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BRIEF COMMUNICATION

Effect of arsenic on some physiological parameters in bean plants

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Abstract

The objective of the study was to investigate the effect of different arsenic concentrations on some physiological parameters of bean (*Phaseolus vulgaris* L.) cultivars Plovdiv 10 and Prelom in the early growth phases. Seedlings, grown in sand with Hoagland-Arnon nutrient solution in a climatic box, were treated with 0, 2, 5 mg(As) dm^{-3} as Na_3AsO_4 (pH 5.5). After 5 d of As treatment, the changes in leaf gas-exchange, water potential, chlorophyll and protein contents, peroxidase activity and lipid peroxidation in roots were recorded. Physiological analysis showed a minor negative effect of arsenic at concentration 2 mg(As) dm^{-3}, but at the higher dosage of 5 mg(As) dm^{-3} growth, leaf gas-exchange, water potential, protein content and biomass accumulation were reduced in both cultivars. The peroxidase activity and lipid peroxidation increased considerably at 5 mg(As) dm^{-3}, which is a typical reaction of the plants to a presence of oxidative stress.

Additional key words: As stress, chlorophyll content, growth, leaf gas-exchange, lipid peroxidation, peroxidase, water relations.

In the last few years heavy metals have received considerable attention as a consequence of the increased environmental pollution from industrial, agricultural, energetic, and municipal sources (Adrino 1986). They function as stress factors causing physiological disorders in plants (Clijsters and Van Assche 1985, Moustakas *et al*. 1994). Arsenic is not a heavy metal, but it is related to them. In plants, arsenic is accumulated mainly in the root system, to a lesser degree in the aboveground organs, and causes physiological changes and damages (Marin *et al.* 1992, Wells and Gilmor 1997), and reduction of the crop productivity (Stepanok 1998). Arsenic inhibits the growth and fresh and dry biomass accumulation (Stoeva *et al*. 2003/4). Arsenic is not a redox metal. Nevertheless, there is significant evidence that exposure of plants to inorganic arsenic does result in the generation of ROS, which is connected with arsenic valance change, a process that readily occurs in plants (Flora 1999, Lynn *et al*. 1998). Arsenic caused a reduction of the photosynthesis rate (Miteva and Merakchiyska 2002, Stoeva and Bineva 2003, Stoeva *et al*. 2003/4). In our previous investigation with maize we found that the rate of CO_2-fixation in young plants treated with arsenic decreased by about 20 % and functional activity of PS2 was reduced significantly (Stoeva *et al*. 2003/4). Arsenic damaged the chloroplast membrane and disorganized the membrane structure (Miteva and Merakchiyska 2002). It has been demonstrated recently that catalase and glutathione-S-transferase in *Zea mays* were all stimulated upon exposure to arsenic (Mylona *et al*. 1998). The increase in lipid peroxidation, superoxide dismutase (SOD) activity (Hartley-Whitaker *et al.* 2001) and peroxidase (POD) activity (Miteva and Peycheva 1999) were correlated with increasing As stress. According to them, arsenic accumulated in the plant tissue stimulates peroxidase synthesis during the early phases of plant development, long before the visible changes take place. There is insufficient information on the response of bean cultivars to arsenic pollution. The objective of this study was to investigate the effect of different arsenic concentrations on some physiological parameters in young bean plants of two cultivars and to compare their tolerance.

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Abbreviations: E - transpiration rate; g_s - stomatal conductance; LP - lipid peroxidation; MDA - malondialdehyde; PAR - photosynthetically active radiation; P_N - net photosynthetic rate; POD - peroxidase; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase; TBA - thiobarbituric acid, Ψ - water potential.

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Bean plants (*Phaseolus vulgaris* L. cvs. Plovdiv 10 and Prelom) were grown as sandy cultures in a Hoagland-Arnon nutrient solution, enriched with microelements. They were grown in a climatic box under irradiance of 200 μmol(PAR) m^{-2} s^{-1}, 14-h photoperiod, day/night temperature of 24 ± 2/18 ± 2 ºC, and relative air humidity of about 70 %. Fifteen days after emergence the plants were treated with As in the form of Na_3AsO_4 in concentrations 0 (control), 2 and 5 mg dm^{-3} (pH 5.5, adjusted with HCl).

