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Evaluation of arsenic tolerance of oat (*Avena sativa* L.)

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Abstract We evaluate the tolerance of oat (*Avena sativa* L.) to arsenic in two experimental approaches. In early stages of ontogenesis, we evaluated the effect of arsenic (As 10 $mg.l^{-1}$ solution) on root growth of seedlings of 22 oat varieties. The tested varieties showed considerable variability in response to As, the tolerance index (TI) ranged from 68.18-126.67%, with the highest tolerance shown by the Valentin variety and the lowest by the Ivory variety. The Václav variety reached TI=125.53%. We also evaluated the growth of this variety in soil contaminated with 10, 20 and 50 mg $As.kg^{-1}$. Within the given experiment, the variety Václav showed a high tolerance in the entire range of As doses (TI>100%). The stimulatory effect of As on tested parameters is explained by mechanism of hormesis. Knowing the tolerance of varieties to As is a presumption for successful cultivation of this crop in As-contaminated soil and at same time the use of this crop in remediation of As-loaded soils.

Key words Arsenic, oat, tolerance, genotypic variability

1. INTRODUCTION

Heavy metals and metalloids are important environmental contaminants. Increased accumulation of these elements in soil causes a decrease in yields of agricultural crops and threatens individual links of food chain. The solution to the given problem lies on the one hand in the application of suitable remedial measures technologies and also in search and breeding of tolerant plant varieties.

Arsenic (As) is a dangerous environmental contaminant. Common soil As concentrations range between 1-50 $mg.kg^{-1}$, with an average value for world soils of 4.4 $mg.kg^{-1}$ for podzols and 9.3 $mg.kg^{-1}$ for organic soils, and a median value of 7.2 $mg.kg^{-1}$ for Slovakia soils (Dragun, 1998). Arsenic occurs in environment in various organic and inorganic compounds. Of inorganic compounds, these are mainly arsenates-As(V) and arsenites-As(III), while arsenites are more prevalent in anaerobic soil and are more toxic than arsenates (Finnegan and Chen, 2012). In plants, mainly arsenic compounds occur, as the received forms of As(V) are reduced to forms of As(III) by means of arsenic reductase (Xu et al., 2007).

There is no evidence that arsenic (As) is essential for plant growth, although small amounts of arsenic can stimulate plant growth and increase plant biomass (Onken and Hossner, 1995). In addition, small yield increases have been observed at low levels of As, especially for tolerant plants such as corn, potatoes, rye, and wheat (Carbonell et al., 1998; Gulz et al., 2005; Jacobs et al., 1970). However, with increasing concentration As becomes eventually very toxic for all plants, causing oxidative stress, chlorosis, necrosis, inhibition of growth and finally death (Zhao et al., 2009).

Oat belongs among the important agricultural crops with universal use, but its tolerance to heavy metals and metalloids is relatively poorly studied. The aim of this study is to evaluate the tolerance of selected oat varieties to arsenic within two experimental approaches.

2. MATERIAL AND METHODS

2.1 Experiment 1

Seeds of 22 varieties of oat (*Avena sativa* L.) were pre-germinated in Petri dishes in dark at 23°C for 48 hours. Subsequently, viable, approximately identical sprouts were transferred to a new medium with a volume of 20 ml in the following variants: distilled water (control) and As solution (5 mg/l).

The solutions of arsenic (AsIII) were prepared from certified reference material for arsenic (Sigma-Aldrich, Darmstadt, Germany). After 48 hours of cultivation, the length of roots was measured using a ruler and the fresh weight (FW) of the roots was measured, too. The tolerance index (TI) was calculated as a ratio of the mean fresh weight (FW) of plants grown in the presence of arsenic, and the mean FW of the control plants expressed as a percentage (Wilkins, 1978). The experiment was set up in three replicates.

