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ЕФЕКТ НА ЗАСОЛЯВАНЕТО ВЪРХУ АКТИВНОСТТА НА АНТИОКИСЛИТЕЛНИ ЕНЗИМИ В ЛИСТА И КОРЕНИ ОТ ФАСУЛ (*PHASEOLUS VULGARIS L.*)
SALINITY EFFECT ON ANTIOXIDANT ENZYMES IN LEAVES AND ROOTS OF BEANS (*PHASEOLUS VULGARIS L.*)

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Резюме

Проучен беше ефектът на солевия стрес върху активността на някои антиокислителни ензими в листа и корени от фасулеви растения (*Phaseolus vulgaris* L.), отгледани при контролни условия (хранителен разтвор) и в условия на засоляване (100 mM NaCl и Na_2SO_4, добавени към хранителния разтвор). Беше отчетено, че в листата на засолените растения активността на изследваните ензими се повишава (АПО, МДХАР, ДХАР), а в корените се намалява. При третирането с натриев сулфат беше отчетена по-висока ензимна активност в листата на изследваните растения в сравнение с тези, засолени с натриев хлорид. Отчетено беше също, че третирането с Na_2SO_4 понижава в по-голяма степен активността на ензимите.

В резултат на солевия стрес и при двата вида засоляване активността на ензима каталаза (КАТ) беше повишена в корените и понижена в листата в сравнение с нетретираните растения.

Наблюдаваните разлики в ензимните активности показват специфична реакция при различните органи на фасулевите растения, както и в зависимост от вида на приложените соли.

Abstract

The effect of salt stress on the activity of antioxidative enzymes was studied in leaves and roots of bean plants (*Phaseolus vulgaris* L.), grown under control (nutrient solution) and salt stress (nutrient solution containing 100 mM NaCl and Na_2SO_4) conditions. In the leaves of salt-stressed plants, ascorbate connected enzymes (APX, MDHAR and DHAR) demonstrated increased activity and at the same time their activity was decreased the in roots. The increase of the enzyme activity was more pronounced under sodium sulfate compared with the chloride treatment in the bean leaves. In salt-stressed roots the Na_2SO_4 application reduced the activity at a higher degree. As a result of the two salt treatments of bean plants the CAT activity were increased in the roots and decreased in the leaves as compared with the control.

The observed differences in the enzyme activities show the specific reaction in the different organs of the bean plants as well as the dependence on the kind of the applied salts.

Ключови думи: антиокислителни ензими, солеви стрес, *Phaseolus vulgaris L.*
Key words: Antioxidant enzymes; salt stress, *Phaseolus vulgaris L.*

Съкращения: АКВ - активирани кислородни видове, АПО - аскорбат пероксидаза, КАТ - каталаза, ДХАР - дехидроаскорбат редуктаза, МДХАР - монодехидроаскорбат редуктаза.
Abbreviations*:* ROS - reactive oxygen species; APX - ascorbate peroxidase; CAT - catalase; DHAR - dehydroascorbate reductase; MDAR - monodehydroascorbate reductase.

INTRODUTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality, especially in arid and semi-arid climates (Khan, 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. Soil salinization in Bulgaria is spread in form of spots widely and mainly in irrigated regions with intensive agriculture (Trendafilof, 2001; Ivanova, 2006).

The high salinity levels of soil and irrigation water are known to affect many physiological and metabolic processes, leading to yield reduction (Nemoto, 2002). Inhibition of plant growth and even plant death by salinity is due to a reduction in water availability, sodium ion accumulation and mineral imbalances. All these factors manifest themselves in morphological, physiological and metabolic modifications in plants (Sairam, 2004).

In addition, salt stress also leads to oxidative stress through an increase in the production of reactive oxygen species (ROS) which can damage membrane lipids, proteins and nucleic acids (Mittler, 2002).

Higher plants possess enzymatic and non-enzymatic mechanisms to cope with oxidative damage. The ascorbate-glutathione cycle is one efficient way to dispose of H_2O_2 and makes use of the non-enzymatic antioxidants ascorbate and glutathione in reactions catalyzed by the antioxidant enzymes (Noctor, 1998). Enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), peroxidase (POD), and glutathione reductase (GR) are the principal ROS scavenging systems. SOD dismutates superoxide radicals to hydrogen peroxide, whereas CAT and peroxidases dismutate H_2O_2 into water and oxygen. GR and MDHAR are involved in the regeneration of ascorbate (Reddy et al., 2004).

Most salinity studies have involved the determination of crop responses to NaCl salinity (Cavalcanti, 2007; Telesinski, 2008). However, there is reason to suspect that responses to SO_4^{-2} salinity may differ from those observed in a Cl‘ salt system. There have been comparatively few studies examining plant responses to situations where Na_2SO_4 salinity dominates. However, Na_2SO_4 is present in higher concentrations than NaCl in soil and groundwater in many parts of the world (Rogers et al., 1998). Also, there is very limited information about the antioxidative responses of roots (Khan, 2002; Gapinska, 2008), which are the first organs directly exposed to salinity.

Therefore, in order to understand better the biochemical mechanism of salt response, the aim of this study was to determine the activities of investigated enzymes in different organs of beans.

