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OXIDATIVE CHANGES AND PHOTOSYNTHESIS IN OAT PLANTS GROWN IN AS-CONTAMINATED SOIL

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Summary. The effect of different arsenic concentrations on some physiological parameters in oats cultivar Hanza 152 was studied. Arsenic was applied as H_3AsO_4 at concentrations of 40, 80 and 160 mg (As) per kg soil. The plants were grown in pots with 5 kg soil/pot.
Physiological analysis showed a small negative effect of arsenic at concentration of 40 mg (As) per kg soil, but the higher dosages of 80 and 160 mg (As) per kg soil, generated stress in oats plant and as a consequence leaf gas-exchange was suppressed (14% and 25% for photosynthesis rate and 13% and 20% for transpiration intensity). The chlorophyll fluorescence ratio F_v/F_m decreased. The chlorophyll and protein content also decreased. The peroxidase activity and lipid peroxidation increased, considerably at 160 mg arsenic, per kg soil, which is a typical plant reaction to the oxidative stress.

Key words: arsenic, chlorophyll fluorescence, chlorophyll, leaf gas-exchange, lipid peroxidation, peroxidase activity

***Abbreviations*:** ADP – Adenosine diphosphate, As – arsenic, ATP – Adenosine triphosphate; E – transpiration rate; F_o – initial fluorescence; F_v – variable fluorescence; F_m – maximum fluorescence; g_s – stomatal conductance; LP – lipid peroxidation; MDA – Malondialdehyde; POD – peroxidase; PAR – Photosynthetic activity radiation; P_N – net photosynthetic rate; ROS- Reactive oxygen species; SOD – Superoxide dismutase; TBA – Thiobarbituric acid. FWC – full water capacity.

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Introduction

Heavy metal pollution of soils received considerable attention as a consequence of the increased environmental pollution from industrial and agricultural source. Arsenic (As) is a widespread natural element, which is not a bioorganic element to plants and animals. Arsenic is not a heavy metal, but it accompanies them. The increased soil content of arsenic is a result of the technogenic contamination.

Arsenic is accumulated mainly in the root system, to a lesser extent in the overgrown organs, inhibits the growth and fresh and dry biomass accumulation (Stoeva et al., 2003) and causes physiological disorders (Wells and Gilmor, 1997), as well as reduction of the crop productivity (Stepanok, 1998). To some extent its effect is due to the suppression of the high-affinity phosphate/arsenic uptake system (Meharg and Macnair, 1992). Arsenic acts as a phosphate analogue and is transported across the plasma membrane via phosphate transport systems (Ullrich-Eberius et al., 1989). Once inside the cytoplasm, it competes with phosphate. For example, it replaces phosphate in ATP to form unstable ADP-As, and leads to the disruption of energy flows in cells (Meharg, 1994).

However the biochemical responses of plants to As stress are insufficiently studied (Hartley-Whitaker et al., 2001). 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 valence change, a process that readily occurs in plants (Flora, 1999; Lynn et al., 1998). ROS can directly damage proteins, amino acids and nucleic acids and cause peroxidation of membrane lipids (Dat et al., 2000). To combat these effects, enzymatic and nonenzymatic antioxidants are mobilized to quench ROS. It has been demonstrated recently that in *Zea mays,* catalase and SOD were all stimulated after exposure to arsenic (Mylona et al., 1998). Arsenic accumulated in the plant tissue stimulates peroxidase synthesis during the early phases of plant development, long before the appearance of visible changes (Miteva and Peycheva, 1999; Stoeva et al., 2003).

As a result of many negative effects on plants, arsenic caused a reduction of the photosynthesis rate (Miteva and Merakchiyska, 2002). Arsenic damaged the chloroplast membrane and disorganized the membrane structure. The damages of chloroplasts structure during the treatments with high arsenic level imply functional changes of the integral photosynthetic process**.** According to these authors, the changes in the structure and functional activity of the photosynthetic apparatus of plants that they observed were symptoms that they could also be detected under the influence of other stress factors as water and temperature stress (Barcelo et al., 1988).

The objective of the study was to investigate the effect of different arsenic concentrations in soil on some physiological parameters of oats plants.