2.2 Experiment 2 – pot experiment

In the pot experiments, plastic containers (diameter 15 cm) were filled with 300 g dry peat substrate (Klasmann KTS 2, Klasmann-Deilmann GmbH, Germany, pH KCl. 6.7, gravimetric soil water content max. 70%, total arsenic (As) content 1.13 mg.kg^{-1}, water soluble arsenic (As) content 0.15 mg.kg^{-1} soil, nitrogen (N) content 1.65 g.kg^{-1}, carbon (C organic) content 81.59 g.kg^{-1}, phosphorus content (P) 0.38 g.kg^{-1}, potassium (K) content 0.43 g.kg^{-1}), to which 20 seeds were sown. After sowing, arsenic in doses of 10, 20 and 50 mg.kg^{-1} of soil (As10 to As50) was applied to the soil substrate. The solutions of arsenic (AsIII) were prepared from certified reference material for arsenic (Sigma-Aldrich, Darmstadt, Germany). The plants were then regularly watered (every third day), with the water volume corresponding to maximum 80% of the soil's water-holding capacity (150 ml). The pot experiment was conducted in a growth chamber (average night temperature 12°C, average day temperature 23°C, humidity 60–70%; radiation intensity of 400 µmol.m^{-2}.s^{-1}). The experiment was set up in three replicates. After 12 days of growth in contaminated soil, the following parameters were measured: shoot length, fresh weight FW and dry weight (DW) of shoots and photosynthetic pigments content in leaves.

2.2.1 Determination of Growth Parameters and Tolerance Index

The length of the roots was measured with a ruler. After measurement the FW of roots, the DW was determined after drying the roots in an oven for 48 hours at 60°C.The tolerance index (TI) was calculated as a ratio of the mean dry weight (FW) of shoots grown in the presence of arsenic, and the mean FW of shoots of control plants expressed as a percentage (Wilkins, 1978).

2.2.2 Determination of Photosynthetic Pigments

The contents of photosynthetic pigments: chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), and carotenoids were determined spectrophotometrically (spectrophotometer UV-2601, Shimadzu, Japan) in fully developed third assimilating leaves at three wavelengths: 470 nm, 646 nm, and 663 nm. Acetone (80%) was used for pigment extraction. The amount of pigments (chlorophylls and carotenoids) was determined according to Lichtenthaler (1987) in six replicates in each variant of the experiment.

2.2.3 Statistical Analyses

The obtained results were statistically analysed using XLSTAT software. Basic statistical characteristics (arithmetic mean, standard deviation) were determined. The differences between variants of the experiment were examined using t-test or the Kruskal–Wallis test, followed by Dunn's post hoc test (p<0.05).

3. RESULTS AND DISCUSSION

The roots of tested oat varieties showed variability in response to arsenic. Changes in growth parameters are shown in Table 1.

Table 1. Effect of arsenic (As) on growth parameters of root of selected cultivars of oat

