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RESEARCH PAPERS

Effect of Cadmium and Arsenic on Chlorophyll Fluorescence of Selected Soybean Cultivars[1]

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Abstract—The chlorophyll fluorescence imaging technique is a valuable tool for studying the impact of heavy metal stress in plants. The toxic effects of cadmium (50 mg/kg soil) and arsenic (5 mg/kg soil) on growth and the photosynthetic apparatus of two soybean cultivars (*Glycine max* (L.) Merr. cvs. Bólyi 44 and Cordoba) were assessed. After 10 days of growth in the contaminated soil, fresh and dry weights of shoots and maximum quantum yield of photosystem II (F_v/F_m) for the three types of leaves (UL—unifoliate leaf, TL1—first fully expanded trifoliate leaf, TL2—newly expanding trifoliate leaf) were determined. No statistically significant change in the growth parameters was recorded. In the youngest leaves (TL2) of cultivar Bólyi 44, arsenic caused decrease in F_v/F_m by 8.6%. In the cultivar Cordoba we recorded the arsenic impact, conversely, having the highest inhibition rate of fluorescence in the oldest leaves (UL decrease of 5.62%). A similar difference in trend of changes in F_v/F_m as the impact of cadmium was also recorded. With the Bólyi 44 variety, the TL2 leaves showed most sensitive response (a decrease of 10.75%); while in the case of Cordoba variety TL2 leaves showed the highest tolerance (a decrease of 1.2%). The results suggest possible genotypic differences in defense strategy against cadmium and arsenic in the different types of leaves.

Keywords: Glycine max, arsenic, cadmium, growth, chlorophyll fluorescence, leaf type

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INTRODUCTION

The problem of environmental pollution is still current, and the most watched polluters include heavy metals and metalloids (arsenic). Cadmium (Cd) and arsenic (As) are relatively rapidly transported to the leaves through the plant roots and xylem. Their toxic effect results in reduced biomass production, the inhibition of photosynthesis and transpiration, and many other metabolic processes [1].

Many studies have attempted to clarify the mechanism of heavy metal and metalloid toxicity in plants [2–5]. However, relationships between growth inhibition and physiological processes under these conditions are still discussed, mainly because of the fact that the threshold of heavy metal/metalloid injury as well as the tolerance to an excess of these elements are highly dependent on the plant species and cultivars [6]. Dynamics of structure and function in growing tissues also result in very different responses to stress in the leaves at particular developmental stages [7]. Spatial distribution has been documented for chlorophyll and protein content [4, 8], accumulation of defense components [2] as well as for activity of vacuolar H^+-ATPase [4]. Tissue and age-dependent differences in the complexation of cadmium and zinc were observed in *Thlaspi caerulescens* [9].

Although a reduction in chlorophyll content is regarded as an indicator of metal injured plant [10], changes in the chlorophyll content cannot be in general regarded as specific biomarkers for monitoring of heavy metal stress in plants [11] also due to the fact that it may not cause the degradation of chlorophylls [2]. Due to emerging oxidative stress, however, failure of photosystem II occurs relatively early after exposure to the effects of the various stress factors [12]. Many studies have indicated that Cd and As can inhibit the flow of photosynthetic electrons, destroy the antennae pigments, down-regulate PSII proteins, as well as inhibit the water-splitting complex of the oxidizing site of PSII [13–15].

The measurement of chlorophyll fluorescence is a sensitive and non-invasive method for assessment of plant sensitivity to various stress factors [12, 16]. The importance of the method lies in the fact that it can detect changes on the photochemical capacity of PSII before these changes affect plant growth. Out of the

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Abbreviations: DW—dry weight; FW—fresh weight; F_v/F_m—the ratio of variable ($F_v = F_m - F_0$) to maximal (F_m) fluorescence representing the maximal photochemical quantum yield of PSII; TL1—first fully expanded trifoliate leaf; TL2—newly expanding trifoliate leaf; UL—unifoliate leaf.

