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ANTIOXIDANT RESPONSE SYSTEM AND CHLOROPHYLL FLUORESCENCE IN CHROMIUM (VI)- TREATED *ZEA MAYS* L. SEEDLINGS

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The effects of different concentrations of Cr(VI) (1 μM, 10 μM, 100 μM) applied for 7, 14 or 21 days on initiation of high lipid peroxidation level (POL) and consequent changes in the enzymatic-antioxidant protective system and minimization of photosystem II (PSII) activity were studied in maize seedlings. Chromium(VI) caused an increase in the electrical conductivity of the cell membrane, and malondialdehyde (MDA) content (a peroxidation product) reflected peroxidation of membrane lipids leading to the loss of the membrane's selective permeability. It also induced distinct and significant changes in antioxidant enzyme activity. Versus the control, superoxide dismutase (SOD, EC 1.15.1.1.), catalase (CAT, EC 1.11.1.6.) and peroxidase (POD, EC 1.11.1.11.) activity in maize seedling roots and leaves was progressively enhanced by the different Cr(VI) doses and stress periods, except for decreases in SOD and POD activity in leaves exposed to 100 μM Cr(VI) for 21 days. The different Cr(VI) concentrations changed chlorophyll (chl) content differently. The 10 μM and 100 μM doses of Cr(VI) decreased the chl *a*/*b* ratio and quenched the chl *a* fluorescence emission spectra. These effects reflect disturbance of the structure, composition and function of the photosynthetic apparatus as well as PSII activity.

Key words: Chromium(VI), lipid peroxidation, antioxidant protective system, chlorophyll fluorescence, maize.

INTRODUCTION

Chromium is a transition metal located in group VI B of the periodic table and occurs in the workplace predominantly in two valence states: hexavalent chromium [chromium(VI)] and trivalent chromium [chromium(III)], which display quite different chemical properties. Hexavalent chromium compounds find extensive application in diverse industries. The toxicity of Cr(VI) results from the action of this form itself as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr(VI) to Cr(III) inside the cell (Shanker and Pathmanabhan, 2004). Chromium is not considered an essential element for plant nutrition. Both forms, Cr(III) and Cr(VI), may be taken up by plants. Uptake of Cr(III) seems to be passive, while that of Cr(VI) is considered to be active (Barceló and Poschenrieder, 1997).

Chromium interferes with several metabolic processes, and its toxicity to plants is exhibited in reduced growth and phytomass, foliar chlorosis, stunting and finally plant death (Vajpayee et al., 2000; Gikas and Romanos, 2006; Zou et al., 2006). This heavy metal causes oxidative damage to biomolecules such as lipids and proteins (Shanker et al., 2004), modifies the activity of antioxidant enzymes and some other enzymes such as nitrate reductase and ribonuclease (Vartika et al., 2004; Labra et al., 2006), and alters plant water status (Pandey and Sharma, 2003).

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Abbreviations: MDA – malondialdehyde; SOD – Superoxide dismutase; CAT – Catalase; POD – Peroxidase; F_0 – minimum fluorescence in dark-adapted leaves; F'_0 – minimum fluorescence in light-adapted leaves; F_m – maximum fluorescence in dark-adapted leaves; F'_m – maximum fluorescence in light adapted leaves; F_v – maximum variable fluorescence in dark-adapted leaves; F'_v – maximum variable fluorescence in light-adapted leaves; F_s – steady-state fluorescence yield at $q_P > 0$; F_v/F_m – maximum photochemical efficiency of PSII; ΦPSII – quantum yield of PSII electron transport; F'_v/F'_m – efficiency of excitation energy capture by open PSII reaction centers; ETR – electron transport rate; QA – primary electron acceptor of PSII; QB – secondary electron acceptor of PSII; q_P – coefficient of photochemical quenching; q_N – coefficient of nonphotochemical quenching.

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Chromium has been reported to stimulate the formation of free radicals (FR) and reactive oxygen species (ROS) such as superoxide radicals (O_2·-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH) either by direct electron transfer involving metal cations or as a consequence of metal-mediated inhibition of metabolic reactions (Sinha et al., 2005). Under natural growth conditions, plants are adapted to minimize the damage induced by ROS. However, O_2 toxicity emerges when ROS production exceeds the quenching capacity of the protective system (Dai et al., 1997). This occurs under various stress conditions due to dysfunction of the photosynthetic and respiratory electron flow (Meloni et al., 2003; Rakwal et al., 2003). ROS accumulation limits the activity of several enzymes and causes disturbance of membrane structure and function (Mallick and Mohn, 2000).

