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BRIEF COMMUNICATION

Effects of arsenic on phosphorus content in different organs and chlorophyll fluorescence in primary leaves of soybean

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Abstract

The effect of arsenic (32 - 96 μM) on the phosphorus content and Chl fluorescence was studied in soybean (*Glycine max* Merril) grown in the nutrient solution with and without phosphorus. The increased concentration of As led to the decrease in P content in plant organs. Parameters of Chl fluorescence of soybean leaves in the presence of these As concentrations did not show significant changes.

Additional key words: *Glycine max*, photosystem 2, quantum yield.

Pollution of the soil and atmosphere by arsenic (As) is conditioned by the industry development. Copper smelters and thermal plants are great pollutants of the atmosphere. According to Smirnov and Muravin (1977) natural As content in the soil is 5 mg(As) kg^{-1}(soil). As, an analogue to phosphorus, is absorbed from the soil by P transporters. As inhibits P uptake in barley (Asher and Reay 1979) and *Arabidopsis* (Dunlop *et al*. 1997). In many of plant species, arsenates and arsenites have an affinity for thiols, such as glutathione. Furthermore, phytochelatins are formed as a response to As (Schmöger *et al*. 2000). Hartley-Whitaker *et al*. (2000a, 2000b) confirmed that As-tolerant *Holcus lanatus* L. had higher phytochelatins concentrations than As-intolerant species. Hence, similar to heavy metals, As also mobilises the so-called nonenzymic antioxidants, such as glutathione, ascorbates, and phytochelatins. In order to investigate As effects on the uptake and distribution of phosphorus (P) in plant organs and chlorophyll (Chl) fluorescence in the soybean leaves, different As concentrations were used: 2.4, 4.8, 6.0, and 7.2 g(As) m^{-3}, corresponding to 32, 64, 80 and 96 μM As.

After the 5-d germination in the dark, plants of soybean (*Glycine max* Merril. cv. ZP S015) were transferred into pots with the nutrient medium of pH 7.0 (Hoagland and Arnon 1950). Plants were grown in growth chambers at a 12-h photoperiod, irradiance of 300 μmol m^{-2} s^{-1} (*Sylvania* cool white lamp *P9GT12-CEW-VHO*, Danvers, USA) and day/night temperature of 24/20 °C. Plants grown on the complete medium were the control ones. The P content in the other three variants was half or double of that in the control or zero. Plants grown with and without P were exposed to 32, 64, 80 and 96 μM Na_2HAsO_4 for 5 d.

Roots, stems, cotyledons, and leaves was finally dried to the constant mass and homogenised by grinding. The P amount was determined by the official method of Association of Official Agricultural Chemists (A.O.A.C.) (Horwitz 1960). Chl fluorescence of soybean primary leaves was measured by the *PAM 101/103* fluorimeter (*Walz*, Effeltrich, Germany). Parameters of Chl fluorescence were defined after Maxwell and Johnson (2000). The analysis of variance (*ANOVA*) for all variables was carried out by the *MStatic* programme.

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Abbreviation: Chl - chlorophyll; F_m - maximum fluorescence; F_0 - initial fluorescence; F_v - variable fluorescence; NPQ - non-photochemical quenching, PS2 - photosystem 2; q_P - photochemical quenching.
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The applied concentrations of As, especially from 64 to 96 μM, decreased the P content in all plant organs of soybean with different P amounts in the nutrient solution (Table 1). Higher molar P/As ratios reduced As-toxicity in all plant organs of soybean. The P content in leaves and cotyledons of P-deficient soybean plants significantly decreased in the presence of As, indicating the P stored in these organs was used for building plant biomass. A statistically highly significant P decrease in the presence of As in plants with 0.05, 0.1 and 0.2 M KH_2PO_4 in the nutrient solution in relation to P-deficient plants pointed out that the As uptake was similar to the P uptake. In the studies with As and Al (Milivojević *et al*. 2000) the deficiency and decrease of P content in the medium led to the increase in toxicity of these elements. Investigations of the effect of As on some physiological parameters of maize in the early growth stage showed that applied As decreased the growth, leaf and biomass accumulation, induced lipid peroxidation and increased peroxidase activity. It also decreased the Chl, carotenoid, and protein contents and F_v/F_m ratio indicated lower photosynthetic efficiency (Stoeva *et al*. 2003/4). The study of growth parameters, ATP and chlorophyll contents in *Pisum sativum* seedings after 9-d exposure to sodium arsenate showed that the low concentration of arsenate elevated the ATP content per fresh matter of the cotyledons and shoot, but the higher concentrations were without any significant effect (Paivoke 2003). The observed negative correlation between growth and ATP concentration may imply that the arsenate impacted indirectly via ATP on the growth of seedlings.

