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EFFECT OF SOIL SALINITY ON ACTIVITY OF ANTIOXIDANT ENZYMES AND CONTENT OF ASCORBIC ACID AND PHENOLS IN BEAN (*PHASEOLUS VULGARIS* L.) PLANTS

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Abstract

Soil salinity is the one of the most important abiotic factors influencing the growth, development and yields of crops. However, it is difficult to determine exact concentrations of salt which cause soil salinity. Salinity threshold levels depend on a crop species, variety, developmental stage and environmental factors. This paper presents the results of an experiment on the effect of different soil concentrations of NaCl soil on several oxidation stress parameters, such as catalase and peoxidase activity, content of ascorbic acid, phenols and flavonoids in bean plants.

A laboratory pot experiment was carried out on samples of light silty loam containing 1.2% of humus. Pots were filled with 1 kg soil samples each, to which NaCl solution was added in doses 10, 30 and 50 mM kg^{-1}. Each pot was seeded with 7 seeds of cv. Aura bean. The plants grown in soil without NaCl were the control. On days 14, 21 and 28 green parts of plants were collected for determinations of catalase and peroxidase activity by colorometry as well as the content and flavonoids, phenols, ascorbic acid and chloride concentration by Mohr's method.

The results show that chloride concentration in bean plants increased at higher of NaCl concentration in soil. The activity of the antyoxidative enzymes such as catalase and peroxidase in bean plants, on sampling days, was higher as the chloride concentration in plants increased. Non-enzymatic antioxidants: flavonoids, phenols and ascorbic acid content during the experiment showed different changes with relation to the chloride content, but in all the trials ascorbic acid content was significantly positively correlated whereas the content of phenols was significantly negatively correlated with the chloride content in plant tissues.

Key words: salinity, oxidative stress, bean, flavonoids, phenols.

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WPŁYW ZASOLENIA GLEBY NA AKTYWNOŚĆ ENYZMÓW ANTYOKSYDACYJNYCH ORAZ ZAWARTOŚĆ KWASU ASKORBINOWEGO I FENOLI W ROŚLINACH FASOLI (*PHASEOLUS VULGARIS* L.)

Abstrakt

Jednym z ważniejszych abiotycznych czynników wpływających na wzrost, rozwój i produktywność roślin jest zasolenie podłoża. Trudno jest jednak określić, w przypadku jakiego stężenia soli mówi się o zasoleniu podłoża. Graniczna jego wartość jest uzależniona od gatunku, a w nawet odmiany, etapu rozwoju rośliny oraz od wielu towarzyszących czynników środowiska. W pracy zaprezentowano wyniki doświadczeń mających na celu określenie, w jaki sposób dodatek do gleby NaCl o różnych stężeniach oddziałuje na wybrane parametry stresu oksydacyjnego: aktywność katalazy i peroksydazy oraz zawartość kwasu askorbinowego, fenoli i flawonoidów w roślinach fasoli.

Doświadczenie wazonowe w warunkach laboratoryjnych przeprowadzono na próbkach gliny pylastej lekkiej o zawartości próchnicy 1,2%. Glebę podzielono na 1 kg naważki, którymi, po wcześniejszym dodaniu wodnych roztworów NaCl w dawkach 10, 30 i 50 mM kg^{-1}, napełniono wazony. Do każdego wazonu wysiano po 7 nasion fasoli odmiany Aura. Próbę kontrolną stanowiły rośliny rosnące w glebie bez dodatku soli. W 14., 21. i 28. dniu doświadczenia pobrano zielone części roślin i oznaczono w nich kolorymetrycznie aktywność katalazy i peroksydazy oraz zawartość flawonoidów, fenoli i kwasu askorbinowego, a także zawartość chlorków miareczkową metodą Mohra.

Stwierdzono, że zawartość chlorków w roślinach fasoli wzrastała wraz ze zwiększaniem stężenia NaCl w podłożu. Aktywność enzymów antyoksydacyjnych: katalazy i peroksydazy w roślinach fasoli, w poszczególnych terminach pomiarów, wzrastała wraz ze zwiększaniem się w nich ilości chlorków. Zawartość nieenzymatycznych antyutleniaczy w roślinach fasoli: kwasu askorbinowego, flawonoidów i fenoli wykazywała w trakcie trwania doświadczenia zmienne zależności w stosunku do ilości w nich chlorków, jednakże w całym doświadczeniu zawartość kwasu askorbinowego była istotnie dodatnio, a fenoli istotnie ujemnie skorelowana z ilością chlorków w tkankach roślinnych.

Słowa kluczowe: zasolenie, stres oksydacyjny, fasola, flawonoidy, fenole.

