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ARTICLE

Comparative study on sensitivity of different physiological properties of *Spirodela polyrrhiza* (L.) Schleiden to Cr (VI) treatments

Viktor Oláh[1]*, György D. Tóth[1], Erzsébet Szöllősi[1], Tibor Kiss[1]

[1]Department of Botany, Faculty of Sciences and Technology, Debrecen University, Debrecen, Hungary

ABSTRACT The aim of this study was to compare the sensitivity of various growth and biochemical traits in axenic S. polyrrhiza cultures under chromium (VI) stress. Frond number, fresh weight of plants, number and elongation of roots, frond area, photosynthetic pigment content and anthocyanin accumulation of plants responded sensitively to Cr (VI) treatments in the applied concentration range. Anthocyanic abaxial leaf surface and anthocyanin content of plants showed extremely high sensitivity to Cr (VI) reaching multifold levels to that of control values.
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Several methods have been developed to screen the heavy metal induced alteration of plant metabolism uptake and distribution of heavy metals in plant tissues as well (Kertész et al. 2005; Kertész et al. 2006). Standardized ecotoxicity test methods frequently use duckweed species due to their advantages such as rapid vegetative reproduction, sensitivity to toxicants, easy culturing under axenic conditions (Lakatos et al. 1993). *Spirodela polyrrhiza* (L.) Schleiden the largest representative of Lemnaceae family (Arales) is widely used test plant too. In contrast with Lemna spp its fronds have more than one root and accumulate anthocyanin on the abaxial leaf surface which changes sensitively under stress. In testing the effects of contaminants growth parameters of duckweed are the most commonly used but other traits such as root elongation, pigment content could also be applied (EPS 1/RM/37). The aim of this study was to compare the sensitivity of various growth and biochemical traits in axenic S. *polyrrhiza* cultures under chromium (VI) stress.

Materials and Methods

Axenic *Spirodela polyrrhiza* (L.) Schleiden plants were used. Tests were performed under controlled conditions (Mészáros et al. 1998). Each treatment was performed with 12-12 fronds in 100 cm^3 Erlenmeyer's flasks containing 50-50 cm^3 Hutner's media. Chromium (VI) was applied in 0, 25, 50 and 100 µM l^{-1} concentrations under static conditions in 4 replicates for 5 days. Growth was characterized by frond number and fresh weight of cultures (Oláh et al. 2008). Cultures were scanned using a flatbed scanner (600 dpi). Digital images of plants were used for calculation of frond area by means of Adobe Photoshop 7.0. Horizontal view of plants was digitally recorded in front of graph paper for subsequent count of roots and measurement of root length. Photosynthetic pigment content and anthocyanin content were measured spectrofotometrically (Oláh et al. 2008). Anthocyanic abaxial surface area of plants was also analyzed by same way as leaf area measurement.

*Corresponding author. E-mail: olahviktor@gmail.com

Results and Discussion

Control cultures of S. *polyrrhiza* showed rapid growth doubling their frond number by the end of tests. Cr (VI) strongly decreased the growth of plants (Table 1.). Even 25 µM Cr (VI) lowered frond numbers by 20%. Fresh weights of cultures reflected even stronger growth inhibition by the end of tests compared to frond numbers. Even the lowest concentration decreased in biomass by 30% and growing concentrations caused further decrease (Table 1). Total frond area of cultures responded to Cr (VI) treatments with similar sensitivity as fresh weight in higher concentrations (Table 1). Root length was sensitive to Cr (VI) but did not change on a dose-dependent manner proving not to be a good indicator to Cr (VI)

Table 1. Sensitivity of measured growth parameters expressed in the percent of control values at the 5th dav of Cr (VI) treatments.

	frond number	fresh weight	root elongation	number of roots	frond area
25 µM	82	69	72	50	75
50 µM	81	65	69	37	64
100 µM	71	55	69	33	59

Table 2. Alterations in the pigment content of plants expressed in the percent of control values at the 5th day of Cr (VI) treatments.

	anthocyanic surface	Chl-*a*	Chl-*b*	Carotenoids	Anthocyan content in A_{530}/g freshweight
25 µM	404	64	76	85	201
50 µM	410	53	64	67	246
100 µM	380	43	56	59	359

treatments. However, number of roots showed extremely high sensitivity to Cr (VI) treatments. Even the lowest concentration resulted in 50% drop of root numbers (Table 1). Amounts of photosynthetic pigments decreased gradually parallel to increasing Cr (VI) concentrations (Table 2). Concentration of chl-*a* and chl-*b* showed higher sensitivity to Cr (VI) as carotenoids which reflected the role of latter ones in photoprotection and antioxidative defense under heavy metal stress. In the course of tests accelerated anthocyanin accumulation was observed on the lower surface of Cr (VI) treated plants. Surface analyses and spectrophotometric measurements confirmed these observations (Table 2). Even the lowest Cr (VI) concentration caused fourfold increase in purple (anthocyanic) abaxial surfaces of total frond area compared to control values but 50 and 100 µM of Cr (VI) did not result in further increase (Table 2). Spectrophotometric analyses indicated anthocyan accumulation on a dose-depended manner. 25 µM Cr (VI) nearly doubled the content of anthocyanins in plants and higher concentrations caused further increases up to 359%. The results reflected that anthocyanins may play a role in protection against oxidative stress in *S. polyrrhiza*.

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