Cultivar	Length of roots (cm) Control	Length of roots (cm) As	FW of roots (g) Control	FW of roots (g) As
Swbetania	88.90 ± 10.11	83.71 ± 10.25 *	0.063 ± 0.009	0.060 ± 0.006
Bingo	87.02 ± 10.14	79.68 ± 10.69 *	0.043 ± 0.007	0.051 ± 0.004 *
Valentin	89.62 ± 10.67	86.40 ± 12.06	0.039 ± 0.007	0.049 ± 0.007 *
Pomoro	89.54 ± 9.48	86.44 ± 10.91	0.066 ± 0.005	0.070 ± 0.009
Václav	92.74 ± 10.79	76.51 ± 9.29 *	0.047 ± 0.006	0.059 ± 0.004 *
Tatran	100.17 ± 10.62	89.17 ± 11.22 *	0.062 ± 0.008	0.062 ± 0.006
Pushkinskij	90.26 ± 11.24	86.25 ± 10.41 *	0.050 ± 0.010	0.060 ± 0.008 *
Vok	91.12 ± 11.13	91.54 ± 10.21	0.045 ± 0.006	0.057 ± 0.004 *
CD SOL Fl	88.38 ± 12.71	78.51 ± 11.27 *	0.050 ± 0.009	0.050 ± 0.007
V2 6/19	83.93 ± 10.00	87.75 ± 12.51 *	0.068 ± 0.008	0.067 ± 0.013
Prokop	99.50 ± 9.05	96.34 ± 10.00	0.050 ± 0.008	0.055 ± 0.008
PS 243	60.60 ± 7.37	73.70 ± 11.40 *	0.043 ± 0.008	0.052 ± 0.004 *
Viliam	88.02 ± 11.59	69.31 ± 8.99 *	0.063 ± 0.008	0.052 ± 0.004 *
V2 11/19	84.46 ± 9.46	66.54 ± 10.70 *	0.048 ± 0.005	0.048 ± 0.005
Ac Percy	85.87 ± 9.41	79.71 ± 9.64 *	0.051 ± 0.006	0.047 ± 0.007
V2 1/19	92.36 ± 11.92	85.74 ± 11.85 *	0.043 ± 0.009	0.041 ± 0.006
Bison	83.80 ± 10.12	48.93 ± 9.56 *	0.054 ± 0.007	0.042 ± 0.004 *
Ozon	76.74 ± 11.91	49.18 ± 13.46 *	0.043 ± 0.005	0.037 ± 0.002
Aragon	111.79 ± 16.61	88.36 ± 18.98 *	0.057 ± 0.006	0.052 ± 0.005
Bay Yan2	100.93 ± 23.46	84.43 ± 14.21 *	0.05 ± 0.006	0.045 ± 0.009
Ivory	81.79 ± 13.30	52.45 ± 11.30 *	0.032 ± 0.010	0.024 ± 0.003 *
Racoon	78.12 ± 11.87	63.30 ± 11.18 *	0.044 ± 0.004	0.030 ± 0.005 *

Data are presented as means ± SD from three biological replicates. * the level of significance of the differences against the control at p<0.05 (t-test). FW – fresh weight.

A significant stimulatory effect of As on root elongation was shown in the case of varieties V2 6/19 and PS 243 (root elongation by 4.55% and 21.62%). In the case of the other varieties, root growth was inhibited (Table 1), with the strongest inhibition in the case of the variety Bison. The tolerance indices determined for fresh biomass of roots were in the range of 74.03-127.56. The varieties Valentin, Vok, Václav, PS 243 and Pushkinskij showed the highest tolerance to As, and the lowest tolerance had varieties Ivory, Bison, Viliam, Racoon and Ozon (Fig. 1). The stimulatory effect of As on plant growth was also pointed out by Onken and Hossner (1995).

The Václav variety showed also a high tolerance to As doses of 10, 20 and 50 mg.kg of soil substrate and the tolerance indices reached values >100% (Table 2). Doses of As10 and As20 stimulated shoot growth by 5.82% and 11.92% and increased fresh biomass of shoots by 11.92% and 12.32%. The dry matter content increased significantly (by 13.65% and 14.57%) due to higher doses of As (As20 and As50) (Fig. 2).

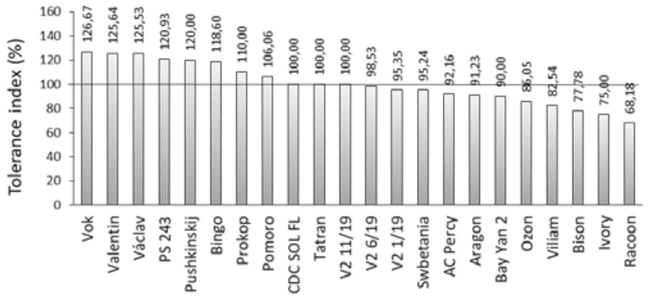

Fig. 1. Tolerance index for arsenic based on fresh weight of roots of selected cultivars of oat

Table 2. Tolerance indexes based on fresh weight (FW) and dry weight (DW) of oat shoots cv. Václav.

