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NEGATIVE EFFECT OF METALLOID STRESS ON WHEAT

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ABSTRACT

Arsenic (As) belongs to heavy metals and its accumulation in plants, besides damaging the organism itself, represents a potential health risk to animal and human consumers. Therefore, contamination of soils and waters with this compound is a serious environmental problem. In this work we focused on investigating a negative impact of As on selected parameters of growth of wheat plants (*Triticum aestivum* L. cv. Genoveva) grown in hydropony. In the stage of first assimilation leaves we applied a solution of heavy metal (1 mg.kg^{-1} As^{3+}) on wheat plants. For plants grown under hydropony conditions we observed different plant parameters such as length, weight, amount of fresh and dry biomass. Further we analyzed accumulation of hydrogen peroxide and products membrane lipid peroxidation as indicators of oxidative stress. In addition to these we also measured the content of photosynthetic pigments, maximal quantum yield and proline in plant tissue. Our data indicate reduction of the biomass of shoots forthcoming as a result of exposure of stressed plants to As. Decline of biomass accumulation was accompanied by increase of hydrogen peroxide in plant tissue. In contrast, level of lipid peroxidation was suppressed in stressed shoots. Contents of photosynthetic pigments soundly decreased. Interestingly, fluorescence (Fp=Fm) in stressed wheat shoots increased. Similarly in tested shoots the content of proline was increased. The results indicate that the applied dose of As has a negative impact on the growth and photosynthetic performance of stressed plants. A better understanding of the mechanisms responsible for As resistance and toxicity in plants requires further investigation.

Keywords: Arsenic, Photosynthesis, Heavy metal, Stress, Wheat

INTRODUCTION

Arsenic (As) is a non-essential element that is largely phytotoxic and seriously affects the growth and development of plants (**Caille *et al.*, 2005; Jedynak *et al.*, 2010**). Such toxicity leads to a variety of biochemical and physiological damages (**Singh *et al.*, 2007**). In the environment, As can exist in inorganic and organic form. Of the inorganic forms, oxidized arsenate is more prevalent in aerobic conditions, whereas the more reduced arsenite is the predominant form in anaerobic environment (**Ahsan *et al.*, 2010; Wang *et al.*, 2010; Grasielle *et al.*, 2013**). Plants are able to take up As^{V} through phosphate (P^{V}) transporters, while As^{III} through aquaporin channels (**Zhao *et al.*, 2009; Wang *et al.*, 2010**). As^{V} and As^{III} promote damages in plants by different mechanisms. As^{V} is able to replace phosphate and restricting ATP production, whereas As^{III} is able to bind sulfhydryl groups and interferes with plant metabolism and leads to cellular dysfunction (**Zhao *et al.*, 2009; Grasielle *et al.*, 2013**). Roots are usually the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (**Garg and Singla, 2011**). At sufficiently high concentrations, As interferes with critical metabolic processes, which can lead to death. Most plants possess mechanisms to retain much of their As burden in the root. However, a proportion of the As is translocated to the shoot and other tissues of the plant in genotype-dependent manner (**Finnegan and Chen, 2012**). **Liu *et al.* (2005)** noticed that metal sensitivity and toxicity to plants are influenced by not only the concentration and the toxicant types, but also by the life-stage or biological process (germination, seedling survival, vegetative growth). For example, seed germination and the early seedling growth are the more sensitive stages to metal pollution because some of the defense mechanisms have not developed.
The aim of our study was to gain insight into changes in the morphological, physiological and biochemical parameters of wheat plants grown in hydroponic conditions with addition of As like a stressor.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of wheat (*Triticum aestivum* L. cv. Genoveva) were surface sterilized in 10 % (v / v) sodium hypochlorite solution (Savo) for 15 minutes, then rinsed three times with distilled water and germinated on Petri dishes (Ø 15 cm) with a double layer of filter paper (Whatman no. 1) in sterile distilled water. When the roots reached the length of 6-8 mm, germinated seeds were transferred to 4 hydroponic containers (2 for control + 2 for stress) in that they continued to grow in Hoagland solution for 5 days under 25 °C, humidity 60-70%, of the light period of 12 hours light / 12 hours dark, the maximum radiation intensity of 500 µmol.m^{-2} s $^{-1}$. As stress (in form of As_2O_3 solution) was applied to the 2 containers after 4 days at a concentration of 1 mg.kg^{-1} As^{3+} for 24 hours. A stock solution of As (As_2O_3), was prepared by **Tamarins *et al.* (1988)**. After 5 day of growth control and stress plants were analysed.

Measurement of length, and weight of fresh and dry biomass

Plant shoots were separated from roots with scalpel and used for the length measurement with ruler. Fresh weight of shoots was measured on an analytical balance (Explorer Pro EP 114 CM). After determination of fresh weight shoots of wheat plants were dried at 105 ° C to constant weight (**Erdelský and Frič, 1979**), and then weighed on an analytical balance.

