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Changes in chlorophyll spectral characteristics in rye seedlings grown under heavy metal stress

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Introduction

The studies on the effects of heavy metals on the photosynthetic apparatus have risen the question about possible mechanisms of their toxicity towards its integrity and functional aspects. One of the toxic effects resulting from excess supply of heavy metals is a change in chloroplast pigments, especially in chlorophyll. Reduced accumulation of chlorophyll in Cd, Cu, Hg, Pb, Mn and Ni-treated plants has been well documented (Siedlecka *et al*. 2001, and refs. therein).

Moreover, different heavy metals are able to substitute Mg as a central ion in the porphyrin ring of chlorophyll or bacteriochlorophyll. This phenomenon was observed *in vivo* for algae, lichens, water plants and *in vitro* for isolated pigments (Puckett 1976, DeFilippis 1979, Kowalewska *et al*. 1987, 1989, Küpper *et al*. 1996, 1998, Teuchner *et al*. 1997).

Here we present the results of the experiments on the effects of selected heavy metals (Cd, Cu, Hg, Ni, Pb, Zn) on some efficiency parameters of the photosynthetic apparatus in heavy metal-treated rye seedlings. The possibility of heavy metal substitution in the porphyrin ring of chlorophylls in land plants is also discussed.

Materials and methods

Winter rye (*Secale cereale* L cv. Pastar) seeds were germinated for 2-3 days at +25°C and 95% humidity in a thermostated chamber and then transferred to full strength Hoagland nutrient medium (Hoagland and Arnon 1950). After 4 days of growth at PPFD of 150 µmol $m^{-2}s^{-1}$, 16/8 h day/night period and temperature of 23/16°C, seedlings were transferred to a fresh medium containing one of the heavy metals at a concentration of 500 µM. Heavy metals were used in the following chemical forms: Cd in the form of $CdSO_4$ $8H_2O$; Cu in the form of $CuSO_4$ $5H_2O$; Hg in the form of $HgCl_2$; Ni in the form of $NiSO_4$ $7H_2O$; Pb in the form of $Pb(NO_3)_2$ and Zn as $ZnSO_4$ $7H_2O$. After 7 days of growth in the presence of heavy metals in the nutrient medium, second leaves were taken for all subsequent analyses.

Total chlorophyll and carotenoid content was estimated in 80% acetone leaf extracts after Lichtenthaler and Wellburn (1983). Carotenoid separation and quantification was performed by HPLC in acetonitryl:methanol:water (72:8:3 *v/v*) on Nucleosil 5µC18 column with detection at 441 nm (Thermo Separation Products model Spectra Series UV100).

Chlorophyll *a* induction kinetics parameters were performed using modulated fluorescence technique with PAM 101 Chlorophyll Fluorometer (H. Walz, Germany) as described earlier (Krupa *et al*. 1993, and refs. therein).

Chloroplasts were isolated from second rye leaves after Krupa *et al*. (1987). Chlorophyll content was estimated according to Arnon (1949).

Absorption spectra *in vivo* were measured in the second leaf middle segment or in isolated chloroplasts suspension on Hewlett Packard 8453 spectrophotometer in the range of 350-800 nm. Fluorescence induction and emission spectra were taken from intact leaves or isolated chloroplasts at room temperature using Shimadzu RF5001PC spectrofluorometer.

For inspection of the presence of heavy metal-substituted chlorophylls leaf samples were dried and extracted according to the procedure of Küpper *et al*. (1996). Chlorophyll extracts were then subjected to spectral measurements.

Results and discussion

To study the effects of heavy metals on spectral properties of photosynthetic pigments one must start with basic measurements of their contents in treated plants. In case of our studies Ni, Cd and Cu diminished contents of total chlorophyll in second rye leaf to 33, 51 and 85% of control values, respectively. The analysis of metal influence on carotenoids showed that, in general, the most susceptible was the accumulation of neoxanthin and violaxanthin with β-carotene pool increasing from 5.8% in control leaves to 23.9% in Cd-treated plants. These preliminary results suggested that the efficiency of the photosynthetic apparatus might be disturbed by changes in pigment contents. The basic measure F_v/F_m (variable to maximum fluorescence) ratio shows the quantum efficiency of open photosystem II centres. Basically, this ratio has not changed substantially despite metal treatment. However, when we analyzed so-called F_N/F_A ratio being the measure of "non-active" (F_N) to "active" (F_A) fluorescence coming form photosystem II (Marder *et al*. 1995). It has been noted that F_N may come from "dead" PSII, or even from pigments totally disconnected with PSII or chemically changed, *i.e*. by heavy metal substitution of Mg in the porphyrin ring. It appeared that all heavy metals tested substantially increased F_N/F_A ratio with Cu and Ni being the most active. The increase in values ranged from 1.5 times for Hg, through 1.7 for Cd and Zn, to 2.2 and 2.4 for Ni and Cu, respectively. The high values of this ratio suggest the presence of free or metal-substituted chlorophyll molecules. Therefore, we have attempted to perform spectral characteristics of chlorophylls both *in vivo*, in intact leaves, and *in vitro* in isolated chloroplasts and chlorophyll extracts. Recently, the effects of Mg substitution in chlorophyll porphyrin ring by heavy metals has been demonstrated by Küpper *et al*. (1996, 1998). However, these experiments were performed on algae or water plants having very close or even direct contact with metals in the nutrient medium, thus facilitating their effects on physiology and metabolism. When it comes to land higher plants they posses very efficient detoxication mechanisms located mostly in roots. Depending on plant species and heavy metal, only about 1% of total metal content in leaf can be found in chloroplasts (Krupa 1999). Thus, the modifications of chlorophyll chemical and spectral properties by heavy metals might differ from those in algae or water plants.

Our preliminary results on the effects of the most toxic heavy metals like Cd, Cu, Hg, Ni, Pb and Zn presented here show that these metals may affect the spectral properties of chlorophylls in metal-treated rye seedlings. The measurements of fluorescence emission spectra of intact leaves, with special attention paid to 708 nm band showing the extent of aggregation of the major light-harvesting complex (LHCII) (Krupa *et al*. 1992), proved an increased aggregation of LHCII complexes in plants treated with Cu and Zn. The analysis of the shift in Q_y band has revealed the possibility of Cu and Ni substitution of Mg in chlorophyll porphyrin ring. Spectral analysis of the organic solvent extracts from treated plants confirmed our earlier observations that Cu, Ni, Hg and eventually Zn may substitute Mg leading to long wavelength shift in chlorophyll absorption spectra. Our preliminary calculations show that about 10% of the total chlorophyll pool in chloroplasts of metal-treated rye plants was subjected to the above phenomenon. Moreover, we postulate that heavy metals like Cd or Pb may interact indirectly with carotenoid pigments, affecting the protein environment of the photosynthetic antenna. Thus, the effects of heavy metals on the photosynthetic apparatus of higher with special attention paid to chlorophylls are much more complex than just simple substitution by other metals the central Mg ion in the porphyrin ring.

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