The fresh masses of the shoots and roots were measured after 5 d of As treatment. Plant material was rinsed in deionised water and blotted. The dry masses were measured by drying the shoots and roots at 75 °C to constant mass. The leaf area was measured with a leaf area meter *NEO-2 (Technical University*, Sofia, Bulgaria). The net photosynthesis rate (P_N), transpiration rate (E), and stomatal conductance (g_s) of the intact leaves were measured with a portable infrared gas analyser *LCA-4* (*Analytical Development Company Ltd,*. Hoddesdon, England), equipped with a *PLCB-4* chamber. The measurements were made under irradiance of 800 μmol (PAR) m^{-2} s^{-1}, temperature of 26 ± 2 °C, an external CO_2 concentration of 400 μmol mol^{-1}, and relative air humidity of 70 %. The relative water content (RWC) in leaves was determined according to Morgan (1986). The water potential (Ψ) in leaves was measured with pressure chamber (*ELE-International,* England). Chlorophyll (Chl) and carotenoids (Car) were extracted with 80 % acetone and the pigments were determined spectrophotometrically (*Specol 11, K. Zeiss,* Jena, Germany) at wavelengths 663 nm (Chl *a*), 645 nm (Chl *b*) and 470 nm (Car) and calculated according to Lichtenthaler and Wellburn (1983). For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test, which determines malondialdehyde (MDA) content, was applied (Heath and Packer 1968). The amount of MDA-TBA complex (red pigment) was measured by means of its specific absorbance at 532 nm. Non-specific absorbance at 600 nm was also subtracted (De Vos *et al*. 1989). The data were calculated using the coefficient of absorbance of 155 mM^{-1} cm^{-1}. Each plant extract was assayed twice. Peroxidase (POD; EC 1.11.1.7) activity was determined according to Herzog and Fahimi (1973). The roots were homogenized in 0.05 M Tris-glycine buffer (pH 8.3) containing 170 g dm^{-3} sucrose. The POD activity was expressed as ΔA_{470} g^{-1}(f.m.) min^{-1}. Protein content in the extracts was determined according to Lowry *at al*. (1951). The plant material was homogenized in a boron buffer (pH 8.7) in a refrigerated centrifuge at 5 000 *g* for 15 min. The solution absorbance was determined in the presence of Folin reagent at wavelength of 750 nm. The protein amount was determined using a standard curve obtained with albumin. Three independent experiments, each with 5 repetitions per treatment, were conducted. There were 5 plants in each pot. The results showed similar tendencies therefore, data from one representative experiment were given. The significance of the differences between control and each treatment was analyzed by Student's *t*-test.

The term "toxic concentration" is used in the literature for a heavy metal concentration that significantly inhibits the metabolic activity without inducing plant death (Clijsters and Van Assche 1985). The As treatment resulted in symptoms of phytotoxicity and in a considerable inhibition of the initial growth of young bean plants. There was chlorosis and necrosis of leaf tips, as well as necrosis and reduction of the number of ramifications in root systems of treated plants.

In our experiments (Table 1) the accumulation of fresh biomass of As-treated (5 mg(As) dm^{-3}) plants was inhibited by 30 % (cv. Prelom) and 33 % (cv. Plovdiv 10). The changes in the dry biomass followed the same tendency. The dry/fresh mass ratio increased as well, more significantly at concentration 5 mg As (52 % cv. Plovdiv 10, and 19 % cv. Prelom).The growth of the shoot and the root were significantly reduced (33 and 30 % in cv. Plovdiv, and 24 and 28 % in cv. Prelom) in the case of As concentration of 5 mg dm^{-3}. Reduction of the leaf area was also more considerable in higher As concentration - 38 % below the control in cv. Plovdiv 10, and 32 % below the control in cv. Prelom. The results showed that the concentration of 2 mg As influenced all parameters less negatively.

Table 1. Effects of bean root immersion in arsenic (As) solutions of different concentrations (0, 2 and 5 mg dm^{-3}) on growth parameters. All parameters were measured 5 d after application. Means ± SE, n = 15, * - P < 0.1, ** - P < 0.01, *** - P < 0.001.