Photosystem II (PSII) is believed to be the most susceptible. The overall effect of Cr ions on photosynthesis and excitation energy transfer could also be due to Cr(VI)-induced abnormalities in chloroplast ultrastructure – a slightly lamellar system with widely spaced thylakoids and a few grana (Rocchetta et al., 2006). Chromium is known to inhibit photosynthesis and PSII is known to be the main target for this negative action (Davies et al., 2002). However, the relationship between chromium and the primary reactions of photosynthesis is not well described. In recent years the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. Chlorophyll fluorescence subtly reflects the primary reactions of photosynthesis. Intricate relationships between fluorescence kinetics and photosynthesis help researchers to understand photosynthetic biophysical processes. Chlorophyll fluorescence technique is useful as a noninvasive tool in ecophysiological studies, and has been used extensively in assessing plant responses to environmental stress (Calatayud et al., 2004; Miyashita et al., 2005; Guo et al., 2005; Vernay et al., 2007).

The aim of the present study was to investigate the effects of different concentrations of Cr(VI) (1 μM, 10 μM, 100 μM) on lipid peroxidation level, the activities of antioxidant enzymes (SOD, CAT, POD) as well as photosynthetic parameters (chlorophyll content, chlorophyll fluorescence) in maize after 7, 14 and 21 days of exposure.

MATERIALS AND METHODS

PLANTS, GROWTH CONDITIONS, TREATMENTS

Seeds of drought-tolerant maize (Jindan No. 2) supplied by the Agricultural Research Center (Tianjin, China) were allowed to germinate in Petri dishes in the dark at constant 25°C. After 3 days, seedlings of uniform appearance were selected and transferred to a hydroponic system and then grown in a climate chamber under a 14 h photoperiod and day/night 25/20°C and 55/75% relative humidity (RH). Groups of 15 seedlings were placed on a polystyrol plate in a pot containing 2 l modified half Hoagland's nutrient solution (Stephan and Prochazka, 1989) containing 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50 μM H_3BO_3, 1 mM $MgSO_4$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.1 μM $(NH_4)_6Mo_7O_{24}$ and 10 μM Fe-EDTA, pH 5.5. The seedlings grew in this solution for 1 week, then were treated with different concentrations of Cr(VI) (1 μM, 10 μM and 100 μM) for 21 days. Chromium(VI) was given as potassium dichromate ($K_2Cr_2O_7$). The Cr solutions were prepared in deionized water and were added to full strength Hoagland's nutrition solution. Full strength Hoagland's solution without Cr(VI) was used for the control plants. The seedlings treated with different concentrations of Cr(VI) grew in the same climate chamber. The nutrient solutions were continuously aerated and changed regularly every 5 days until the seedlings were harvested.

ESTIMATION OF LIPID PEROXIDATION

The level of lipid peroxidation products in Cr(VI)-stressed maize was expressed as malondialdehyde (MDA) according to Buege and Aust (1978). After 7, 14 and 21 days of Cr(VI) exposure, fresh leaves or roots (0.2 g) in each treatment were homogenized in 5 ml 10% trichloroacetic acid (TCA) with a pestle and mortar. The homogenates were centrifuged at 4,000 g for 10 min. To 2 ml aliquot of the supernatant, 2 ml of 0.6% thiobarbituric acid (TBA) in 10% TCA was added. The mixture was heated at 100°C for 15 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm and 450 nm. Lipid peroxidation was expressed as MDA content (μM/g FW).

ANTIOXIDANT ENZYME ASSAYS

Enzyme extraction and preparation were carried out at 4°C. Fresh leaf (0.2 g) was harvested, washed with deionized water and homogenized with a pestle and mortar with 5 ml chilled sodium phosphate buffer (50 mM, pH 7.8). The homogenates were centrifuged at 10,500 g for 20 min and the supernatant was stored at 4°C prior to enzyme analyses.

Superoxide dismutase activity was estimated according to a modification of Beauchamp and Fridovich's (1971) method. The reaction mixture (3 ml) contained 1.5 ml 0.05 M sodium phosphate buffer (pH 7.8), 0.3 ml 130 mM methionine, 0.3 ml 750 μM nitroblue tetrazolium chloride (NBT), 0.3 ml 0.1 mM EDTA-Na_2, 0.3 ml 20 μM riboflavin, 0.01 ml

enzyme extract, 0.01 ml 4% (w/v) insoluble polyvinylpyrrolidone (PVPP) and 0.28 ml deionized water. The reaction was started by placing the tubes below two 15 W fluorescent lamps for 10 min, and stopped by keeping the tubes in the dark for 10 min. Absorbance was recorded at 560 nm. One unit of SOD enzyme activity was defined as the quantity of SOD enzyme required to produce 50% inhibition of NBT reduction under the experimental conditions, and specific enzyme activity was expressed as units per mg fresh weight of leaf.