	Tolerance index (%)		
	As10	**As20**	**As50**
Fresh weigth	111.91	112.31	108.77
Dry weigth	112.16	113.65	114.57

The increased content of dry matter corresponded to the increased content of photosynthetic pigments, but a significant increase in Chl*a* (by 44.87%), Chl*b* (by 36.40%) and carotenoids (by 49.30%) was only observed due to the influence of the highest dose of As50 (Fig. 3).

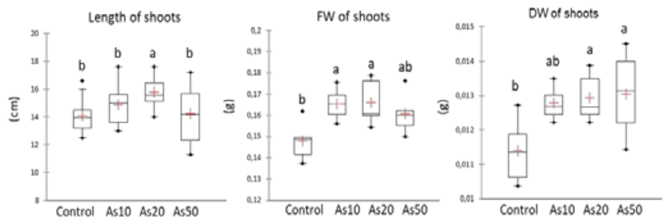

Fig. 2. Effect of arsenic (As) on the growth parameters of oat cv. Václav. Data are presented as means ± SD from three biological replicates. The differences between cultivars were determined using the Kruskal–Wallis test followed by Dunn's post hoc test for multiple comparisons. Different letters on the top of bars indicate a significant difference (p<0.05) between variant of experiment. FW – fresh weigth, DW – dry weigth.

An increased accumulation of photosynthetic pigments due to lower AsIII (1- 2 mg.l^{-1}) doses was also recorded in the leaves of wheat (Li et al., 2007) and onion (Sushant and Ghosh, 2010). In shoots, hormesis is often associated with enhanced photosynthesis activity, which improves plant development (Agathokleous, 2021; Jia et al., 2015). Modulation of the proportion between chlorophylls *a* and *b* (Chl *a*/*b* ratio) can improve photosynthesis efficiency in plants that face environmental challenges, such as low N availability, excess light, and metal (metalloid) exposure (Agathokleous, 2021). Increased concentration in carotenoids could act as part of the protection mechanism against As-induced oxidative stress (Gusman et al., 2013).

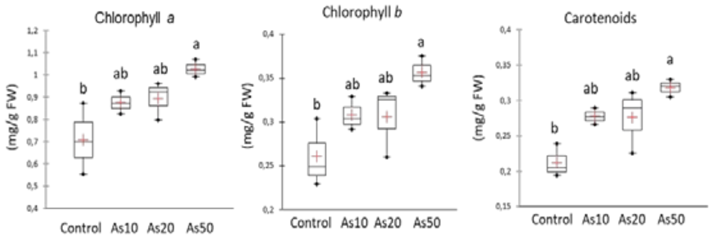

Fig. 3. Effect of arsenic (As) on the photosynthetic pigment contents in leaves of oat cv. Václav. Data are presented as means ± SD from three biological replicates. The differences between cultivars were determined using the Kruskal–Wallis test followed by Dunn's post hoc test for multiple comparisons. Different letters on the top of bars indicate a significant difference (p<0.05) between variant of experiment.

4. CONCLUSION

In early stages of ontogenesis, the tested oat varieties manifested high variability in response to arsenic. The tolerance index ranged from 68.18 to 126.67%. The lowest tolerance was shown by the Racoon variety and the highest by the Vok variety. The Václav variety showed high tolerance even when growing in As contaminated soil. Doses of 10 and 20 mg.kg^{-1} of soil had a stimulating effect on growth and the content of photosynthetic pigments. As a result of dose of 50 mg As.kg^{-1}, we also did not notice growth inhibition, this dose increased the dry matter content of shoots and the content of photosynthetic pigments. We explain the stimulating effect of As by mechanism of hormesis. The given study needs to be expanded by testing the tolerance of other oat varieties in As contaminated soil. The evaluation of As accumulation by tissues can contribute to the evaluation of their remedial potential for As contaminated soils.

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