Material and methods

Plant and growth conditions

The experiment was carried out with spring oats, cv Hanza 152, grown in a greenhouse. The dried soil was homogenized and sieved with a 2 mm sieve. Each pot was filled with oven-dried Fluvisol soil (5 kg soil/pot), with pH 7.2. Arsenic was applied in soil as a water solution of H_3AsO_4 at final concentrations of 40, 80 and 160 mg (As).kg^{-1} dry soil. The applied concentrations were taken from scientific publications (Miteva, 1998 – 100, 150 mg.kg^{-1} As in beans; Jiang et al., 1994 – up to 250 mg.kg^{-1} As in barley, and others) and from unpublished results of ours. Oats belongs to the group of agricultural crops tolerant to As contamination (Adrino, 2001). In oats the arsenic transportation in the stems is limited, and being located in the roots, its effect on the growth and metabolism is weaker (Carbonell-Barrachina et al., 1997). After the treatment the soil in the pots was moistured to full water holding capacity and was left for a month to reach equilibrium.

Seeds were sown and after the emergence 15 equally developed plants were picked out.The soil moisture was kept at 60–70% FWC. During the vegetation period the plants were irrigated with tap water. Each treatment was replicated 2 times in 4 pots**,** (120 plants). When the plants reached the phase of stem extension, they were used for physiological analysis.

Leaf gas-exchange

The net photosynthesis rate, transpiration rate and stomatal conductance of the youngest fully developed intact leaves were measured with a portable infrared gas analyzer LCA-4 (Analytical Development Company Ltd,. Hoddesdon, England), equipped with a PLCB-4 chamber. The measurements were made in the chamber (giving 11 cm^2 leaf area) under irradiance of 800 µmol photons.$m^{-2}.s^{-1}$, temperature of 26±2°C, external CO_2 concentration of 400 µmol.mol^{-1}, and a relative air humidity of 70%.

Chlorophyll Fluorescence

Chl fluorescence was measured by the Pulse Modulated Chlorophyll Fluorometer MINI-PAM (H.Walz, Effeltrich, Germany). Prior to fluorescence measurements the plants were dark-adapted for 1 h at room temperature (22°C). The values of the initial (F_o) and maximum fluorescence (F_m) were recorded at measuring irradiance of 0.15 µmol.$m^{-2}.s^{-1}$ and saturating pulse (SP) – 5 000 µmol.$m^{-2}.s^{-1}$, for 0.8 s.

Photosynthetic pigments

Total chlorophyll (Chl) and carotenoids (Car) content in the oats plants were extracted by 80% water acetone and determined spectrophotometrically at wavelengths 663 nm

(Chl *a*), 645 nm (Chl *b*) and 470 nm (Car), after centrifugation of the extract at 3 000×g for 5 min (Welschen and Bergkotte, 1994) and calculated according to the Lichtenthaler and Wellburn (1983) formulae.

Lipid peroxidation

For the measurement of lipid peroxidation in roots, the thiobarbituric acid (TBA) test, which determines malondialdehyde (MDA) level, was applied (Heath and Packer, 1968). The amount of MDA-TBA complex (red pigment) was measured by its specific absorbency at 532 nm. Non-specific absorbency at 600 nm was also subtracted (De Vos et al., 1989). The data were calculated using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Peroxidase activity

Peroxidase (POD) activity (EC 1.11.1.7) was determined according to Herzog and Fahimi (1973). The roots were homogenized with a china homogenizer in 0.05 M Tris-glycine buffer (pH 8.3) containing 17% i.e. 0.45 M sucrose. The POD activity was expressed as ΔA_{470} g^{-1} (FM).min^{-1}.

Protein content

Protein content in the extracts was determined according to Lowry et al. (1954). The plant material was homogenized with a china homogenizer in a boron buffer (pH 8.7), in a refrigerated centrifuge at 5 000×g for 15 min. The solution absorbency was determined at the presence of Folin reagent, at wavelength of 750 nm.The protein amount was determined following a standard curve obtained with albumen.

Statistical analysis

Analyses were done in five replications. The shown results are mean values ±SE of 120 plants.Experimental data were processed sttistically by the Student's t-test.