Catalase activity was assayed according to the method of Beers and Sizer (1952). Catalase activity was determined by UV-Vis spectrophotometry (UV-2550, Shimadzu Japan) at 25°C in 2.8 ml reaction mixture containing 1.5 ml 200 mM sodium phosphate buffer (pH 7.8), 1.0 ml deionized water and 0.3 ml 0.1 M H_2O_2 prepared immediately before use. The reaction was initiated by the addition of 0.2 ml enzyme extract. CAT activity was measured by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. Activity was expressed as units per minute per g leaf fresh weight; one catalase activity unit was defined as a 0.1 change in absorbance at 240 nm.

Peroxidase activity was determined following a modification of Kato and Shimizu's (1987) method. The reaction mixture in a total volume of 25 ml 100 mM sodium phosphate buffer (pH 6.0) containing 9.5 µl H_2O_2 (30%) and 14 µl guaiacol was prepared immediately before use. The reaction was initiated by the addition of 1 ml enzyme extract to 3 ml reaction mixture, and the increase in absorbance was monitored at 470 nm at 0.5 min intervals up to 2 min with a UV-Vis spectrophotometer (UV-2550, Shimadzu Japan). POD activity was defined as units per g fresh weight (one peroxidase activity unit was defined as a 0.1 change in absorbance at 470 nm).

LEAF PIGMENT DETERMINATION

After 7, 14 and 21 days of Cr(VI) exposure, leaf samples from each treatment were homogenized in 5 ml 80% acetone at 4°C, and 15 ml acetone was added to each tube. The tubes were stored in the dark at 4°C for 48 h prior to spectrophotometry. The pigment samples were filtered for determination. Absorbance was measured at 645 and 663 nm with a UV-Vis spectrophotometer (UV-2550, Shimadzu Japan). Chl *a*, chl *b* and the chl *a*/*b* ratio were calculated according to Zarco-Tejada et al. (2005).

CHLOROPHYLL FLUORESCENCE MEASUREMENT

Chlorophyll fluorescence quenching analysis was carried out at room temperature with a portable fluorometer (LI-6400, LI-COR, Lincoln, U.S.A.). The 3rd mature leaves from the bottom were darkened for 2 h prior to measurement. Minimum (dark) fluorescence (F_0) was obtained upon excitation of leaves with a weak beam. Maximum fluorescence (F_m) was determined following a 600 ms pulse of saturating white light. The yield of variable fluorescence (F_v) was calculated as $F_m - F_0$. Following 2 min of dark readaptation, actinic white light (430 µmol photons m^{-2} s^{-1}) was switched on and saturating pulses (8000 µmol photons m^{-2} s^{-1}) were applied at 60 s intervals for 15 min in order to determine the maximum fluorescence yield during actinic illumination F'_m, the level of modulated fluorescence during brief interruption of actinic illumination in the presence of far red light F'_0, and chlorophyll fluorescence yield during actinic illumination F_s.

Using both light and dark fluorescence parameters, we calculated the following: (1) maximum efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m); (2) efficiency of excitation energy capture by open PSII reaction centers, $F'_v/F'_m = (F'_m - F'_0)/F'_m$; (3) the photochemical quenching coefficient, $q_P = (F'_m - F_s)/(F'_m - F'_0)$, which measures the proportion of open PSII reaction centers (Van Kooten and Snel, 1990); (4) the nonphotochemical quenching coefficient, $q_N = 1 - (F'_m - F'_0)/(F_m - F_0)$; and (5) the quantum yield of PSII electron transport, $\Phi PSII = (F'_m - F_s)/F'_m$.

STATISTICAL ANALYSIS

For statistical validity, each treatment was made in 5 replicates for estimating enzyme activity and photosynthetic parameters, and 10 replicates for root length and plant height. The data were expressed as means ± standard error (SE) and were analyzed by ANOVA using Sigma statistical software (Jandel Scientific Corporation). Significance of differences was checked with the t-test. Statistical significance was set at $p < 0.05$.

RESULTS

MACROSCOPIC SYMPTOMS

Some leaves of the chromium-treated plants exhibited chlorosis and became necrotic after 14 days of exposure to 100 µM Cr(VI), and the roots became yellow and root growth stopped almost completely. The 10 µM Cr(VI) treatment inhibited seedling growth slightly. At the 1 µM Cr(VI) dose, seedlings grew better than the control during the first 14 days and were similar to the control after 21 days of exposure.