Parameters	cv. Plovdiv 10 control	2 mg(As) dm^{-3}	5 mg(As) dm^{-3}	cv. Prelom control	2 mg(As) dm^{-3}	5 mg(As) dm^{-3}
Shoot length [cm]	32.46 ± 0.76	26.18 ± 0.45*	21.45 ± 0.88***	26.15 ± 0.66	22.50 ± 0.55*	19.92 ± 1.22**
Root length [cm]	19.54 ± 0.98	17.35 ± 0.65*	13.60 ± 0.42**	18.20 ± 0.98	16.95 ± 0.92*	13.20 ± 0.88**
Fresh mass [g $plant^{-1}$]	10.54 ± 1.15	9.75 ± 0.57*	7.05 ± 0.58**	7.31 ± 0.65	6.85 ± 0.56*	5.11 ± 0.44**
Dry mass [g $plant^{-1}$]	1.40 ± 0.56	1.10 ± 0.95*	0.85 ± 0.65**	0.74 ± 0.05	0.73 ± 0.04	0.65 ± 0.05*
Fresh/dry mass ratio	0.10 ± 0.02	0.11 ± 0.03	0.12 ± 0.03**	0.10 ± 0.01	0.11 ± 0.04	0.12 ± 0.04***
Leaf area [cm^2]	186.88 ± 11.42	154.55 ± 9.62	116.15 ± 9.45***	124.53 ± 10.13	105.62 ± 9.65	84.15 ± 7.28***

Table 2. Effects of bean root immersion in arsenic (As) solutions of different concentrations (0, 2 and 5 mg dm^{-3}) on transpiration rate (E), stomatal conductance (g_s), relative water content (RWC), water potential (Ψ), net photosynthesis rate (P_N), P_N/E ratio, total chlorophyll (Chl) and carotenoids (Car) contents, lipid peroxidation (LP), peroxidase activity (POD) and soluble protein content (SP). All parameters were measured 5 d after application. Means ± SE, $n = 5$, * - $P <$ 0.1, ** - $P < 0.01$, *** - $P < 0.001$.

Parameters	cv. Plovdiv 10 control	2 mg(As) dm^{-3}	5 mg(As) dm^{-3}	cv. Prelom control	2 mg(As) dm^{-3}	5 mg(As) dm^{-3}
RWC [%]	96.60 ± 2.98	95.20 ± 1.85*	94.60 ± 1.88**	96.20 ± 1.98	95.95 ± 2.02*	94.80 ± 2.80**
E [mmol(H_2O) m^{-2} s^{-1}]	2.45 ± 0.15	2.28 ± 0.57**	1.95 ± 0.58** *	2.41 ± 0.53	2.35 ± 0.36*	1.95 ± 0.44*
g_s [mol m^{-2} s^{-1}]	0.06 ± 0.003	0.05 ± 0.002*	0.04 ± 0.002**	0.06 ± 0.00	0.04 ± 0.01	0.04 ± 0.01*
P_N [μmol(CO_2) m^{-2} s^{-1}]	8.64 ± 0.35	7.52 ± 0.45*	6.28 ± 0.82***	8.35 ± 0.71	7.15 ± 0.65**	6.11 ± 0.22***
Ψ [-MPa]	0.24 ± 0.02	0.38 ± 0.02*	0.51 ± 0.02***	0.25 ± 0.01	0.39 ± 0.01	0.52 ± 0.01***
P_N/E	3.52 ± 0.02	2.84 ± 0.25***	2.56 ± 0.31***	3.46 ± 0.40	2.37 ± 0.32*	2.51 ± 0.28***
Chl *a*+*b* [mg g^{-1}(d.m.)]	10.56 ± 1.15	9.06 ± 0.92*	8.25 ± 0.68**	10.80 ± 0.45	9.46 ± 0.42*	8.99 ± 0.49**
Car [mg g^{-1}(d.m.)]	3.45 ± 0.32	3.05 ± 0.35**	2.80 ± 0.99**	3.60 ± 0.26	3.20 ± 0.25**	3.05 ± 0.35**
Chl/Car	3.07 ± 0.42	2.92 ± 0.86	2.83 ± 0.30*	3.11 ± 0.32	2.99 ± 0.32	2.90 ± 0.42
LP [nmol(MDA) g^{-1}(f.m.]	11.35 ± 1.10	13.80 ± 1.25*	17.46 ± 1.31***	11.20 ± 0.98	14.20 ± 1.45*	16.62 ± 1.32***
POD [ΔA_{470} g^{-1}(f.m.) min^{-1}]	215.00 ± 18.0	258.00 ± 22.0**	370.00 ± 21.0***	205.00 ± 12.0	247.00 ± 16.0**	339.00 ± 20.0***
SP [mg g^{-1}(f.m.)]	10.54 ± 1.05	8.85 ± 0.94*	7.15 ± 0.46**	10.25 ± 1.11	9.16 ± 0.62*	7.86 ± 0.31**

