types caused stress in the young bean plants. The study recorded decline in stomata conductance and photosynthetic capacity which could be attributed to the growth inhibition of the treated plants.

Ключови думи: фасул, фотосинтеза, пролин, липидна пероксидация, солеви стрес.
Key words: bean, growth, photosinthesis, proline, lipid peroxidation, salt stress.

INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality, especially in arid and semi-arid climates (Khan and Panda, 2008). It may occur naturally or as result of management practices. Salinity not only decreases the agricultural production of crops, but also, affects the associated ecological balance of the area. Salinity has been classified as ‘‘Primary’’ or ‘‘Secondary’’ salinity (Manchanda and Garg, 2008).

The problem of salinity might be a result of poorly drained soil, the presence of high water tables and salinization groundwater resources (Manchanda and Garg, 2008). Also, overuse and misuse of fertilizers leads to salinity buildup. The type and amount of fertilizers applied to soil, affect its salinity. Some fertilizers contain high levels of potentially harmful salts, such as potassium chloride or ammonium sulphate. In nursery and greenhouse production incorrect watering combined with the solubilization of fertilizers can lead to increased ionic stress causing (Khayyat et al., 2009). Minor changes in electrical conductivity and salinity in a hydroponic system can delay a crop or cause poor yields.

Soils containing elevated levels of NaCl and Na_2SO_4 are naturally present in many areas in Bulgaria. Also, soil salinization is spread in form of spots widely in irrigated regions with intensive agriculture (Trendafilof, 2001).

The high salinity levels of soil and irrigation water leading to inhibition of plant growth, yield reduction and even plants death (Khayyat et al., 2009). Salinity affects plants through osmotic effects, ion-specific effects and oxidative stress (Munns and Tester, 2008). The main consequences of plant exposure to salt stress are water deficit and ion excess, which lead to several morphological, physiological and metabolic modifications in plants (Khan and Panda, 2008; Nawaz et al., 2010). Osmotic effects are due to salt-induced decrease in the soil water potential. Salinity ions may affect membrane selective permeability and interfere with uptake of other ions, thus altering tissue contents of a range of elements (Hu and Schmidhalter, 2005).

Reduction in biomass, photosynthetic capacity, changes in leaf water potential have been reported to have a cumulative effect attributed to salinity stress (Gama et al., 2007). 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 CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate (Munns, 2002). Salt stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids (Mittler, 2002; Khan and Panda, 2008). This processes lead to overproduction of lipid peroxidation, associated with membrane deterioration (Khan and Panda, 2008).

Many physiological responses in plants are function to maintain or increase water uptake, reduce water loss and mitigate osmotic and ionic stress (Sairam and Tyagi, 2004; Nawaz et al., 2010). A large number of plant species accumulate prolinå in response to salinity stress and that accumulation may play a role in combating salinity stress. However, data do not always indicate a positive correlation between the osmolyte accumulation and the adaptation to stress (Asharf and Harris, 2004; Mansour et al., 2005).

Most salinity studies are focused on plant responses to NaCl stress, but also in many cases Na_2SO_4 salinity may be a major problem There have been comparatively few studies examining plant responses to situations where Na_2SO_4 salinity dominates. According to our previous result Na_2SO_4 was more phytotoxic to bean plants than equimolar NaCl (Kaymakanova and Stoeva, 2008). A similar tendency was observed in *Cornus stolonifera* (Renault et al., 2001) and *Helianthus annuus* (Manivannan et al., 2008). On the contrary, Nguyen at al. (2006) found that NaCl treatment of *Picea mariana*, *Picea glauca* and *Pinus banksiana* cause more damages than in plants treated with Na_2SO_4 in the same molar concentration. In *Phragmides australis* growth was more adversely affected by NaCl than by iso-osmotic Na_2SO_4 (Pagter et al., 2009).

The objectives of this study were to estimate the effects of iso-osmotic solutions of Na_2SO_4 and NaCl, on growth, gas exchange and pigments content, accumulation of proline and malondialdehyde in bean plants (*Phaseolus vulgaris* L.).