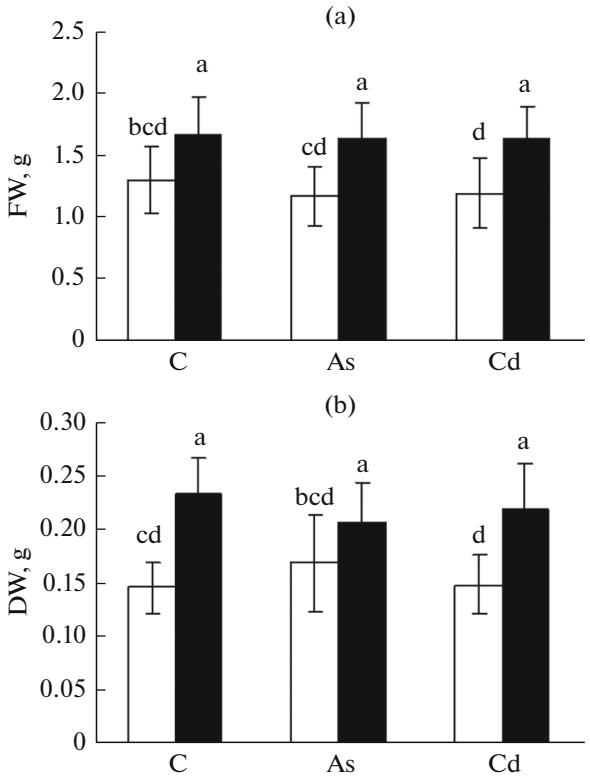

Fig. 1. Effect of cadmium (Cd) and arsenic (As) on fresh weight (FW) (a) and dry weight (DW) (b) of shoots of two soybean cultivars (Bólyi 44 and Cordoba). □—cv. Bólyi 44, ■—Cordoba. C—control untreated plants. Different letters indicate significant differences between means ($P < 0.05$).

several parameters of chlorophyll fluorescence measurement, evaluation of maximum quantum yield of fluorescence ranks among the most used parameters in plant physiology, and determines the maximum photochemical capacity of PSII. It is calculated as a proportion of the variable fluorescence (F_v) on maximum fluorescence (F_m) [16]. The inhibitory effect of Cd and As on chlorophyll fluorescence efficiency was confirmed by several authors [17–19], but the changes are reported often at high concentrations of metal (metalloid) disregarding the type (age) of leaves or the plant genotype.

The aim of our study was to characterize two soybean varieties in terms of tolerance to Cd and As ions on the basis of changes in growth parameters and the parameters of chlorophyll fluorescence (F_v/F_m) of leaves. We also evaluated the impact of the plant genotype and type of leaves on the monitored parameters.

MATERIALS AND METHODS

Plant material and growth conditions. The seeds of two soybean cultivars (*Glycine max* (L.) Merr. cvs. Bólyi 44 and Cordoba) were sown into a mixture of Bora peat soil (pH 7.5) and the pearlite at a ratio of 4 : 1 (w/w). The pot experiment was conducted in a climate box in order to provide the constant conditions of the experiment (temperature of 20°C, humidity 60–70%, illumination periods of 12 h light/12 h dark, the intensity of radiation of 400 μmol/m^{2}s). The plants were grown to the stage of creating the first assimilation leaves. In this stage the solutions of Cd ($Cd(NO_3)_2 \cdot 4H_2O$, 50 mg/kg of soil substrate) and As (As_2O_3, 5 mg/kg of soil substrate) were applied. The stock solution for As was prepared according to the method described in [20]. The plants in control variants were watered with distilled water. The given concentrations of arsenic and cadmium were used due to the predicted toxicity of these elements [3].