INTRODUCTION

Soil and water salinity have an increasing importance in agriculture (SUDHAKER et al. 2001). Salinity is one of the most important abiotic factors influencing the growth, development and yield of plants (CHAPARZADEH et al. 2004, RAHNAMA, EBRAHIMZADEH 2005) and causes its considerable losses in crops (SMIRNOFF 1998). The influence of high concentrations of salt in soil on the growth and metabolism of plants often manifests as deformations and decay of leaves. The excessive content of salt in soil solution also impedes germination, particularly in case of sensitive plants.

However, it is difficult to state precisely what concentration of salt causes soil salinity. Salinity thresholds depend on the plant's species, cultivar, development stage and environmental factors.

One of the biochemical responses of plants to biotic and abiotic stresses is the production of reactive oxygen forms (RAHNAMA, EBRAHIMZADEH 2005). Re-

active oxygen forms also occur during the physiological metabolic activity of plants (photosynthesis, respiration) and are under control of a complex antioxidative system (DIXON, PAIVA 1995, YAMASAKI et al. 1997). This system consists of enzymes, e.g. superoxide dismutase, catalase, peroxidases, and low-molecular compounds, e.g. ascorbate, glutathione, β-carotene, α-tocopherol, or phenolic compounds (MALENĆIĆ et al. 2003).

The aim of the present work has been to establish how soil salinity affects some oxidative stress parameters, such as catalase and peroxidase activity and the content of ascorbate, phenols and flavonoids in bean plants.

MATERIALS AND METHODS

A laboratory pot experiment was carried out on black earth from the Gumieńce Plain. In the arable-humus horizon this soil is light silty loam with 1.2% humus content, highly abundant in available phosphorus and moderately to highly abundant in available potassium and magnesium. Soil collected from a field was passed through a 2 mm sieve and brought to 60% maximum water capacity. Then it was divided into 1 kg weighed samples, which received aqueous NaCl solutions in the doses given in table 1 and put in the pots. In each pot, 7 seeds of cv. Aura bean (produced by CNOS-VIL-MORIN from Poznań) were seeded. The control trial consisted of bean plants growing on soil with an addition of bitter salt.

During the experiment the plants were illuminated by a sodium lamp Son-T Agro 400W (Philips) at the radiation intensity measured on level of the soil equal 90 $\mu E \cdot m^2 \cdot s^{-1}$ PAR (photosynthetic active radiation). The photoperiod was set as 12 hours of light and 12 hours of darkness.

In days 14, 21 and 28 of the experiment, green parts of the bean plants were collected for assays of the activity of catalase and peroxidase as well as the content of flavonoids, phenols and ascorbic acid. For this purpose, the plant samples were homogenized with an appropriate buffer for antioxidant enzymes, 80% acetone for phenols, 80% methanol for phenols and 2% oxalic acid for ascorbic acid. Afterwards, the samples were centrifuged at 14800 rpm. The supernatants thus obtained were tested for the selected biochemical parameters. Methods elabotrated by the following researchers were applied: LÜCK (1963) for catalase activity, CHANCE and MACHLY (1955) for peroxidase activity, WOISKY and SALATINO (1998) for the content of flavonoids, and SINGLETON and SAMUEL-RAVENTOS (1999) for the content of phenols content. The content of ascorbic acid was evaluated according to Polish Norm PN-90/A-75101/11. All the analyses were performed without any changes or modifications to the above procedures. In addition, the content of chlorides in plant tissues was determined using Mohr's titration method (KREŁOWSKA-KUŁAS 1993).

The experiment was established in a completely randomized design with three replications. The results were processed statistically using ANOVA. The least significance differences (LSD) were determined by Tukey's test at α=0.05. The statistical analyses were performed executed independently for every sampling day and experimental variants.

Correlation coefficients were calculated between the content of chlorides versus the activity of catalase and peroxidase and the content of phenols, flavonoids and ascorbate. Non-linear quadratic regression curves whose determination coefficients were above 0.850 were plotted to assess how on the subsequent sampling dates the stress parameters changes according to the increased Cl^- content in plants.

RESULTS AND DISCUSSION

Bean plants growing in soil with an addition of 10 mM $NaCl \cdot kg^{-1}$ soil were similar to the control plants except their height. The development of plants growing in soil with larger NaCl concentrations was distinctly inhibited. Plants growing in soil with 50 mM $NaCl \cdot kg^{-1}$ soil developed so poorly that on day 28 of the experiment it was impossible to obtain any plant material for analyses (Table 1).