MATERIALS AND METHODS

Plant growth and treatments: Bean seeds (*Phaseolus vulgaris* L.), cultivar Cher Starozagorski, were germinated at 26^0C in perlite media. After that, they were transferred in pots, with modified half-strength Hoagland nutrient solution and grown in a climatic room under controlled environmental conditions. The conditions, maintained during the experiments, were the following: light duration – 14 hours, light intensity (PAR) 250 μmol m^{-2} s^{-1}, temperature – 22 ± 2^{o}C and relative air humidity – $60 \pm 5\%$. Seedlings were grown until emergence of the first true trifoliate leaf, at which time salt stress treatment was initiated. Plants were exposed to salinity by adding iso-osmotic concentrations of NaCl (100 mM) and Na_2SO_4 (67 mM) to the growth media according to modified method of Pagter et al. (2009). Seven days after salt treatment, four plants were harvest and analyzed.

Plant analysis: The fresh and dry weight of plants and the leaf area (electronic area meter – NEO-3, TU-Sofia) were measured. Mean relative growth plant (RGR) was calculated according to Radford (1967). The photosynthetic parameters were measured with a portable infrared gas analyzer *LCA-4* (*Analytical Development Company Ltd.,* Hoddesdon, England), equipped with a *PLCB-4* chamber. Lipid peroxidation was measured as amount of malondialdehide (MDA) (Heath and Packer, 1968). Proline was determined calorimetrically according to the method of Bates et al. (1973).

Statistical analysis. Data presented are means ± standard deviation of five replicates.

RESULTS

The summary of the analysis for the biometrical parameters studied during the experiment showed that salinity stress had adverse effect on the biomass yield and leaf area of bean plants. The decline observed in plants weight showed almost a similar trend for both salinity types. The relative growth rates (RGR) was highly significant and declined considerably after salt treatment of bean plants. The effects of salt stress on leaf area were similar to that on growth parameters in both treatments (Table 1).

Reduction of the biomass in beans under saline condition was indicative of several growth limitations. Some authors reported that in *Phaseolus vulgaris* salinity also reduced shoot and root weights (Bayuelo-Jimenez et al., 2003; Gama et al., 2008; Kaymakanova and Stoeva, 2008).

Salt stress in beans caused significant differences in gas exchange parameters (Table 2). On the 7th day of treatment P_N was reduced to 63 % in comparison with the control without differences between salinity type. The data with respect to the transpiration intensity (E) and stomata conductance of salt-treated plants follow the same tendency as that in the photosynthesis. Conversely, our previous studies found that the same photosynthetic parameters of stressed bean plants (*Phaseolus vulgaris* L.) were more affected by Na_2SO_4 salinity compare to equimolar NaCl (Kaymakanova et al., 2009).

The decline in photosynthesis observed in case of iso-osmotic salinity could be attributed to stomata factors, but also to non-stomata factors or direct damages of photosynthetic apparatus (Tavakkoli et al., 2010). This response could have been due to an inhibition of stomata opening by an elevated apoplastic Cl^- concentration (Silva et al., 2008) or high rate of Na^+ (Barhoumi et al., 2007). In the same way, it has been hypothesised that lowered water potential in roots could trigger signal from roots to reduce g_s (Pagter et al., 2009). Brougnoly and Lauteri (1991) also indicated that reduced photosynthetic carbon assimilation was attributed to reduced stomata conductance.

The results (Table 3) demonstrate that the content of chlorophyll *a* after the two salt treatments diminishes, and this trend is more obvious after treatment with NaCl. The most probable explanation of this result after exposure of plants to iso-osmotic salinity is the more damaging effect of the Cl^- ions for pigment molecule. Total chlorophyll content in both salt-stressed plants declined too. It was established that Car decreased to a lesser extent than Chl.