MATERIALS AND METHODS

Growth of plants. The bean plants (*Phaseolus vulgaris,* cv. Lody) were grown in a greenhouse in pots on perlite and S Hoagland nutrient solution was added in the trays. The treatment was for 7 days with 100 mM NaCl and 100 mM Na_2SO_4, starting at the appearance of the first trifoliate leaf unfolded. The volume of the nutrient solution was kept constant and salt concentrations as well as pH were controlled every second day.

Assays of antioxidant enzyme. The extract preparation was performed according to published procedures (Gцtz and Schrцder, 2005). Extracts were stored at -80°C until used**.** Spectrophotometric assays were employed to determine **CAT** (EC 1.11.1.6) according to Verma and Dubey, 2003; **APX** (EC 1. 11. 1. 11) according to Vanacker et al., 1998; **MDHAR** (EC 1. 6. 5. 4) and **DHAR** (EC 1. 8. 5. 1) according to Foyer, 1993. Protein contents was estimated according to Bradford (1976).

Statistical analysis. Data presented are means ± standard deviation of six replicates. The data means were compared by the least significance differences test (L.S.D) using SPSS program.

RESULTS AND DISCUSSION

Oxidative stress is one consequence of salinity that may be responsible for much of the damage observed in the field and our experiments. We investigated the immediate enzymatic responses towards salinity-induced oxidative stress in different organs of bean plants.

APX is the most important peroxidase in H_2O_2 detoxification in aerobic cells, catalysing the reduction of H_2O_2 to water using the reducing power of ascorbate (Jebara, 2005). In plants CAT has been identified to be predominant in the detoxification of H_2O_2 (Noctor, 2002). DHAR and MDHAR are involved in enzymatic regeneration of ascorbate. Therefore, it may be supposed that CAT and APX, both responsible for detoxification of H_2O_2, are probably equally important in the detoxification in plant organs (Yasar, 2008). These enzymes were also reported to be important in salt tolerance in cotton (Meloni, 2003), and maize genotypes (Neto, 2006).

The coordination among enzymatic activities, antioxidant substrate flux, and gene expression in roots might be different from that of leaves (Cavalcanti, 2007), even though these two organs share almost the same enzymatic machinery.

In our study, CAT activity was found to be much higher in roots than in leaves of beans under salt stress. The increase in enzyme activities was more pronounced after NaCL application. Some reports have demonstrated that leaf CAT is very sensitive to salt stress (Cavalcanti, 2007). Our results are in agreement with these of Eyidogan (2007) but different from Yasar (2008).

In salt treated plants (NaCl and Na_2SO_2) ascorbate connected enzymes (APX, MDHAR and DHAR) demonstrated enhanced activity in leaves and at the same time the activity were depressed in roots. There are published reports that support the results (Jebara, 2005) but also papers with opposite data (Chaparzadeh, 2004).

In this context, our results are showing a salt-induced root CAT activity, contrasting with the observed response of leaves, where the CAT activity was very sensitive to salt exposure and inhibited. Considering that salinity did not affect the CAT activity in roots of beans, our results suggest a less active ascorbate–glutathione cycle in roots.

Таблица 1. Ефект на засоляването върху активността на някои ензими при фасул. Активността е измерена в мkat/mg протеин. Представени са средните стойности от шест измервания

Table 1. Effect of salinity on the enzyme activities in bean plants. Activity is expressed in мkat/mg protein. Each value represents of six measurements and SD determined

Параметри/ Parameters	CAT	APX	MDHAR	DHAR
Листа /Leaves				
Контрола /Control	670,95±6,95 [a]	0,61±0,10 [a]	0,49±0,05 [a]	1,64±0,25 [a]
NaCl	519,31±57,26 [b]	1,24±0,29 [b]	0,56±0,10 [a]	4,18±0,38 [c]
Na_2SO_4	148,16±72,05 [c]	0,84±0,18 [a]	0,96±0,39 [b]	2,91±0,78 [b]
Корени/ Roots				
Контрола/ Control	76,04±17,84 [a]	0,94±0,20 [a]	0,71±0,14 [a]	1,80±0,57 [a]
NaCl	196,58±35,63 [b]	0,75±0,20 [b]	0,40±0,13 [c]	0,64±0,49 [b]
Na_2SO_4	189,69±45,98 [b]	0,23±0,06 [c]	0,54±0,17 [b]	0,42±0,24 [c]

Within the same column values followed by the same letter (a, b or c) are not different for P< 0.05.

Our experiments further suggest that the induction of APX in the leaves of bean plants subjected to salt stress may be mediated by the overproduction of H_2O_2 under conditions of catalase deactivation. On the other hand, the low leaf CAT activity suggests that the enzyme suffered irreversible damage to its structure and/or that very low rates of de novo synthesis occurred.

During salinity treatment, MDHAR and DHAR have shown different activities against the oxidative stress as compared to the control, but whereas DHAR reacts similarly to the APX activities, MDHAR remains almost constant and is induced only at low rates.

The results obtained in the present work clearly demonstrate that the application of both salts to the root medium resulted in oxidative responses in bean plants. The indicators of this physiological state were the modified activity of antioxidant enzymes. The observed difference in the enzyme activity shows salts and organs specific reaction in the bean plants.

In conclusion, bean roots and leaves present distinct mechanisms of response to salinity stress.

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