Table 1

Doses NaCl added into soil

Concentration	NaCl	
	($mM \cdot kg^{-1}$)	($g \cdot kg^{-1}$)
I	10.00	0.585
II	30.00	1.755
III	50.00	2.925

The average Cl^- ionic content in the tissues of bean plants growing in soil without addition of salt for the whole experiment ranged from 2.000 to 2.632 $\mu g \cdot g^{-1}$ f.w.

The content of chlorides tissues of bean plants (Table 2) increased alongside an increasing NaCl concentration in the soil and on the subsequent sampling days. The largest accumulation of Cl^- ions, about fourfold more than the control, was observed on day 28 of the experiment at 50 mM NaCl. This finding finds confirmation in the literature, where increasing concentration of chlorides due to higher NaCl doses in soil have been observed in *Thellungiella halophilla* (M'RAH et al. 2006) and *Catharanthus roseus* (ELKAHOUI et al. 2005).

Table 2

Chloride concentration in bean plants growing in soil with different amounts of NaCl (μg $Cl^- g^{-1}$f.w. plant)

Dose of NaCl added into soil (mM kg^{-1})	14th day	21st day	28th day
0	2.000±0.095	2.632±0.110	2.580±0.087
10	3.372±0.101	4.639±0.114	5.577±0.116
30	4.308±0.150	8.067±0.189	8.901±0.131
50	-	-	9.820±0.270

In most cases, introduction of NaCl to soil caused significant changes occurring during the experiment in the activity of the enzymes analysed as well as in the content of the compounds determined in bean plants (Table 3).

While analysing dependences among the Cl^- content in plants and the assayed oxidative stress parameters, it was discovered that on day 14 an increased Cl^- content in bean plants coincided with a growing activity of the enzymes, especially peroxidase, as well as a higher total content of flavonoids (Figure 1). This observation was confirmed by the correlation coefficients, which proved that activity of catalase and peroxidase as well as the content of flavonoids were significantly positively correlated with chlorides in plants (Table 4). With respect to peroxidase, this correlation persisted in the subsequent days of the experiment, which is in agreement with the report by Jebary et al. (2005). A rise in the peroxidase activity as a result of soil salinity has also been verified in *Morus alba* (Sudhaker et al. 2001), *Glicyne max* (Ghorbanli et al. 2004) and *Lycopersicon esculentum* (Rahnama, Ebrahimzadeh 2005). However, the activity of catalase on days 21 and 28 of the experiment fluctuated, first increasing and then declining as the ionic concentration Cl^- content in bean plants rose. Besides, a significantly positive correlation between the catalase activity and chlorides in bean plants appeared on day 28. Chaparzadeh et al. (2004) suggested a stimulating influence of NaCl on the catalase activity in green parts of *Calandula officinalis*. Also Rahnama, Ebrahimzadeh (2005) observed a rise in the catalase activity in *Lycopersicon esculentum* plants.

The content of flavonoids in bean plants was stable on day 21, but on day 28 it was found to decrease as the content of Cl^- ions in plants rose, thus being negatively correlated with the content of chlorides. A similar tendency was observed for the total content of phenols. On day 14 the total phenolic content slightly fell as the concentration of Cl^- in plants increased. Moreover, on all sampling days, the total content of phenols and the content of chlorides in plants were significantly negatively correlated. The fact

Table 3

Changes of biochemical parameters in bean plants growing in soil with different amounts of NaCl

Parameter	Dose of NaCl added into soil (mM kg^{-1})	14th day	21st day	28th day
μM H_2O_2 · (g f.w. plant · min)$^{-1}$				
Catalase	0	1.087	2.590	2.252
	10	1.649	1.557	2.078
	30	2.732	2.581	2.066
	50	-	-	3.656
	$LSD_{0.05}$	0.211	0.321	0.125
μM purpurogaline · (g f.w. plant · 4 min)$^{-1}$				
Peroxidase	0	0.463	0.659	0.610
	10	1.267	0.516	0.574
	30	2.915	1.385	1.129
	50	-	-	1.274
	$LSD_{0.05}$	0.133	0.129	0.137
mg quercetine · g^{-1} f.w. plant				
Flavonoids	0	0.168	0.564	1.098
	10	0.283	0.470	1.200
	30	0.351	0.557	0.978
	50	-	-	0.235
	$LSD_{0.05}$	0.054	0.043	0.032
mg gallate · g^{-1} f.w. plant				
Phenols	0	0.076	0.079	0.148
	10	0.073	0.074	0.099
	30	0.060	0.064	0.084
	50	-	-	0.058
	$LSD_{0.05}$	0.004	0.003	0.002
mg ascorbate · g^{-1} f.w. plant				
Ascorbate	0	0.297	0.205	0.249
	10	0.281	0.315	0.388
	30	0.287	0.468	0.291
	50	-	-	0.361
	$LSD_{0.05}$	0.041	0.029	0.021