The measured parameters of Chl fluorescence (Table 2) did not vary significantly under As treatments. Changes of maximum quantum yield of photosystem 2 (Φ) and photochemical quenching of fluorescence (q_P) under the highest As concentrations point out to the changes of the redox state of the plastoquinone pool. Non-photochemical quenching (NPQ) of fluorescence (related to photoprotective processes in antennae of chloroplast thylakoids) was not significantly modified by As application.

Since As in higher concentrations in the nutrient solution significantly reduced the P accumulation in root, leaf and stem, in certain cases even below 0.3 %, it can be spoken about the deficiency of the phosphorus. The measured parameters of Chl fluorescence did not significantly change in primary leaves of soybean, pointing out that there were no effects of As on the photosynthetic electron transport. Abadia *et al*. (1987) found out that a low phosphorus content in the leaf had

Table 1. Amount of phosphorus [% (d.m.)] in organs of 11-d- old soybean plants grown under different As concentrations (0 - 96 μM) in phosphate-sufficient (+P), half of control phosphate (1/2P), double of control (2P) and phosphate-deficient (-P) nutrient solution. Values are means of three determinations from 2 - 3 plants.

As [μM]	Leaf				Stem				Cotyledon				Root			
	+P	1/2P	2P	-P	+P	1/2P	2P	-P	+P	1/2P	2P	-P	+P	1/2P	2P	-P
0	1.50	1.39	2.05	0.82	0.95	0.72	1.85	0.72	0.97	0.86	1.13	0.83	1.30	1.29	1.95	0.81
32	0.84	0.88	1.57	0.54	0.94	0.77	1.00	0.48	0.75	0.72	1.00	0.65	1.25	0.88	0.92	0.83
64	0.29	0.83	0.87	0.50	0.56	0.72	0.78	0.46	0.42	0.43	0.31	0.45	0.65	0.38	0.91	0.50
96	0.29	0.46	0.50	0.56	0.15	0.10	0.46	0.54	0.31	0.59	0.46	0.33	0.28	0.29	0.46	0.52
$LSD_{0.05}$	0.31				0.29				0.44				0.32			
$LSD_{0.01}$	0.43				0.41				0.62				0.44			

Table 2. Effects of arsenic in phosphate-sufficient nutrient medium on parameters of induction and quenching of fluorescence. F_v/F_m or F_v/F_o (maximum quantum yield of PS2 in the dark); F_v'/F_m' (maximum quantum yield of PS2 in the light); Φ (maximum quantum yield of PS2 at the equilibrium photosynthesis); q_P (photochemical quenching of Chl fluorescence); NPQ (non-photochemical quenching of Chl fluorescence) (Maxwell and Johnson 2000).

As [μM]	Parameters of fluorescence					
	F_v/F_m	F_v'/F_m'	F_v/F_o	Φ	q_P	NPQ
0	0.804	0.524	4.111	0.320	0.560	4.111
32	0.796	0.426	3.897	0.190	0.435	3.897
80	0.797	0.442	3.927	0.230	0.515	3.927
96	0.806	0.584	6.296	0.510	0.880	6.296
$LSD_{0.05}$	0.060	0.130	2.440	0.230	0.290	2.440
$LSD_{0.01}$	0.100	0.190	3.700	0.350	0.440	3.700

only small effects on the content of pigment-protein complexes of the thylakoids and electron transport in the light reactions of photosynthesis, while Rao and Terry (1989) detected that the P deficiency in soybean reduced the photosynthetic assimilation of CO_2. We assume that certain concentrations of As caused P-deficiency without affecting the photochemical reactions in soybean leaves during the trial. This is in accordance with the conclusions of Abadia *et al*. (1987) about a weak influence of low leaf P content on light reactions of photosynthesis. Our opinion is that the observed phenomenon indicates an early phase of As-induced P-deficiency in soybean.

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