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Effect of a short period of phosphate deprivation on anti-oxidative enzymatic activities in bean plants.

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Introduction

Excess or deficiency of nutritional elements are among the abiotic conditions inducing an increase of active oxygen species (AOS) formation and oxidative stress in plant tissues (Cakmak 1994). Phosphate deficiency in the growth medium and severe decline in inorganic phosphate concentration in bean plant tissues results in an increased AOS production (Malusa' et al. 2002). Increase of AOS production under phosphate deficiency conditions induces a rise in the activity of some scavenging enzymes (Juszczuk et al. 2001). The aim of the study was to evaluate the effect of a transient phosphate deprivation on some of the principal anti-oxidative enzymatic activities in roots and leaves of bean plants.

Materials and Methods

Plant material

Seeds of *Phaseolus vulgaris* L. (cv Bianco di Bagnasco) were germinated in the dark for four days and than the seedlings were transferred to 4 l continuously aerated containers (20 plants in each container) filled with a phosphate-sufficient (+ P) Knop nutrient solution containing 3 mM $Ca(NO_3)_2$, 1.5 mM KNO_3, 1.25 mM $MgSO_4$, 1 mM KH_2PO_4 and 0.04 mM Fe-EDTA. After 6 days, (when plants were 10 day-old), part of the plants were transferred to a phosphate-deficient (-P) Knop medium, in which KCl replaced KH_2PO_4. Plants were grown in controlled conditions under 16/8 h (day/night) photoperiod, with a photon flux density of 400 μmol m^{-2} s^{-1}, 25/20 °C (day/night) temperatures and 75-85% relative humidity. Roots and shoots from both treatments were harvested and immediately used for analyses at 10, 14 and 18 days of growth.

Inorganic phosphate (Pi) determination

Plant tissues were extracted with 10% (v/v) trichloroacetic acid at 4°C and inorganic P content was measured according to Fiske e Subbarow (1925).

Enzyme activities determinations

Catalase (CAT) extraction was performed with a potassium phosphate buffer 50 mM (pH 7,8), containing Na_2EDTA 0,1 mM, PMSF 1 mM, and 1% polyvinylpolypyrrolidone (PVPP) (p/v). Catalase activity was measured according to the method of Chance and Maehly (1955).

Ascorbate peroxidase (APX) was extracted using a potassium phosphate buffer 50 mM (pH 7,0) containing 1 mM ascorbic acid to avoid the inactivation of the enzyme during the extraction and the activity was measured according to Wang et al. (2004).

Glutathione reductase (GR) was extracted with a potassium phosphate buffer 1 mM (pH 7,5), containing Na_2EDTA 0,4 mM and isoascorbic acid 9,94 mM. The activity was determined according to Gillham and Dodge (1986).

Dehydroascorbate reductase (DHAR) extraction was performed using a buffer solution made with Tris-HCl 50 mM (pH 7,4), NaCl 100 mM, EDTA 2 mM, $MgCl_2$ 1 mM and the enzyme activity was determined according to Chen and Gallie (2006).

Statistics

The results were statistically evaluated by t-test analysis separately for each sampling date.

Results

Plant growth and inorganic phosphate content

Initial symptoms of phosphate deficiency appeared in plants after 8 days of culture on the phosphate-deficient medium. The removal of phosphate from the growth medium caused a decrease of inorganic phosphate (Pi) concentration in both leaf and root tissues. Pi concentration in +P plants was on average, for the experimental period, 17.5 and 30.3 mg g^{-1} FW in shoots and roots, respectively. Plants deprived of P for 8 days contained 6.3 and 8.0 mg g^{-1} FW in leaves and roots, respectively; the content of 18-days-old bean plants was thus about ¼ that of the control.

Enzymatic activities

Catalase activity in leaves decreased during the growing period in both P-sufficient and P-deprived plants, while it increased in root tissues (Tab. 1). P deprivation provoked a significant decrease of CAT activity in leaves of 14-day-old plants, but no differences were measured after prolonged P deprivation. The enzyme activity in roots was not affected by the withdrawal of P from the culture medium (Tab. 1).

Tab.1: Specific activity of catalase (U*mg prot-1) in leaves and roots of bean plants (*Phaseolus vulgaris* L.) grown with complete nutrient medium (+P) and in a medium deprived of phosphorus (-P). Data represent the mean of three independent experiments. Asterisk shows significance for $p \leq 0,05$.