The decreased content of plastid pigments could probably be a result of the mineral deficiency and destructive processes in chloroplasts. According to Barhoumi et al., (2007) salinity caused membrane disappearance, tilakoid disorganization and appearance of vesicles and numerous plastoglobulin in thilakoid membranes of pigments.

Таблица 1. Промени в растежа на фасулеви растения след 7-дневно засоляване с изоосмотични разтвори на NaCl (100 mM) и Na_2SO (67 mM). FM_{pl} – свежа маса на растенията (g), DM_{pl} – суха маса на растенията, LA – листна площ (cm^2), RGR – относителна скорост на растежа. Стойностите са осреднени ±S.E. (n=5). * <0.5 ** <0.1

Table 1. Changes in growth of bean plants treated for 7 days with iso-osmotic solutions of NaCl (100 mM) and Na_2SO_4 (67 mM). FM_{pl} – fresh mass of plants (g), DM_{pl} – dry mass of plants, LA – leaf area (cm^2), RGR – relative growth rate. Data presented are mean ±S.E. (n=5). * <0.5 ** <0.1

Parameters	Control	*100 mM NaCl*	*67 mM Na_2SO_4*
FM_{pl}	17.03±0.9	10.26±1.03 **	9.40±0.89 **
DM_{pl}	2.65±0.22	1.21±0.12 **	1.12±0.08 **
LA	577.1±10.00	366.0±7.12 **	296.2±4.32 **
RGR_{pl}	177.±6.01	64±0.02 **	52.9±0.06 **

Таблица 2. Ефект от засоляването върху параметрите на листния газообмен на фасулеви растения след 7-дневно засоляване с изоосмотични разтвори на NaCl (100 mM) и Na_2SO (67 mM). P_N – скорост на фотосинтезата [μmol (CO_2) m^{-2} s^{-1}]; E – скорост на транспирацията [mmol (H_2O) m^{-2} s^{-1}]; g_s – устична проводимост [mol m^{-2} s^{-1}]. Стойностите са осреднени ±S.E. (n=5). * <0.5 ** <0.1

Table 2. Effect of salinity on the leaf gas-exchange parameters in bean plants treated for 7 days with iso-osmotic solutions of NaCl (100 mM) and Na_2SO_4 (67 mM). P_N – Net photosynthetic rate [μmol (CO_2) m^{-2} s^{-1}]; E – Transpiration rate [mmol (H_2O) m^{-2} s^{-1}]; g_s – stomata conductance [mol m^{-2} s^{-1}]. Data presented are mean ±S.E. (n=5). * <0.5 ** <0.1

Parameters	Control	*100 mM NaCl*	*67 mM Na_2SO_4*
Pn	9.10±0.63	3.34±0.43 **	3.38±0.23 **
E	1.89±0.01	1.18±0.02 *	1.27±0.06 *
g_s	0.06±0.00	0.02±0.02 **	0.02±0.01 **

Таблица 3. Съдържание на фотосинтетичните пигменти във фасулеви растения след 7-дневно засоляване с изоосмотични разтвори на NaCl (100 mM) и Na_2SO (67 mM). Chl. a – хлорофил a; Chl. b – хлорофил b; Car – каротиноиди. Стойностите са осреднени ±S.E. (n=5). * <0.5 ** <0.1

Table 3. Content of photosynthetic pigments in bean plants treated for 7 days with iso-osmotic solutions of NaCl (100 mM) and Na_2SO_4 (67 mM). Chl. a – chlorophyll a; Chl. b – chlorophyll b; Car – carotenoids. Data presented are mean ±S.E. (n=5). * <0.5 ** <0.1

