Effect of sulphite ions on the proline and polyamine content in the bean *Phaseolus vulgaris*

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Abstract

The influence of sulphite (0.0125-0.2%) on changes in the levels of free proline, protein bound proline and hydroxyproline and of free polyamines in the roots and leaves of two cultivars of beans (*Phaseolus vulgaris*) differing in sensitivity to these ions was investigated. It was found that both cultivars reacted similarly, the response of the seedlings of the more sensitive cultivar occurring at lower concentrations of sulphite. The observed changes in the content of imino acids and polyamines are discussed.

Key words: proline, hydroxyproline, polyamines, environmental pollution, sulphite, Phaseolus vulgaris

INTRODUCTION

In plants subjected to the action of stress factors there occurs an increase in the level of free proline. This phenomenon occurs under the influence of drought (Barnett and Naylor 1966), low temperature (Teschke 1979), salinity (Storey and Wyn 1975) and gaseous air pollution (Karolewski 1985). So far, however, the dose: response relation between the stress factor and the level of accumulated proline as well as the sensitivity of the plant to its action has been little studied. Results of some studies indicate that the level of accumulation of free proline under the influence of SO₂ is the greater the more tolerant the plants are (Erickson and Dashek 1982) and conversely data indicating that more sensitive plants accumulate more proline (Karolewski 1984a, b). Proline is generally believed to be an imino acid performing several

protective functions in plants under the influence of stress factors (Britikov 1975).

A different group of compounds, which are believed to have a protective function in the reaction of plants to the action of stress factors, are the polyamines (Naik and Srivastava 1978, Altman 1982). Changes in their content have been observed also by Pierre and Queiroz (1981) in plants exposed to SO_2 .

The compounds discussed above probably play an important role in the tolerance of plants to the action of SO_2 (Spedding et al. 1980). It is the purpose of the present study to compare the responses regarding proline and polyamines of two cultivars of beans of different sensitivity to the action of sulphite.

MATERIALS AND METHODS

PLANT MATERIAL

For the experiments used seedlings of two bean (*Phaseolus vulgaris*) cultivars commonly used in Poland, cv. Fana — a dwarf variety of pod bean, with green pods, obtained by crossing 'Favorit Różowa' $\times$ 'Imuna' and cv. Słowianka — a dwarf variety cultivated for dry seeds.

Three-day-old germinating seeds which imbibed water at a temperature of 25°C in darkness have been placed on plates with perforations positioned over 1-liter containers with distilled water. The plants (3 per dish) grew in a hydroponic culture, in a climatized chamber with 12 hour ($9^{\circ\circ}$ - $21^{\circ\circ}$) artificial illumination (about 10 000 lux) from luminescent lamps at a temperature of 25°C and a relative air humidity of 70%. After one day water was replaced with a 1/4 nutrient medium of the normal concentration and after a further 3 days by a 1/2 medium of the norm with following full composition:

macroelements: NH_4NO_3	3.0 mM	microelements: H_3BO_3	48.5 μM
K_2SO_4	0.5 mM	MnSO_4	2.1 μM
NaH_2PO_4	0.5 mM	ZnSO_4	4.1 μM
CaCl_2	2.0 mM	CuSO_4	0.8 μM
MgSO_4	1.0 mM	NaMoO_4	0.9 μM
		$\text{FeCl}_3(-\text{EDTA})$	0.6 μM

DOSING OF SULPHITE

When the first pair of leaves was fully grown (5 days after placing of the germinating seeds on the dishes) the medium was exchanged for distilled water and gradual dosing of sulphite was begun. The sulphite was supplied in the form of a previously prepared concentrated solution of $\text{NaHSO}_3 + \text{Na}_2\text{SO}_3$ at a 4:6 mole ratio, so as to yield pH 7. The solutions in the dishes were

exchanged daily increasing the concentration of sulphite over 7 days so as to expose the plants to maximal concentrations of 0.0125, 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2%. After attaining each of these levels three dishes were left at this level till the end of the experiment. Each day twice for 0.5 h the solutions were aerated. Simultaneously in analogous conditions control plants were cultivated (without sulphite), exchanging daily the distilled water in the dishes.

After that time the dosing of sulphite was stopped, the roots were first rinsed in tap water and then several times in distilled water.

ANALYTIC METHODS

In each of 3 plants (replicates) representing 1 experimental variant the fresh mass of leaves and roots was determined and then the plant material was subjected to lyophilisation at -40°C . The difference in mass before and after lyophilisation was used as measure of the water content expressed as percentage of the control.