	Leaves		Roots	
	+P	-P	+P	-P
10-days	0.549±0.06	0.549±0.06	0.046±0.01	0.046±0.01
14-days	0.318±0.03	0.172±0.01*	0.174±0.02	0.221±0.02
18-days	0.293±0.01	0.301±0.01	0.158±0.02	0.185±0.02

The activity of ascorbate peroxidase in roots was generally higher (two to four-fold) than in leaves (Tab. 2). The deprivation of P from the growing medium provoked an increase in APX activity in leaves after 4 days of growth. No differences were detected in roots of +P and –P plants (Tab. 2).

Tab.2: Specific activity of ascorbate peroxidase (U*mg prot-1) in leaves and roots of bean plants (*Phaseolus vulgaris* L.) grown with complete nutrient medium (+P) and in a medium deprived of phosphorus (-P). Data represent the mean of three independent experiments. Asterisk shows significance for $p \leq 0,05$.

	Leaves		Roots	
	+P	-P	+P	-P
10-days	0.72±0.03	0.72±0.03	3.09±0.19	3.09±0.19
14-days	0.57±0.01	0.85±0.05*	2.14±0.12	1.95±0.15
18-days	0.48±0.02	0.54±0.04	2.84±0.21	3.35±0.08

Glutathione reductase activity decreased in leaves of 14 day-old P-deprived plants significantly in comparison to +P plants (Tab. 3). However, after 18 days of growth, the enzymatic activity

was similar in both –P and +P plants. The deprivation of P provoked a significant decrease of GR activity in roots of both 14 day-old and 18 day-old plants.

Tab.3: Specific activity of glutathione reductase (U*mg prot-1) in leaves and roots of bean plants (*Phaseolus vulgaris* L.) grown with complete nutrient medium (+P) and in a medium deprived of phosphorus (-P). Data represent the mean of three independent experiments. Asterisk shows significance for $p \leq 0,05$.

	Leaves		Roots	
	+P	-P	+P	-P
10-days	0.207±0.001	0.207±0.001	0.208±0.02	0.208±0.02
14-days	0.463±0.12	0.090±0.01*	0.301±0.06	0.111±0.02*
18-days	0.111±0.01	0.108±0.01	0.308±0.02	0.185±0.01*

The deprivation of phosphate from the growing medium induced an increase in the activity of dehydroascorbate reductase in both leaves and roots, either after 4 and 8 days of P withdrawal (Tab. 4).

Tab.4: Specific activity of dehydroascorbate reductase (U*mg prot-1) in leaves and roots of bean plants (*Phaseolus vulgaris* L.) grown with complete nutrient medium (+P) and in a medium deprived of phosphorus (-P). Data represent the mean of three independent experiments. Asterisk shows significance for $p \leq 0,05$.

	Leaves		Roots	
	+P	-P	+P	-P
10-days	0.175±0.01	0.175±0.01	0.153±0.01	0.153±0.01
14-days	0.030±0.002	0.038±0.003*	0.088±0.001	0.102±0.01*
18-days	0.032±0.002	0.049±0.003*	0.054±0.004	0.069±0.001*

Discussion and Conclusions

The withdrawal of P from the growing medium induced a slow decrease of Pi from the plant tissues, but only to an amount of about 25% that of P-sufficient plants. The appearance of dramatic P-deficiency visual symptoms in beans was observed when the Pi content was about 10 times lower than in control (Malusa' et al. 2002). Therefore, even though the plants after deprivation were demonstrating a reduced growth it is possible that the transient lack of P was not sufficient to cause a severe P deficiency.

The reduced availability of Pi in the plant tissues affected the activity of anti-oxidative enzymes mainly after the first days of deprivation, particularly in leaves. It could be suggested that during this period the plant is adjusting its metabolism to cope with the nutritional stress. This is in accordance with the evidence that the activities in root tissues of some scavenging enzymes, such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalases, were found to be only slightly affected by phosphate deficiency (Juszczuk et al. 2001). Our results could thus indicate that phosphate deprivation for a short period of time does not impose a strong oxidative stress to both photosynthetic and non-photosynthetic tissues.

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