The effects of Cr(VI) on root elongation of maize varied with the concentration (Fig. 1a). The 100 µM and 10 µM Cr(VI) doses reduced seedling root length

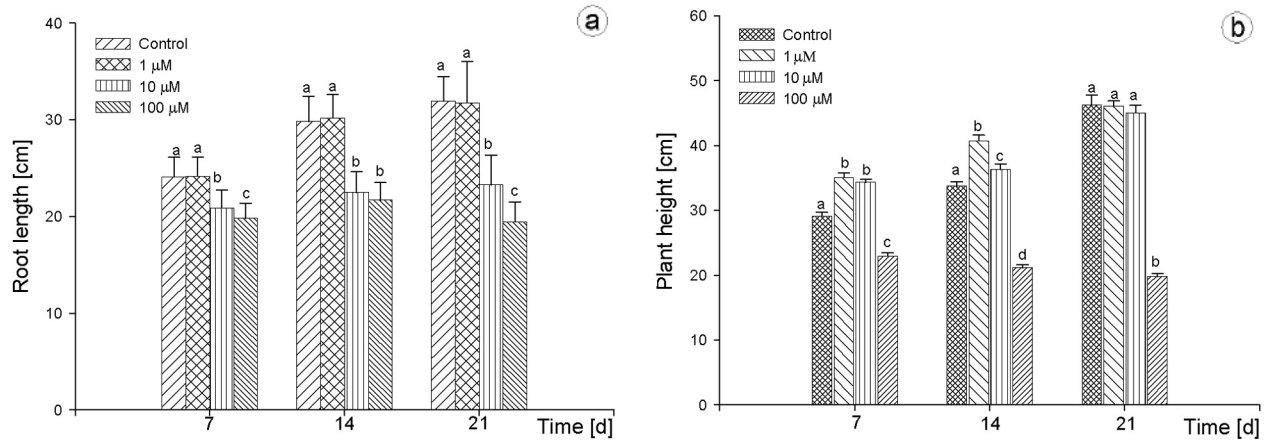

Fig. 1. Effects of different concentrations of Cr(VI) on root length of maize seedlings stressed for 7, 14 and 21 days. (**a**) Root length, (**b**) Plant height. Vertical bars denote SE, n = 10. Values with different letters differ significantly from each other ($p < 0.05$, t-test).

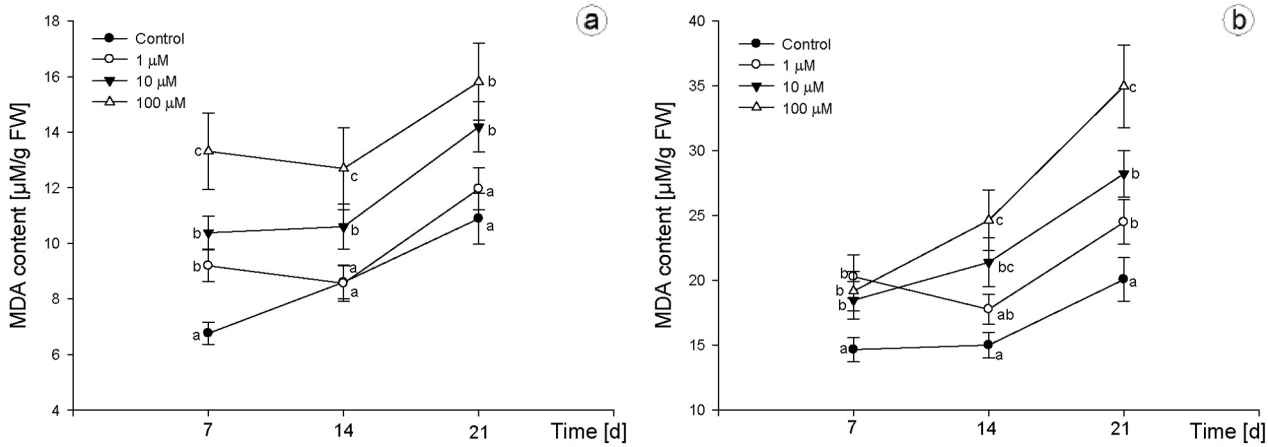

Fig. 2. Effects of different concentrations of Cr(VI) on MDA content of maize seedling roots and leaves stressed for 7, 14 and 21 days. (**a**) MDA content in roots, (**b**) MDA content in leaves. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other ($p < 0.05$, t-test).

significantly. The root length of plants treated with 1 μM Cr(VI) was similar to that in the control during the entire experiment. The 100 μM Cr(VI) dose inhibited plant growth during the whole course of treatment; 10 μM and 1 μM Cr(VI) increased plant height in the first 14 days (Fig. 1b).

EFFECTS OF Cr(VI) ON LIPID PEROXIDATION

Figure 2 shows lipid peroxidation levels in terms of malondialdehyde (MDA) accumulation in maize roots and leaves under the different Cr(VI) treatments. MDA content increased with the Cr(VI) concentration and duration of treatment. After 21 days of exposure to 100 μM Cr(VI), MDA content was 45.2% higher in roots and 74.1% higher in leaves than in the control seedlings.