Results and discussion

It is well known that the enzymes are the most sensitive indexes for the adaptation and response of the plants to stress factors (Miteva and Peycheva, 1999). The established induction of a particular group of enzymes, including peroxidase, is considered to play an important role in heavy metal stress (Mocquot et al., 1996).

Table 1 presents the POD activity in the roots of the As treated plants. The activity was higher at 160 mg kg^{-1} As – 63% above the control. The data indicated that the

Table 1. Effect of arsenic on the peroxidase activity (POD) [ΔA_{470} g^{-1} (FM) min^{-1}], lipid peroxidation (LP) [nmol (MDA) g^{-1} (FM)], soluble protein content (SP) [mg g^{-1} (FM)] and total chlorophyll (Chl) and carotin (Car) levels [mg g^{-1} DM] in oats plants.

Parameters	Control	40 mg As	80 mg As	160 mg As
POD-[ΔA_{470} g^{-1} (FM) min^{-1}]	745±11.6	865±10.8	988±8.45	1215±11.32**
LP-[nmol (MDA) g^{-1} (FM)]	9.95±0.26	12.65±0.44	14.35±1.15*	16.20±1.12***
SP-[mg g^{-1} (FM)]	12.45±0.96	11.35±1.18	10.36±0.83	8.45±0.46**
Chl (a+b) [mg g^{-1} DM]	13.275±0.33	12.963±0.27	11.135±0.23*	10.345±0.18*
Carotenoids [mg g^{-1} DM]	2.973±0.19	2.825±0.22	2.547±0.21*	2.407±0.19**
Chl/Car	4.46±0.21	4.58±0.11	4.37±0.26	4.21±0.19

*$p < 0.1$, **$p < 0.01$, ***$p < 0.001$

POD activity in roots was reversely correlated with shoot growth and biomass reduction (Stoeva et al., 2002). According to Shaw (1995), this induction of POD is a typical reaction of the plants to a presence of oxygen stress. The change in capacity of some enzymes is an early manifestation of stress, induced by toxic metal concentration (Bjorkman et al.,1987).

Since the peroxidase enzyme is related to free radical formations, it is evident that As, applied in a soluble form, induces the appearance and development of free radical reactions. The primary stress reaction and rapid changes appear in plants due to the applied element. The threshold values of As accumulated in plant tissue induce peroxidase synthesis.

The relationship between metal sensitivity and lipid peroxidation was clearly illustrated in response to As stress, as well. Arsenic proved to be highly effective in stimulating lipid peroxidation - an increase of MDA accumulation as a result of As stress was observed (27, 44 and 63% higher than in the control – Table 1). In accordance with Cakmak et al. (1991), we presume that As potentiates or facilitates lipid peroxidation by disorganizing the membrane structure. Enhanced lipid peroxidation, occurring in response to arsenic, indicates that arsenic toxicity resulted in the increased production of ROS.

The soluble protein content in plant cells is an important indicator of their physiological state. The results from Table 1 show that as a result of As stress the soluble protein amount in the oats roots decreased by 9, 17 and 32%, respectively.

The reduced amount of soluble protein in the organs of the As treated oats plants was most probably a result of the reduced biosynthesis or the accelerated catabolic processes, as well. This suggestion is also confirmed by Vassilev (1998) in his study on Cd, according to whom, the protein degradation to amino acids is in fact an adaptation of the cells to the carbohydrate deficiency. On the other hand, the accelerated

catabolism is probably due to the considerable disturbances in the membrane systems, in response to the metal phytotoxicity.

The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthesis rate. It is believed that they are some of the receptor points of the toxic As effect (Stancheva et al., 1999). The data in Table 1 indicate that there is a considerable decrease of Chl and Car content (16 and 14% below the control at 80 mg (As).kg^{-1} and 22 and 19% at 160 mg (As).kg^{-1}. It was established that Car decreased to a lesser extend than Chl. The decreased ratio Chl/Car may be due to the fact that there is an oxidative stress, which is a marker of the tissue ageing, as result of the stress factors of the environment (Hendry et al., 1993). A reduction of the pigment content in the case of increasing levels of heavy metals and metalloids, was also established by Bogoeva (1998).

