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The xanthophyll cycle pigments in *Secale cereale* leaves under combined Cd and high light stress conditions

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Abstract

Leaves of *Secale cereale* seedlings were exposed to high light illumination (1200 μmol m^{-2} s^{-1}) and Cd ions at 5 or 50 μM concentrations. Influence of these stress factors on violaxanthin cycle pigments content was analysed chromatographically. Chlorophyll *a* fluorescence induction was used to analyse response of PSII to stress conditions and contribution of light-harvesting complex (LHCII) in non-photochemical quenching of excitation energy. The Cd-induced all-*trans* violaxanthin isomerization was analysed by HPLC technique in acetonitrile:methanol:water (72:8:3, v/v) solvent mixture. Interestingly, in the control and Cd-treated leaves subjected to high light, photochemical utilization of absorbed energy increased. This indicates plant adaptation to high light stress. In control plants high light caused zeaxanthin formation, however, the presence of Cd in the nutrient solution resulted in reduction of the second step of violaxanthin de-epoxidation process and anteraxanthin accumulation. In this study we have also shown, that non-photochemical quenching can be independent of anteraxanthin and zeaxanthin content. The particular increase in the *cis* isomers fraction in Cd-treated leaves has been explained in terms of a direct metal–pigment interaction as confirmed by Cd-induced all-*trans* violaxanthin isomerization in organic solvent, leading to formation of 13-*cis*, 9-*cis* and 15-*cis* isomers.

Keywords: Xanthophyll cycle; Cd; Heavy metal stress; Xanthophyll isomerization

1. Introduction

Cd is a non-essential environmental pollutant which toxic influence on plants has been known for a number of years. Many experiments have shown that Cd inhibits light phase of photosynthesis. Cd disturbs electron transport chain mainly by influence on donor and acceptor side of Photosystem II (PSII) causing disorganization of the water splitting system [1]. In the experiments with Cd-treated plants the F_v/F_m parameter, which defines the photochemical efficiency of PSII, was rather independent of a metal presence [2]. The photosynthetic electron transport may also decrease due to the degradation of the oligomeric structure of LHCII [3,4]. This chlorophyll–protein complex is the major light-harvesting antenna in photosynthesis, transferring excitation energy to the reaction center of PSII. The content of chlorophylls and carotenoids also decreased in plants in the presence of Cd. This heavy metal may also cause an increase in the rate of dissipation of the excess energy, referred to as non-photochemical quenching and resulting from the thermal energy dissipation. It can be also related to the xanthophyll cycle involving the interconversion of violaxanthin, anteraxanthin and zeaxanthin. Operation of this light-dependent process is usually connected with high light stress and is recognized to be one of the main photoprotection mechanisms against photoinhibition. Overexcitation of the photosynthetic apparatus may be associated with its photo-degradation, owing to the production of reactive oxygen species. Zeaxanthin was implied to be the main quencher of Chl *a* excitation energy under overexcitation conditions. Its singlet state

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energy (S_1) is lower than the energy of the excited singlet state (Q_y) of Chl *a*. In contrast, the singlet state of violaxanthin is slightly higher than the Q_y state of Chl *a*. It suggests that violaxanthin can function as a light-harvesting pigment and directs the excitation energy to chlorophyll [5]. In general, violaxanthin occurs in the conformation all-*trans* in the photosynthetic apparatus but certain fraction of the geometrical isomers 9-*cis* and 13-*cis* has been also detected in intact leaves [6] and in the isolated LHCII [7–9]. In the lipid phase of the thylakoid membrane, violaxanthin is converted by the enzyme violaxanthin de-epoxidase to anteraxanthin, as a first de-epoxidation step, and finally to zeaxanthin [10]. Only the all-*trans* isomer of violaxanthin is a specific substrate of the de-epoxidase enzyme and therefore the transient appearance of the *cis* geometric isomers of this xanthophyll pigment has been discussed in terms of making the pigment available for de-epoxidation, by transferring violaxanthin from the protein environment of the pigment–protein antenna complexes to the lipid phase of the thylakoid membrane, where the enzymatic de-epoxidation takes place [7–9]. Latowski et al. [11] reported that Cd can change the content of the xanthophyll cycle pigments. In the Cd-treated plants the content of zeaxanthin and anteraxanthin was higher than in the control plants, indicating inhibition of the epoxidation by Cd.