Free proline has been determined by the colorimetric method using ninhydrine according to Bates et al. (1973). Absorption of the solutions was measured using a Spekol 11 (GDR) spectrophotometer. The content of the imino acid was expressed in $\mu\text{M g}^{-1}$ of lyophilized dry weight of roots or leaves. The content of this imino acid was also measured in the solution in which the roots were immersed. For this purpose daily throughout the period of plant exposition to sulphite 10 ml samples of the solutions were taken from the dishes including the control ones. The level of bound imino acids was determined colorimetrically, proline using ninhydrine after Bergman and Loxley (1970) and hydroxyproline using p-dimethylaminobenzaldehyde after Stegemann and Stalder (1967). The content of the bound imino acids is expressed in $\mu\text{M g}^{-1}$ of dry weight (acetone powder) of roots and leaves.

Free polyamines have been determined after prior homogenisation of lyophilized material in 5% solution of perchloric acid, ultracentrifugation at $48000 \times g$ for 20 min, reaction with 1-dimethyl-amino-5-naphtalenesulfonyl chloride and thin layer chromatography on silica gel plates L 5/40 μ (Czechoslovakia) developed with chloroform-triethylamine 25:2 (v/v) and extraction with ethyl acetate. Measurement of fluorescence intensity has been made with a fluorescence spectrophotometer MPF-4 Hitachi (Japan) at an excitation wave length of 350 nm and an emission wave length of 495 nm, based on the method described by Flores and Galston (1982). The content of polyamines has been expressed in $\mu\text{M g}^{-1}$ of root or leaves dry weight.

For each variant of the experiment the mean out of 3 replicates (plants) and the standard deviation were calculated. The latter is shown on the figures by vertical lines.

RESULTS

The two bean cultivars used in the study were characterized by various sensitivity of the action of sulphite. This was manifest in the earlier drop in leaf fresh weight (Fig. 1B) and loss of water content (Fig. 1D) in cultivar Fana compared to cv. Słowianka, together with the use of increasingly larger concentrations of sulphite. The corresponding changes in the roots were smaller (Fig. 1A, C).

Increase in the content of free proline in both the studied cultivars occurred in roots at lower sulphite concentrations than in leaves (Fig. 1E, F). An increase in the content of this imino acid, compared to the control in the leaves of the more sensitive cultivar Fana was observed already after using a sulphite concentration of 0.05% and for the less sensitive cv. Słowianka only after a twice as high concentration of 0.1% (Fig. 1F). In the roots an increase in the content of free proline in the sensitive cultivar Fana occurred already at the lowest of the concentrations used in this study, 0.0125%, while in the tolerant cv. Słowianka at a twice higher concentration of 0.025% (Fig. 1E). Also an increase of the concentration of this imino acid in the solution in which the roots of the seedlings were immersed occurred at lower concentrations of sulphite in the more sensitive cultivar (Fig. 2).

The use of successively higher concentrations of sulphite solutions caused initially an increase and then a decline in the content of bound proline in the leaves of both cultivars (Fig. 3B) and in the roots of the tolerant cultivar (Fig. 3A). In the case of the more sensitive cultivar the level of this imino acid in the roots declined compared to the control already at the lowest of the concentrations used, 0.0125%.

In the tolerant cultivar the action of sulphite solutions in successively higher concentrations caused an initial increase and then a lowering of the content of protein bound hydroxyproline both in the roots and in the leaves (Fig. 3C, D). In the sensitive variety the content of this imino acid in the roots and leaves successively declined with an increase in the concentration of sulphite throughout the range used (Fig. 3C, D).

The general course of the changes in the content of the diamine putrescine and of the polyamines spermidine + spermine and cadaverine was characterized initially by an increase and then a decline, both in the roots and in the leaves together with the gradual increase in the concentration of sulphite (Fig. 4). Only in the case of the sensitive cultivar did the action of sulphite cause a lowering of the content of putrescine in the roots at each of the concentrations used (Fig. 4A). Changes in the level of polyamines in the roots were more intensive than in the leaves (Figs. 4C, D and E, F), occurring at lower concentrations of sulphite in the more sensitive cultivar and at higher concentrations in the more tolerant ones.

Fig. 1. Effect of sulphite ion concentration on the fresh weight (A, B), water content (C, D) and free proline (E, F) in the roots (A, C, E) and leaves (B, D, F) of two bean cultivars, a tolerant one — *Phaseolus vulgaris* Słowianka (---) and a sensitive one — Fana (—)

Fig. 2. Effect of sulphite ion concentration on the content of free proline in the exudates from roots of two bean cultivars, a tolerant one – *Phaseolus vulgaris* Słowianka (---) and a sensitive one – Fana (—)

Fig. 3. Effect of sulphite ion concentration on the content of bound imino acids, proline (A, B) and hydroxyproline (C, D) in roots (A, C) and leaves (B, D) of two bean cultivars, a tolerant one – *Phaseolus vulgaris* Słowianka (---) and a sensitive one – Fana (—)