Table 2 shows the changes in the leaf gas-exchange rate in oats plants subjected to As treatment. While in the variant with low arsenic content in soil (40 mg.kg^{-1}), CO_2 assimilation was weakly depressed (3%) and was near the control, the soil levels of 80 and 160 mg(As).kg^{-1} resulted in a reduction of the leaf photosynthetic activity (14 and 25% lower vs. control). The reduced photosynthesis rate can be due to the many factors. The photosynthetic reactions are closely related with stomata behavior (i.e. diminish or cessation of CO_2 uptake) and others to thylakoid (i.e. photosynthetic electron transport, ATP synthesis) (Bienhler et al., 1996). The insufficient water supply in tissues may induce photoinhibition but in some cases plants prevent this by decreasing the rate of the electron transport, as a result of both PS I and PS II activity (Bienhler et al., 1996). On the other hand, the decreased photosynthetis rate under stress conditions could be due both to stomatal and mesophyll limitations. The mesophyll factors could be of a different nature, such as disturbances in the pigment apparatus, light and biochemical reactions from the Calvin cycle. Photosynthesis affected by arsenic was also reported by Marin et al. (1993a).

Table 2.Effect of arsenic on the leaf gas-exchange: net photosynthetic rate (P_N) [µmol (CO_2).m^{-2}.s^{-1}], transpiration rate (E) [mmol (H_2O).m^{-2}.s^{-1}], stomatal conductance (g_s) [mol.m^{-2}.s^{-1}] and variable to initial fluorescence ratio (F_v/F_o), and variable to maximum fluorescence ratio (F_v/F_m) in oats plants.

Parameters	Control	40 mg As	80 mg As	160 mg As
P_N - [µmol (CO_2).m^{-2}.s^{-1}];	5.48±0.41	5.32±0.26	4.75±0.06*	4.15±0.03*
E - [mmol (H_2O).m^{-2}.s^{-1}]	1.95±0.06	1.88±0.02	1.70±0.03	1.66±0.02*
g_s - [mol.m^{-2}.s^{-1}].	0.040±0.003	0.037±0.002	0.035±0.003	0.032±0.003
F_v/F_o	5.88±0.10	5.52±0.12	4.78±0.06	4.20±0.08*
F_v/F_m	0.842±0.01	0.826±0.005	0.810±0.04	0.772±0.01**

*$p < 0.1$, **$p < 0.01$.

The data in Table 2 indicate that as a result of the arsenic contamination the transpiration intensity was reduced by 4, 13 and 20%, compared to the control plants. The stomatal conductance diminished least. The negative effect of As on the transpiration process probably is a result of the disturbed uptake and transport of water in the oats plants, caused by the changes in the root system.

The parameters of the chlorophyll fluorescence are indicators of the photochemical processes in photosystem 2 (PS2), which is considered to be more sensitive to stress than photosystem 1 (PS1), (Bjorkman et al., 1987). The results in Table 2 show that in the treated oats plants the ratios F_v/F_o and F_v/F_m decreased considerably, a fact, which indicates that the functional activity of PS2 was reduced (Georgieva and Yordanov, 1993). At a concentration of 160 mg.kg^{-1} soil these values were 29 and 15% for both of the parameters, respectively. The chlorophyll fluorescence ratio F_v/F_m is correlated with the efficiency of leaf photosynthesis and a decline in this ratio is a good indicator of photoinhibitory damage caused by the incident photon flux density, when plants are subjected to a wide range of environmental stresses (Bjorkman et al., 1987).

Conclusion

The soil contamination of arsenic at concentrations of 40, 80 and 160 mg.kg^{-1} provoked an apparent response in plant behavior.

The arsenic contamination generates oxygen stress in the young outs plants, as a consequence the lipid peroxidation and peroxidase activity were activated, and the content of protein was diminished.

The higher doses generated stress in oats plants, and as a result the photosynthesis rate and transpiration intensity were suppressed.

Arsenic reduced the plastid pigments concentrations and the functional activity of PS II, characterized via parameters of chlorophyll fluorescence – the ratios F_v/F_o and F_v/F_m.

The effect of As contamination was much at the higher concentration.

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