Detection of lipid peroxidation

The levels of generated malondialdehyde (MDA) as product of lipid peroxidation were estimated according to **Dhindsa and Matowe (1981)**. The concentration of MDA was determined in its unit equivalent using a molar extinction coefficient 155 x 10^5 mmol.l^{-1}.

Qualitative detection of H_2O_2

Hydrogen peroxide was detected histochemically directly on the leaf tissue using solution of 1 mg.ml^{-1} (pH 3.8) 3,3'-diaminobenzidine-HCL (DAB-HCL) according to **Thordal-Christensen *et al.* (1997)**. Controls and stress shoots were placed into DAB-HCl and incubated for 3 hours in the dark. Afterwards shoots were washed in distilled water and photographed.

Determination of photosynthetic pigments

The content of photosynthetic pigments was determined by **Lichtenthaler and Wellburna (1985)** as follows: shoots (0.05 g), were homogenized in a ceramic pot with sea sand (0.5 g), magnesium carbonate (0.5 g) and 5 ml of 80% acetone (v / v). The acetone extract was transferred by pipette to a glass porous filter S-3 (connected to a water pump) and washed with acetone to 10 ml of the resulting solution. In this way photosynthetic pigments were quantitatively transferred to a tube. The extract was transferred to a cuvette and the absorbance of the samples was measured spectrophotometrically at wavelengths 663 nm, 646 nm and 470 nm.

Measurement of maximal quantum yield

Measurement on intact leaves was performed with a Handy FluorCam FC 1000-H fluorometer. Prior to fluorescence measurements the plants were dark-adapted for 1 h at room temperature (22 °C). Measuring flash duration was 10 µs – 33 µs. Continuous actinic light was adjustable in duration and power (up to 660 µmol photons $m^{-2}s^{-1}$). Fluorescence signal was detected by a high sensitivity CCD camera.

Measurement of proline content

The amount of free proline in shoot tips (1 cm) was quantified as described in **Sanchez *et al.* (2001)**.

Statistical analyses

All experiments were performed in three independent biological replicates. The obtained data were subjected to statistical analysis using MS Excel. Statistically significant differences were determined by Student's t-test.

RESULTS AND DISCUSSION

In this work we observed changes in the growth of shoots of wheat in relation to the applied metalloid as a stressor. As caused a slight reduction in fresh (Figure 1a) and dry biomass (data not shown) production compared to the control. Similarly, we noticed changes in growth regarding the length of shoots, however these changes were not statistically significant ($p \leq 0.05$) (Figure 1b). **Liu *et al.* (2005)** described in detail the reduction of shoots, height and root length forthcoming as a result of plant exposure to As.

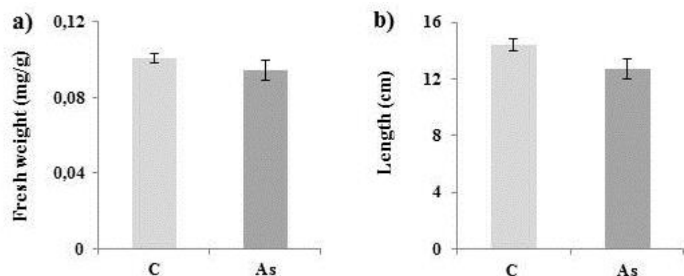

Figure 1 Growth response of wheat shoots to As exposure. a) Fresh weight. b) Length. Abbreviations used: C – non-stressed control, As – stressed plants. Bars indicate ± standard deviation of mean values.

As a result of metalloid effects on plants, the photosynthesis rate was also reduced, as observed previously (**Miteva and Merakchiyska, 2002**). Arsenic damages the chloroplast membranes and disorganizes the membrane structure. The damages of chloroplasts in turn result in functional changes of the integral photosynthetic process (**Stoeva and Bineva, 2003**). In the shoots of the stressed wheat plants the synthesis of photosynthetic pigments was clearly affected. Pigment contents of chlorophyll *a* and *b* (Figure 2a) show a significant tendency to decrease and indicate to less efficient photosynthesis. Regarding the content of carotenoids (Car), we also observed a decreasing trend, but these changes were not statistically sound (Figure 2b). Similarly **Stoeva and Bineva (2003)** and **Rahman *et al.* (2007)** have established that Car were affected to a lesser extent than chlorophylls. Interestingly, the maximal quantum yield (Fp=Fm) in stressed wheat shoots increased (Figure 3a, 3b). This parameter gives information about the photochemical processes in Photosystem II. Previously **Li *et al.* (2008)** observed Fp=Fm decline, and concluded on occurrence of photoinhibitory damage caused by the incident photon flux density, and/or to reversible inactivation or down-regulation of PSII.