EFFECTS OF Cr(VI) ON SOD ACTIVITY

As shown in Figure 3a and b, SOD activity varied with the concentration of Cr(VI). SOD activity in roots was higher in the 100 μM Cr(VI) treatment than at 10 μM and 1 μM Cr(VI) (Fig. 3a), and significantly higher than the control in all treatments after 14 and 21 days of exposure. Versus the control, 1 μM Cr(VI) did not affect SOD activity in leaves significantly; 10 μM and 100 μM Cr(VI) increased SOD activity in leaves after 14 days of exposure (Fig. 3b). SOD activity in the control and the 1 μM and 10 μM

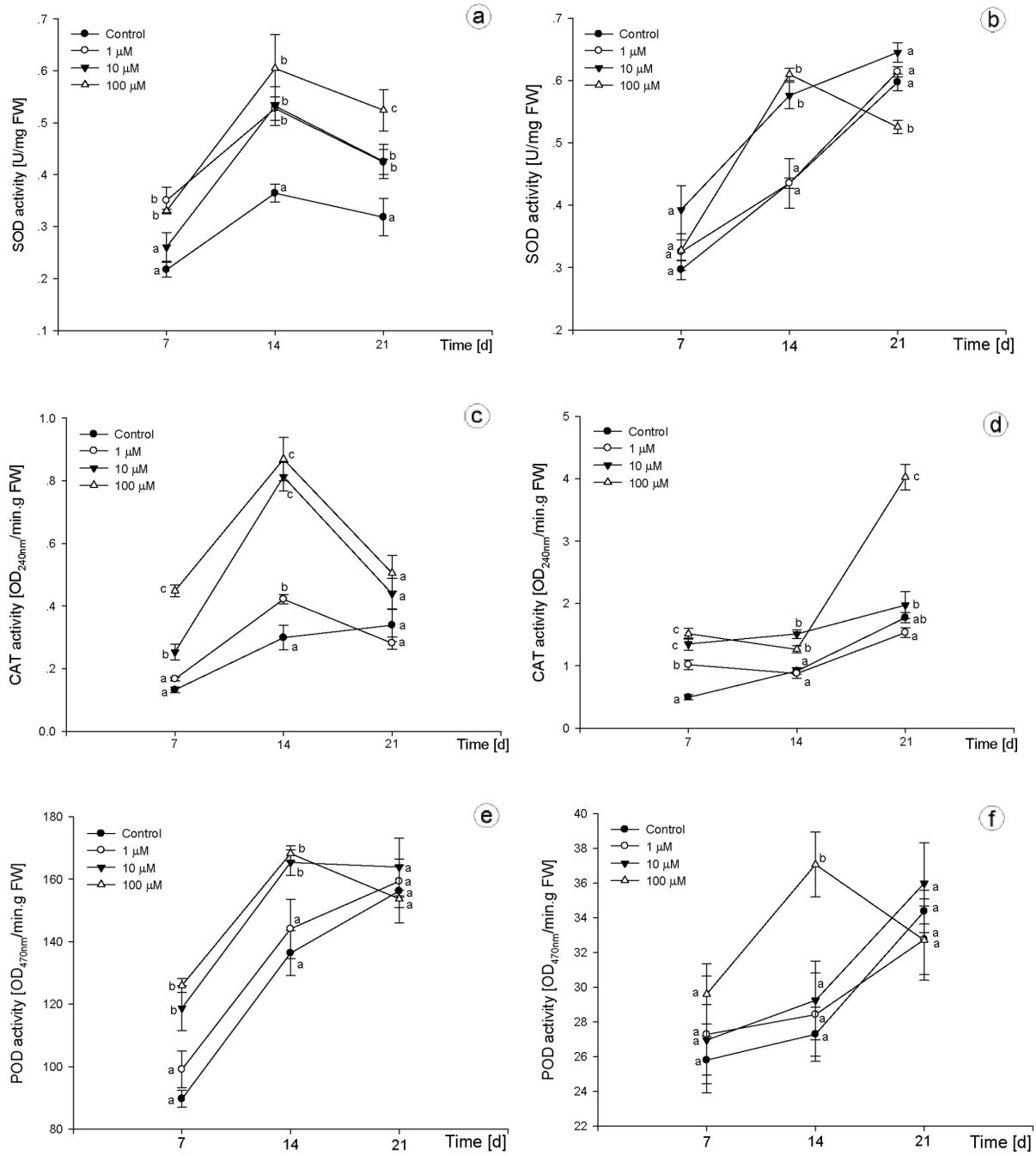

Fig. 3 Effect of different concentrations of Cr(VI) on the activities of three antioxidant enzymes in roots and leaves of maize seedlings stressed for 7, 14 and 21 days. (**a**) SOD in roots, (**b**) SOD in leaves, (**c**) CAT in roots, (**d**) CAT in leaves, (**e**) POD in roots, (**f**) POD in leaves. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other ($p < 0.05$, t-test).