The aim of the present study was analysis of the influence of Cd and high light on the primary photochemical processes of PSII and comparative analysis of the content of the xanthophyll cycle pigments and their isomeric forms.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of winter rye plants (*Secale cereale* L., cv. Pastar) were germinated on wet filter paper in a thermostated darkened chamber (24 °C, 95% relative humidity). Three days old seedlings were cultivated hydroponically (five plants per pot filled with 0.5 l of Hoagland nutrient solution). The nutrient solution was continuously aerated. All plants were grown for 4 days in a climate chamber with 16 h daylight photoperiod of photosynthetic photon flux density 150 μmol m^{-2} s^{-1} and 24/16 °C day/night temperature. Then, the nutrient solution was changed to fresh one and Cd was added as $3CdSO_4 \cdot 5H_2O$ in concentrations: 0 (control), 5 and 50 μM. The measurements were carried out 7 days after the metal additions.

The first leaves of the control and Cd-treated plants were dark-adapted or illuminated with 1200 μmol m^{-2} s^{-1} light intensity for 2 h. Then, the first leaves were cut into three sections of equal length and parameters of chlorophyll *a* fluorescence induction as well as the analysis of the content of carotenoids in the top section were measured.

2.2. Xanthophyll analysis by HPLC

Tops of the first leaves were ground with 1 ml of acetone and homogenates were centrifuged for 3 min. Next, the supernatants were filtered by paper filter. After acetone evaporation from the sample pigments were dissolved in acetonitrile:methanol (72:8, v:v). Xanthophyll pigments were analysed chromatographically by HPLC technique. A C-30 filled, phase-reversed HPLC column (4.6 × 250 mm) was applied with a solvent system acetonitrile:methanol:water (72:8:3, v:v) as a mobile phase with the flow rate of 0.5 ml min^{-1}. A Spectra Series UV100 spectrophotometer (Thermo Separation Product) with absorption set at 441 nm was used as a detector. In addition a diode-array Hewlett Packard spectrophotometer, model HP 8453 was used to record on-line absorption spectra between 280 and 900 nm in 10-s intervals. Chromatograms were integrated using Data Jet Integrator (Thermo Separation Product).

2.3. Violaxanthin isomerization

Violaxanthin was isolated from *Narcissus jonquilla* blossoms and purified chromatographically directly before experiments. Isomerization of all-*trans* violaxanthin was carried out at 24 °C in a solvent mixture of acetonitrile:methanol:water (72:8:3, v:v) at different conditions: in darkness with 1 mol Cd^{2+} per 700 mol violaxanthin incubation (calculated according to the method of thylakoid membranes incubation with 20 μmol Cd^{2+} per 100 μg of chlorophyll [1]), under illumination with 150 μmol m^{-2} s^{-1} light intensity and in both Cd and light presence. All-*trans* violaxanthin was dark-incubated to obtain the blank experiment. Directly after 30-min incubations the reaction mixtures were chromatographically analysed to identify isomeric forms. Details of HPLC pigments analysis were the same as described previously. The violaxanthin isomers were identified on the basis of retention time values and UV–vis absorption spectra described previously [9].

2.4. Chlorophyll fluorescence

Parameters of chlorophyll *a* fluorescence induction were measured with modulated actinic light at 21 °C using PAM 100 Chlorophyll Fluorometer (H. Walz, Effeltrich, Germany) operated by Win Control VI.48 software. Before measurements leaves were dark-adapted for 30 min. Intensity of the actinic light was 456 μmol m^{-2} s^{-1} and saturating light pulses of 9860 μmol m^{-2} s^{-1} intensity with 20 s interval flashes were applied by Schott lamp. F_0 (minimal Chl *a* fluorescence level) was determined by a weak 1.6 kHz photon fluence rate of 10 nmol m^{-2} s^{-1}. The maximal fluorescence level F_m was induced by strong white light pulse with 9860 μmol m^{-2} s^{-1} intensity. The quenching coefficients (q_p and q_n) were calculated according to van Kooten and Snel [12].