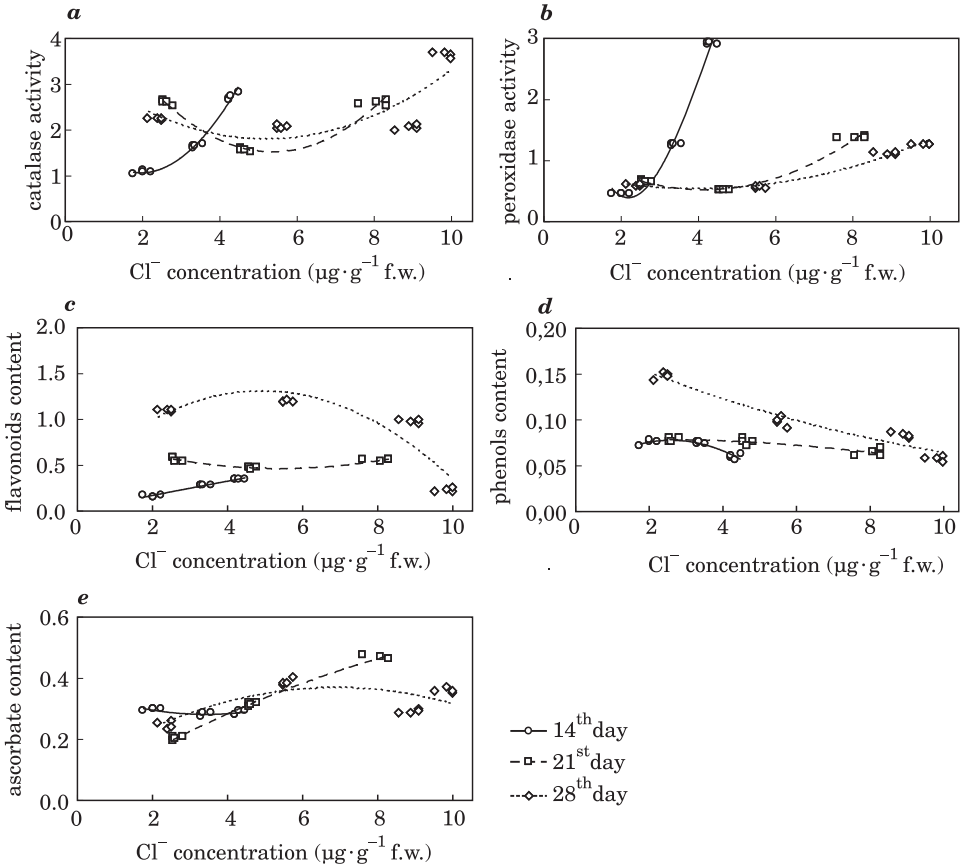

Fig. 1. Relationships between chloride concentration and biochemical parameters in bean plants

Table 4

Correlation coefficients between chloride concentration and biochemical parameters in bean plants

Day of experiment	Catalase	Peroxidase	Flavonoids	Phenols	Ascorbate
14th	0.954*	0.944*	0.984*	-0.769*	-0.506
21st	0.147	0.861*	0.071	-0.878*	0.993*
28th	0.536*	0.903*	-0.694*	-0.963*	0.450
Whole experiment	0.574*	0.237	0.079	-0.510*	0.627*

*significant $p = 0.05$

that the content of phenols in bean plants becomes depressed under soil salinity has also been reported by VERGEER et al. (1995), while NAVARRO et al. (2006) have observed such a relationship in *Capsicum annum* and VERMA and MISHRA (2005) noticed this in *Brassica juncea*.

On days 14 and 28 of the experiment, the content of ascorbate in bean plants remained relatively stable under an increasing Cl^- content in plants, while rising on day 21, when it was significantly positively correlated with the content of chlorides in plants. Increasing concentrations of ascorbate in *Calandula officinalis* caused by soil salinity was noticed by CHAPARZADEH et al. (2004), while SAIRAM et al. (2005) found a decrease in the ascorbate content in *Triticum aestivum* growing in soil with NaCl.

The correlation coefficients computed for the data from the whole experiment revealed that the content of chlorides in bean plants was significantly negatively correlated only with phenols, being significantly positively correlated with the content of ascorbate and the activity of catalase.

CONCLUSION

Growing NaCl concentration in soil caused an increase in the activity of catalase and peroxidase as well as in the content of ascorbate content. In contrast, it depressed the concentration of phenols in bean plants.

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