Cr(VI) treatments increased with treatment time. In the 100 μM Cr(VI) treatment, SOD activity increased during the first 14 days and decreased at 21 days (Fig. 3b).

EFFECTS OF Cr(VI) ON CAT ACTIVITY

In the control plants the specific activity of CAT expressed on a protein basis was higher in the leaves than in the roots, and was also shown to be

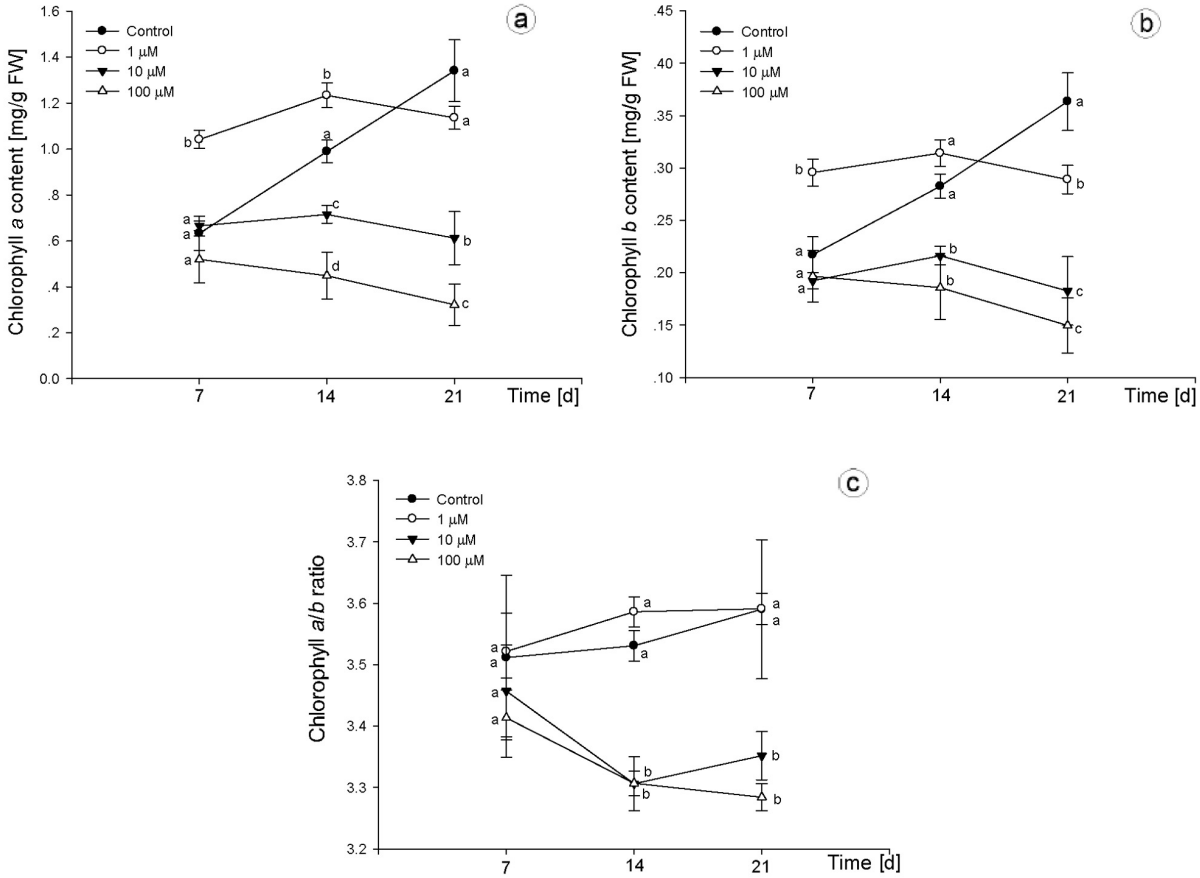

Fig. 4. Effect of Cr(VI) stress on chlorophyll *a*, *b* content and chlorophyll *a*/*b* ratio in maize seedlings stressed for 7, 14 and 21 days. (**a**) Chlorophyll *a* content, (**b**) Chlorophyll *b* content, (**c**) Chlorophyll *a*/*b* ratio. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other ($p < 0.05$, t-test).

very constant during the duration of the experiment (Fig. 3c,d). In the 10 μM and 100 μM Cr(VI) treatments, CAT activity markedly increased in roots in the first 14 days and in leaves during the whole treatment. There was a direct correlation between Cr(VI) concentration and CAT activity in both roots and leaves: higher concentrations of Cr(VI) produced a greater increase in CAT activity (Fig. 3c,d). The percentage increase in CAT activity was higher in roots than in leaves after 7 and 14 days of Cr(VI) exposure.