Fluorescence measurements. After 10 days of growth in contaminated soil, leaves of three types at the same stage of plant development (UL—unifoliate leaf/the oldest leaf, TL1—first fully expanded trifoliate leaf/mature leaf and TL2—newly expanding trifoliate leaf/the youngest leaf) were used to determine the fluorescence of chlorophyll. The fluorescence parameters were measured using kinetic fluorescence camera (Handy FluorCam FC 1000-H, Photon Systems Instruments, Czech Republic). After the light adaptation of leaves for 5 minutes the maximum photochemical efficiency of PSII was determined from the ratio of variable (F_v) to maximum (F_m) fluorescence ($F_v/F_m = (F_m - F_0)/F_m$) [16]. Image processing software integrated with the FluorCam (www.psi.cz) was used to process the captured image sequences. The fluorescence parameters were integrated over the whole leaf area and averages of ten independent measurements were presented for each leaf type and plant genotype.

Measurement of growth parameters. For measurement of growth parameters (fresh and dry weights of shoots), roots were separated from the above-ground parts of the plants. Thereafter, fresh weight (FW) of shoots was determined. Shoot material was dried at 70°C for 72 h, and the dry weight (DW) was determined. Three replicates were used per treatment and 5–8 plants per pot were analyzed.

Statistical analysis. Data were analyzed by XLSTAT software version 2013. The means were compared using Duncan test at 5% level. Two-way analysis of variance (ANOVA) was performed to determine effects of plant genotype, leaf type and their effects on F_v/F_m. Data are expressed as the means of replicates ± standard deviation (SD).

RESULTS AND DISCUSSION

The doses of metal (metalloid) applied in this work are comparable or higher than those commonly detected in soils in Europe: the mean value of As content in soils of Slovakia is 7.2 mg/kg of soil [21] and for Cd is 1.24 mg/kg of soil [22].

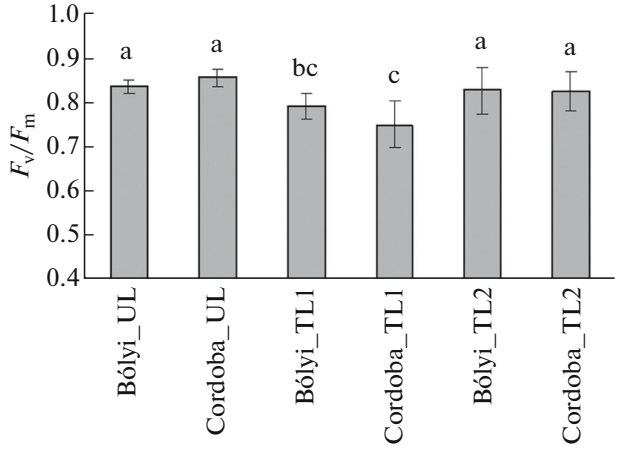

Fig. 2. Maximum quantum yeald of photosystem II (F_v/F_m) at the three types of leaves: UL—unifoliate leaf, TL1—first fully expanded trifoliate leaf, TL2—newly expanding trifoliate leaf of two soybean cultivars (Bólyi 44 and Cordoba). Different letters indicate significant differences between means ($P < 0.05$).

We observed no visual symptoms of toxicity at the given doses of As and Cd. We also did not record a statistically significant change against control for fresh and dry weights of shoots from the tested soybean varieties (Fig. 1). The FW and DW evaluation results point out to a significant difference between the plants of the two cultivars in case of both control and heavy metal-stressed plants (Fig. 1), while slight changes in the growth parameters pointing to a relatively high tolerance of the tested varieties to the applied doses of As and Cd. No effect of Cd and As (50 mg/kg of soil) on soybean shoot growth was observed also by others [23].

The F_v/F_m fluorescence parameter has been frequently used as an indicator of photoinhibition or other injury caused to the PSII complex [24]. Changes in the level of chlorophyll fluorescence of three type of leaves were evaluated: UL—unifoliate leaf, TL1—first fully expanded trifoliate leaf and TL2—newly expanding trifoliate leaf, while we have seen the differences between the types of leaves within each variety (Fig. 2). However, the values do not indicate damage to photosystem II for TL1 leaves, as the F_v/F_m value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species [25]. In case of both varieties in the ratio of F_v/F_m, the UL and TL1 and also TL1 and TL2 leaves differed statistically (Fig. 2) while the lowest values of F_v/F_m were observed in the case of TL1 leaves (Fig. 2). It opposes the results of Jiang et al. [26], who demonstrated the highest down-regulation of the maximum quantum yield (F_v/F_m) in newly expanding soybean leaves. The results of others [27] also pointed to lower values of the parameters of the fluorescence of younger leaves (the newly expanding leaves) than those of the mature leaves (newly-fully expanded leaves). Young leaves of soybean plants