Leaf water potential (Ψ) decreased in the As-treated (5 mg As dm^{-3}) plants in both cultivars (Table 2) while the relative water contents (RWC) decreased only slightly (2 - 3 %). The transpiration rate (E) of both cvs. decreased by about 7 % (2 mg dm^{-3}) and 21 % (5 mg dm^{-3}). The stomatal conductance (g_s) decreased by 36 % in cv. Prelom and 39 % in cv. Plovdiv 10 in case of higher concentration. The photosynthetic rate (P_N) decreased in the As treated plants: at a concentration of 5 mg dm^{-3}, P_N in cv. Plovdiv 10 was 28 % below the control and in cv. Prelom 25 % below the control. The P_N/E ratio was about 28 % below the control. Our results also indicate that in young bean plants the photosynthesis rate inhibition was smaller than that of the growth (Table 1) in two cultivars. This fact confirms the hypothesis of some authors about the different growth and photosynthesis sensitivity to metal phytotoxicity in the early phases of the plant development (Merakchijska and Yordanov 1983). The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthetic rate. It is believed that they are targets of the toxic As effect (Miteva and Merakchiyska 2002). According to them, the limiting step of the heavy metal effect on the plant photosynthesis is a result of the inhibition of chlorophyll (Chl) synthesis. There was a considerable decrease of Chl and carotenoid (Car) contents (22 and 19 % below the control at the 5 mg dm^{-3} in cv. Plovdiv 10 and 17 and 19 % below the control at same concentration in cv. Prelom (Table 2). It was established that Car content decreased to a lesser extent than Chl content.

An increase of malondialdehyde (MDA) accumulation as a result of As stress was observed in both cultivars. This increase was more significant at higher concentration of As (54 and 48 % in cvs Plovdiv 10 and Prelom, respectively; Table 2). In accordance with Cakmak *et al.* (1991), we presume that As facilitates lipid peroxidation by disorganizing the membrane structure. Enhanced lipid peroxidation, occurring in response to arsenic, indicate that arsenic toxicity resulted in the increased production of ROS, which, in turn, caused membrane damage. The induction of antioxidative enzymes, including peroxidase, is considered to play an important role in heavy metal stress (Mocquot *et al.* 1996). It is well documented that such protective enzymes are activated under stress, which stimulate production of oxygen free radicals (Elstner *et al*. 1988). The POD activity in the roots in young bean plants was increased more significant at 5 mg(As) dm^{-3} (72 % above the control in cv. Plovdiv 10 and 48 % in cv. Prelom). The data also indicated, that the POD activity in roots was in negative correlation with shoot growth and biomass reduction. This confirms the opinion of Van Assche *et al.* (1990) and our previous investigations (Stoeva *et al.* 2003). Since the peroxidase activity is related to ROS formation, it is evident that As, applied in a soluble form, induced ROS accumulation. The soluble protein amount in the beans roots decreased by 23 and 32 % at 5 mg(As) dm^{-3}, respectively, in cvs. Prelom and Plovdiv 10. According to Journet *et al.* (1986), the protein degradation is in fact an adaptation of the cells to the sugar deficiency.

On the basis of the results obtained the following conclusion can be drown: *1*) the treatment of the roots with 2 and 5 mg(As) dm^{-3} had a negative effect on the growth, leaf area, biomass accumulation, leaf gas-exchange, pigment content and water potential; *2*) these symptoms of toxicity together with some biochemical characteristics, such as the increased POD activity, lipid peroxidation, and the reduced soluble protein content

confirmed Lee's hypothesis (Lee *et al*. 1976) about the accelerated aging of the young bean plants subjected to metal stress; and *3*) the results indicated that cv. Prelom was more tolerant to As stress than cv. Plovdiv 10 probably due to more efficient defense mechanisms.

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