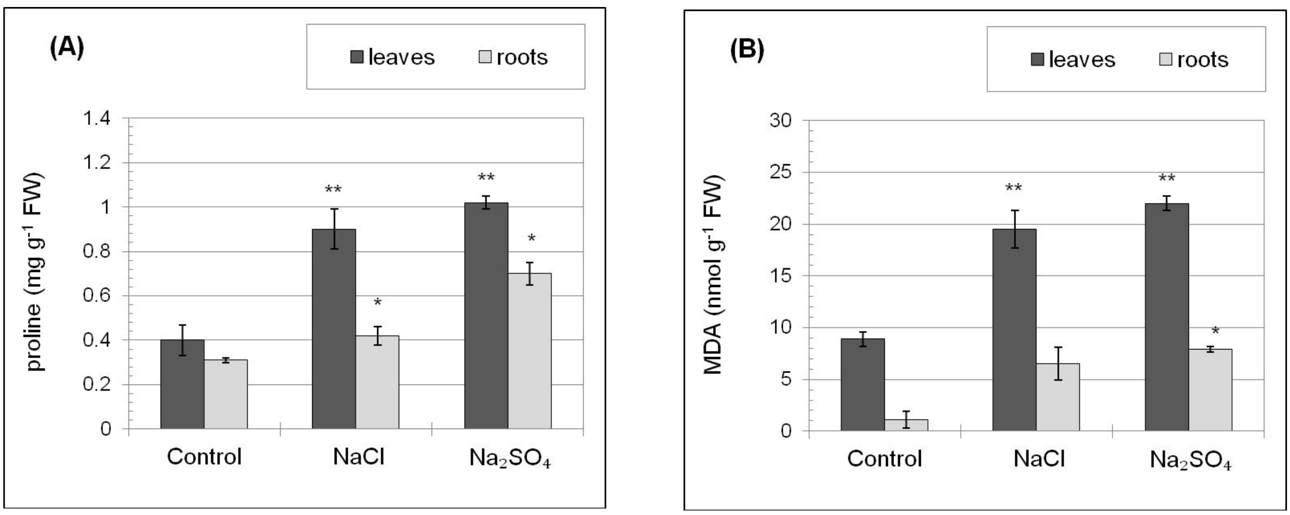
Parameters	Control	*100 mM NaCl*	*100 mM Na_2SO_4*
Chl. *a*	2.25±0.09	1.57±0.03 *	1.97±0.09 *
Chl. *b*	1.04±0.02	0.68±0.02 *	0.72±0.03 *
Car	0.77±0.01	0.60±0.02	0.68±0.06

Proline (Pro) accumulation is supposed to correlate with the adaptation of plants to salinity (Khan et al., 2007; Nawaz et al., 2010). The higher concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition (Khan et al., 2007; Plaza et al., 2009). Our results (Figure 1) implicate that NaCl and Na_2SO_4 stress increases Pro accumulation (2-3 times compared to the control) in the leaves of plants and less in roots. The result demonstrates that both salinity stress can cause higher accumulation of prîline in plants tissue.

The increased levels of proline, under salt stress, have been reported in two wheat cultivars by Khatkar and Kuhad (2000). In our previous study, we demonstrated that bean plants accumulated much more proline in roots after Na_2SO_4 salinity compare to equimolar NaCl (Kaymakanova and Stoeva, 2008).

Lipid peroxidation is often used as an indicator of membrane damage and leakage under salt stress conditions (Khan and Panda, 2008). The results showed that the degree of accumulation of MDA was higher in the roots compared to the leaves, indicating a higher rate of lipid peroxidation in bean plants due to salt stress (Figure 1).

We may conclude that the applied dose of both salt types caused stress in the young bean plants. The growth processes and photosynthetic capacity in the bean plants were suppressed in similar rate after exposure of plants to iso-osmotic salinity.



Фиг. 1. *Промени в съдържанието на пролин (A) и малондиалдехид (MDA) (B) при фасулеви растения след 7-дневно засоляване с изоосмотични разтвори на NaCl (100 mM) и Na_2SO (67 mM). Стойностите са осреднени ±S.E. (n=5). * <0.5 ** <0.1*

Fig.1. *Changes in proline content (A) and malondialdehyde (MDA) (B) in bean plants treated for 7 days with iso-osmotic solutions of NaCl (100 mM) and Na_2SO_4 (67mM). Data presented are mean ±S.E. (n=5). * <0.5 ** <0.1*

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