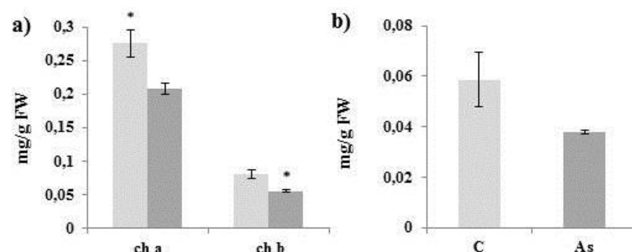

Figure 2 Effect of As on photosynthesis in plant shoots. a) Content of chlorophyll *a* (ch a) and *b* (ch b). Dark columns represent results for As stressed plants. Grey columns represent results for non-stressed plants. b) Content of Car. Abbreviations used: C – non-stressed control, As – stressed plants. Bars indicate ± standard deviation of mean values. The data from analyses are significant as * for $p \leq 0.05$

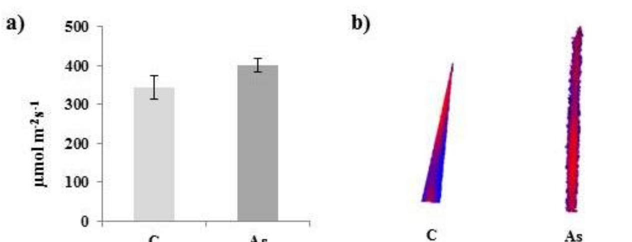

Figure 3 a) Maximal quantum yield in wheat shoots exposed to As treatment. b) Representative fluorescence image of the Fm in shoots of wheat. The analyses were carried out on dark-adapted shoots. Abbreviations used: C – non-stressed control, As – stressed plants. Bars indicate ± standard deviation of mean values.

Under As stress conditions, plants accumulate different reactive oxygen species (ROS) that cause oxidative stress. Consequently, metal stress is often accompanied by an increase in MDA and hydrogen peroxide content is exposed tissue. When exposure to As stress is stronger, ROS generation increases and defense mechanisms may be overwhelmed, leading to cellular damage. This damage might cause loosing of cell barrier function, and endangers the cell integrity leading to cell death (**Lisjak *et al.*, 2009; Finnegan and Chen, 2012**). In our experiment, the applied As concentration caused elevated accumulation of hydrogen peroxide in the stressed plant tissue (Figure 4a). At the same time we detected significant decrease of membrane lipid peroxidation in shoots (Figure 4b), which probably appears to be a result of enhancement of antioxidative enzyme activity (**Yang *et al.*, 2007**).

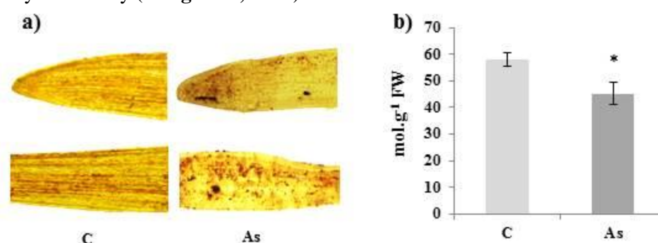

Figure 4 a) Histochemical detection of stress molecules (H_2O_2) in wheat shoots exposed to As (dark spots). b) Levels of MDA in experimental shoots. Abbreviations used: C – non-stressed control, As – stressed plants. Bars indicate ± standard deviation of mean values. The data from analyses are significant as * for $p \leq 0.05$

In experimental plant tissues we also determined the levels of free proline (Figure 5). **Konotop *et al.* (2012)** noticed that in soybean plants grown with the presence of metals the content of proline increases and plays storage or/and protective role. In light of these facts, our data on free proline allow to speculate that plant shoots were stressed under As treatment and elevated proline levels might reflect to similar role in wheat.

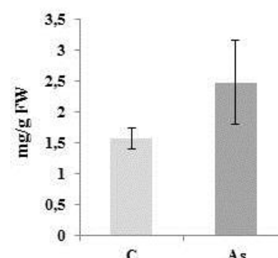

Figure 5 Accumulation of proline in shoots exposed to As stress. Abbreviations used: C – non-stressed control, As – stressed plants. Bars indicate ± standard deviation of mean values.

CONCLUSION

Physiological and biochemical responses of wheat leaves in response to As stress have been investigated. The results of this study indicate a negative impact of As on the growth of stressed plants. To clarify the impact of this environmental (stress) factor during it activity, however, further investigations will be necessary. The combination of detailed physiological and biochemical studies will continue to give us great insights into the mode of action of As in plants. The combination of these methods with more informative physical and biochemical assays and transcriptome and proteome analyses are likely to provide answers to some of the critical questions.

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