EFFECTS OF Cr(VI) ON POD ACTIVITY

During the 21 days of Cr(VI) treatment, POD activity increased concomitantly in maize seedling roots and leaves, except in the 100 μM Cr(VI) treatment at 21 days (Fig. 3e,f). POD activity was higher in roots than in leaves in all three treatments and the control. In the 100 μM Cr(VI) treatment, after 14 days POD activity in roots was 23.4% higher and in leaves 35.8% higher than in the control.

EFFECTS OF Cr(VI) ON CHLOROPHYLL CONTENT

In the control seedlings, chl *a* and *b* content increased with exposure time (Fig. 4a,b). During the first 14 days, pigment content increased in seedlings treated with 1 μM Cr(VI) and decreased in seedlings treated with 10 μM and 100 μM Cr(VI). In the 100 μM Cr(VI) treatment, chl *a* decreased 76.0% and chl *b* decreased 58.8% versus the control at 21 days. The 100 μM and 10 μM Cr(VI) doses markedly lowered the chl *a*/*b* ratio at 14 and 21 days (Fig. 4c).

EFFECTS OF Cr(VI) ON CHLOROPHYLL FLUORESCENCE

The chlorophyll fluorescence parameters of the maize seedlings varied with the Cr(VI) concentration and duration of treatment (Fig. 5a–e). F_v/F_m decreased in a concentration-dependent manner, but increased with time in the control and treated groups. The quantum yield of PSII electron transport (ΦPSII) did not differ between the 1 μM treat-