Fig. 4. Effect of sulphite ion concentration on the content of free di- and polyamines putrescine (A, B), spermidine + spermine (C, D) and cadaverine (E, F) in the roots (A, C, E) and leaves (B, D, F) of two bean cultivars, a tolerant one — *Phaseolus vulgaris* Słowianka (---) and a sensitive one — Fana (—), depending on concentration of these ions

DISCUSSION

Within a range of relatively low concentrations, depending on the degree of sensitivity of the studied bean cultivar, sulphite did not affect the content of free proline in leaves and roots of seedlings. Within this range normal utilization of this imino acid may be assumed. This is indicated by results obtained earlier in the studies on the influence of SO_2 on poplar cuttings (Karolewski 1985). However, the movement of proline to the solution in which the roots were immersed has to be considered at this low concentration.

The observed increase in the content of free proline in exudates parallel with an increase in the sulphite concentration is probably the effect of disturbances in the functioning of the cytoplasmic membrans. This would simultaneously indicate that differences in the sensitivity of the studied cultivars of beans depend among others on a varying degree of injury to the normal functioning of these membranes.

Sulphite caused earlier an increase in the content of free proline (in lower concentrations) in the roots than in the leaves, and correspondingly so in the more sensitive cv. Fana than in the more tolerant Słowianka. The critical concentration of free proline content accumulation could be used as a parameter of sensitivity of plants to the action of sulphite ions.

One of the causes of the intensive accumulation of free proline under the influence of sulphite may be the inhibition of incorporation of this imino acid into proteins. This is confirmed by comparison with corresponding changes in levels of free and protein bound proline in roots and leaves of the two bean cultivars. A similar phenomenon has also been observed by Buhl and Stewart (1983) in plants under the influence of salt.

The observed increase in the content of bound proline in roots and leaves of tolerant bean seedlings at relatively low concentrations of sulphite was accompanied by an increase in the content of bound hydroxyproline. This is an unfavourable phenomenon suggesting an ageing process in the studied plant tissues (Chrispeels et al. 1974). On the other hand, application of sulphite at a higher concentration to the more tolerant seedlings and at all concentrations to the sensitive ones caused a lowering of protein bound hydroxyproline. This is probably caused by proteolysis as was suggested by Malhotra and Sarkar (1979) in the case of plants subjected to the action of sulphur dioxide.

The course of changes in the levels of putrescine and polyamines indicates an increase in the levels of their concentrations under the influence of relatively low concentrations of sulphite from among those used in the study, which would suggest that these compounds possibly play a protective role. Such a function of polyamines, based on the stabilisation of the intracellular pH level is suggested by Pierre and Queiroz (1981), also in seedlings of beans, however subjected to the actions of SO_2 at subnecrotic concentrations of 0.1 ppm. The use of sulphite at correspondingly higher concentrations, depending on the studied cultivar, caused a decline in the level of putrescine and polyamines. It occurred earlier (at lower concentrations of sulphite) in the more sensitive cultivar. The decline in polyamine content is a symptom of unfavourable processes as it is known that the ageing of plants is correlated with a lower level of endogenous polyamines (Kaur-Sawhney et al. 1982).

The results presented in this study indicate that there exists an identical pattern of response of bean seedlings of both cultivars to the action of sulphite ions, except that the more sensitive cultivar reacted at a relatively lower

concentration of sulphite than the more tolerant one. The observed changes in the levels of free proline, of protein bound proline, of hydroxyproline and of di- and polyamines indicate that possibly a defence mechanism is operating with the participation of these compounds, but only when the plants were subjected to sulphite at low concentrations.

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*Wpływ jonów siarczynowych na zawartość proliny i poliamin
u fasoli (*Phaseolus vulgaris*)*

Streszczenie

Badano wpływ siarczynu (0,0125-0,2%) na zmiany zawartości wolnej proliny, proliny i hydroksyproliny związanych z białkiem oraz wolnych poliamin, w korzeniach i liściach siewek dwóch różnych, pod względem wrażliwości na te jony, odmian fasoli (*Phaseolus vulgaris*). Stwierdzono, że obydwie odmiany reagowały podobnie, przy czym reakcja siewek odmiany bardziej wrażliwej następowała przy mniejszych stężeniach siarczynu. Obserwowane zmiany zawartości iminokwasów i poliamin wskazują na istnienie u badanych roślin procesów obronnych przeciw toksycznemu działaniu jonów siarczynowych, ale tylko w stosunkowo niewielkich stężeniach. Siarczyny w większych stężeniach, zależnie od stopnia wrażliwości badanej odmiany, powodowały już zmiany poziomu badanych związków wskazujące na niekorzystne procesy, charakterystyczne dla starzenia się tkanek roślin.