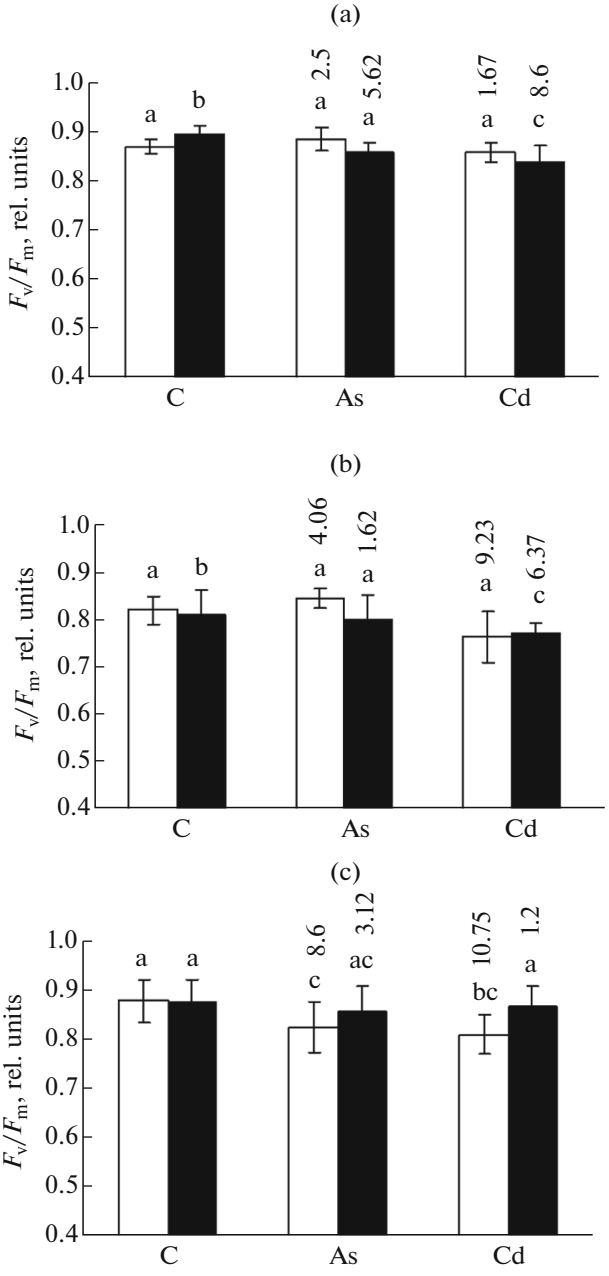

Fig. 3. Effect of cadmium (Cd) and arsenic (As) on maximum quantum yield of photosystem II (F_v/F_m) at the leaves of three types. (a) Unifoliate leaf (UL), (b) first fully expanded trifoliate leaf (TL1), (c) newly expanding trifoliate leaf. (TL2). □—cv. Bólyi 44, ■—Cordoba. C—control untreated plants. Bars marked with the same lower-case letters within the each genotype are not significantly different at $P < 0.05$. Numbers above column reflect the changes against control (%).

could successfully fight against high irradiance in the field by co-operation of the leaf angle, photorespiration, and thermal dissipation depending on the xanthophyll cycle. It is possible that these mechanisms have different efficiency in differently developed young leaves [26].

Analysis of variance for the effects of genotype and the type of leaves on maximum quantum yield of photosynthesis (F_v/F_m) in plants treated with arsenic and cadmium

Source of variation	As		Cd	
	F	*P* value	*F*	*P* value
Plant genotype	2.47563	0.120405	3.405231	0.069477
Type of leaves	15.1391	3.88E-06*	42.7237	1.25E-12*
Interaction	7.09172	0.001622*	7.876568	0.000856*

* Data are significant at $P < 0.05$

By measuring the F_v/F_m ratio, we also evaluated the sensitivity of the test soybean varieties towards Cd and As ions. The measured values of the maximum quantum yield of chlorophyll fluorescence of the control and stressed soybean plants are shown in Fig. 3.

No statistically significant changes were observed on the F_v/F_m ratio in As-treated Bólyi 44 variety for UL and TL1 leaves. In the youngest leaves (TL2) of cv. Bólyi 44, on the contrary, As caused a statistically significant decrease in the F_v/F_m by 8.6% (Fig. 3c). The higher sensitivity of the top leaves to photoinhibition caused by stress factors was also pointed out by others [2, 27]. On the contrary, in case of Cordoba variety we recorded the highest rate of inhibition of fluorescence in the lower leaves (decrease of 5.62%) (Fig. 3a). Higher reduction of F_v/F_m ratio and the corresponding decrease in the level of photosynthetic pigments were also observed in older leaves of barley plants treated with 100 μM Cd [18] and in older cadmium-treated bush been leaves [5]. The distinction between leaves of different age in their photosynthetic apparatus responded to cadmium may be related to the metal content in them and the structural and functional modifications that take place in the cells and tissues during leaf ontogeny [18]. Increased accumulation of Cd in older leaves also represents a mechanism, through which plants avoid toxicity in the most physiologically active portions of the plants by reducing metal translocation to the epigeous portion, and by promoting the re-translocation of toxic metals from shoots to roots [28]. On the other hand, younger leaves may react more strongly than older ones to Cd stress despite a generally lower Cd content in them. Since they had reduced storage capacities in cell walls in comparison to those in older organs, their tissue and cell compartments were probably confronted with Cd stress more directly [29].

Plants generally show relatively large differences in PSII susceptibility to arsenic. No changes in the maximum quantum yield (F_v/F_m) were recorded in safflower grown in As-contaminated soil (90 mg/kg of soil) [19]. Statistically significant decrease of F_v/F_m for oat leaves was recorded only at a dose as high as 160 mg/kg of soil [17]. The parameters of chlorophyll fluorescence of soybean leaves in the presence of arsenic in the nutrition solution (32–96 μM) did not present significant changes either [30].

A similar difference in trend changes of F_v/F_m depending on the leaf age was also observed as the effect of the applied dose of Cd. While in the cv. Bólyi 44 it was the youngest leaves which responded most sensitively to the cadmium ions' (decrease of 1.67% for UL, 9.23% for TL1 and 10.75% for TL2), in the case of the cv. Cordoba the highest tolerance was shown by TL2 leaves (decrease of 8.6% for UL, about 6.37% for TL1 and 1.2% for TL2) (Fig. 3).

The results of the two-way ANOVA test indicate that changes in the F_v/F_m ratio are influenced by the type of leaf and the interaction of the plant genotype and type of leaf (Table).

In conclusion, the results of our study showed differences in the F_v/F_m ratio depending on the type of leaves (the leaf age), while the lowest values of F_v/F_m showed, for both soybean varieties, young fully expanded trifoliate leaves. Changes in the F_v/F_m ratio concerning the cadmium and arsenic impact were dependent on the type of leaf and also the interaction of the type of leaf and plant genotype. The fact that the top leaves have a higher sensitivity to photoinhibition caused by heavy metals or metalloids [2, 27] was confirmed only in the case of the Bólyi 44 variety. When assessing the sensitivity of plants to Cd or As based on the F_v/F_m ratio, it is therefore necessary to consider the type (age) of leaves and also the possible impact of the genotype. Evaluation of the tolerance of the tested soybean varieties to ions of the given metal (metalloid), however, requires a more comprehensive experimental approach based on the biochemical and molecular biological analyses that can detect differences in the mechanisms of tolerance in soybean varieties to cadmium and arsenic ions.

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