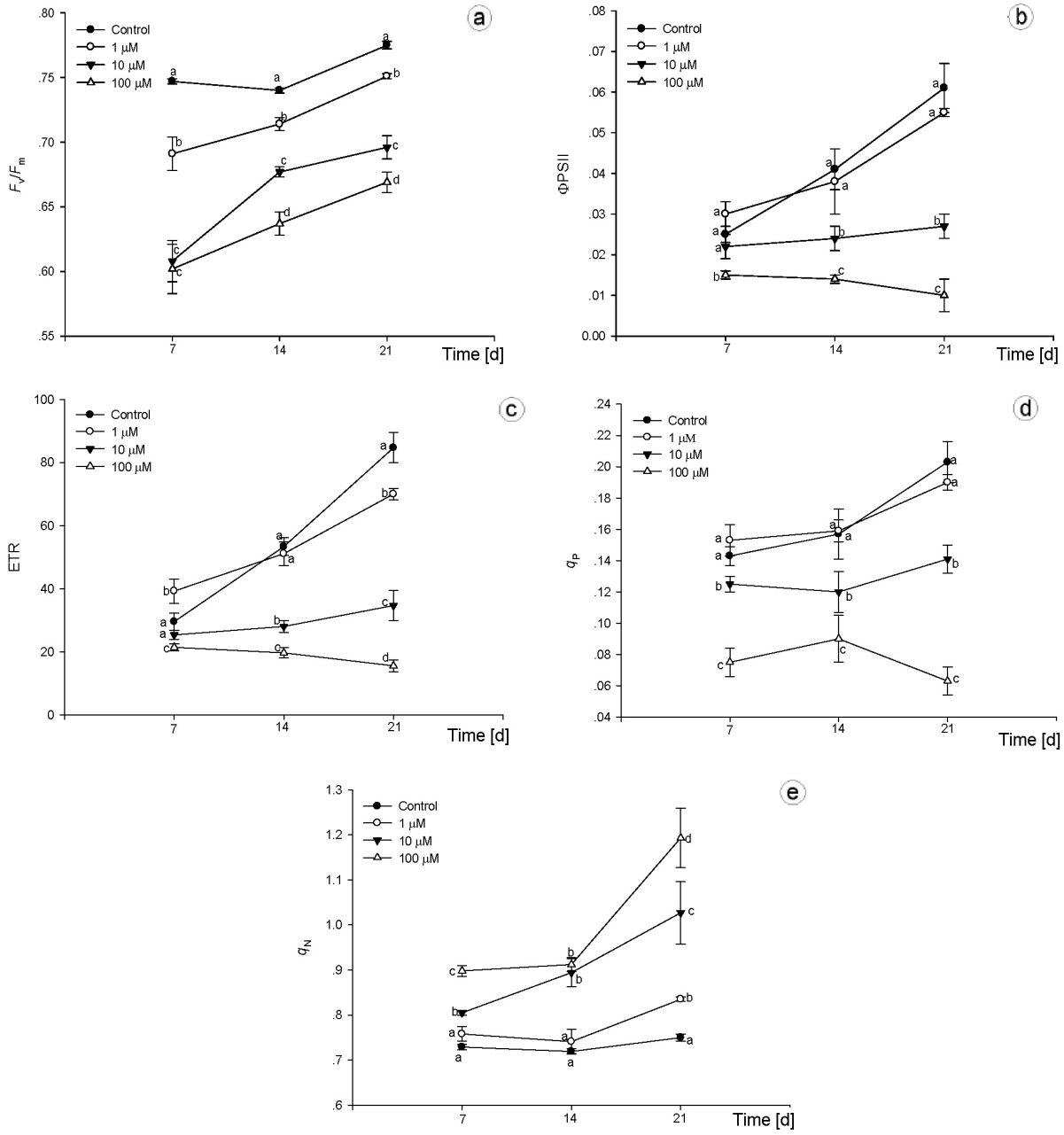

Fig. 5. Effects of different Cr(VI) concentrations on F_v/F_m, ΦPSII, ETR, q_P, q_N in maize seedlings stressed for 7, 14 and 21 days. (**a**) F_v/F_m, (**b**) ΦPSII, (**c**) ETR, (**d**) q_P, (**e**) q_N. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other ($p < 0.05$, t-test).

ment and the control during the whole course of treatment. In the 10 μM Cr(VI) treatment ΦPSII was lower than the control after 14 and 21 days of exposure, and in the 100 μM Cr(VI) treatment it was lower during the whole experiment. At 10 μM Cr(VI), the electron transport rate (ETR) was lower than in the control after 14 and 21 days of exposure, and at 100 μM Cr(VI) it was lower during the whole treatment period. Photochemical quenching (q_P) did not differ from the control at 1 μM Cr(VI), but was significantly lower at 10 μM and 100 μM Cr(VI). Cr(VI)-induced nonphotochemical quenching (q_N) trended inversely to q_P, registering a significant rise in Cr(VI)-treated samples during the entire treatment at the 10 μM and 100 μM doses.

DISCUSSION

In plants, reactive oxygen species are formed during electron transport activities of chloroplasts, mitochondria, peroxisomes and glyoxisomes (Jiang and Zhang, 2002). Lipid peroxidation level is considered the best criterion of damage caused by increasing ROS production. It occurs when ·OH radicals are generated close to cell membranes and attack the unsaturated fatty acid side chains of membrane lipids, resulting in the formation of lipid hydroperoxides (Bestwick et al., 2001). Accumulation of lipid hydroperoxides in membranes disrupts their function and can cause their collapse, leading to leakage and the loss of selective permeability (Saelim and Zwiazek, 2000).

Under stress, ROS production is high, producing oxidative damage. These ROS are extremely reactive and rapidly disrupt normal cell metabolism (Dawes, 2000). Membrane destabilization is generally attributed to lipid peroxidation, due to increased production of active oxygen species. In the present study, enhancement of lipid peroxidation after Cr(VI) treatment (Fig. 3a,b) suggests that Cr caused oxidative damage; it showed a state of oxidative stress most likely due to generation of ROS. Similar observations are reported in *Brassica juncea* L. (Pandey et al., 2005), rice seedlings (Panda, 2007) and *Pistia stratiotes* L. (Sinha et al., 2005).

Plant cells have evolved antioxidant defense mechanisms to combat the danger posed by the presence of ROS. These include enzymatic mechanisms involving antioxidant enzymes such as SOD, POD, and CAT (Meloni et al., 2003). Modification of the plant antioxidant defense system has been reported to enhance tolerance to oxidative stress (Rai et al., 2004; Mishra et al., 2006a,b; Meng et al., 2007; Dazy et al., 2008). Alteration of antioxidant enzymes may be due to the synthesis of new isozymes or enhancement of the activity of pre-existing enzymes for the metabolism of ROS (Kang et al., 1999). The activities of antioxidant enzymes we recorded in maize roots and leaves (Fig. 4–6a,b) indicate that the enzymes are engaged in antioxidant defense. As compared to the control, SOD, CAT and POD activities were altered by the different concentrations of Cr(VI) after 7–21 days of exposure. Similar results were reported for SOD and APX in *Pistia stratiotes* plants exposed to Cr concentrations of 10–160 μM (Sinha et al., 2005) and for SOD, CAT and POD in the aquatic moss *Fontinalis antipyretica* Hedw. exposed to Cr concentrations ranging from 62.5 μM to 6.25 mM (Dazy et al., 2008).

The first line of defense against ROS-mediated toxicity involves SOD, which catalyzes the dismutation of superoxide radicals to H_2O_2 and O_2. The significant increase in SOD activity in roots and leaves at the low concentration (1 μM) of Cr(VI) demonstrates this defense mechanism. Peroxidase and CAT are two potent scavengers of H_2O_2, which minimize its accumulation and diffusion across cell membranes, preventing peroxidative damage to cell constituents. Enhancement in the activities of both POD and CAT was recorded at low Cr(VI) stress in roots as well as in leaves, and this possibly contributed to enhancement of scavenging of H_2O_2 in these plants. After the treatment with 100 μM Cr for 21 days, some decreases in the activities of SOD and POD in roots and leaves and CAT activity in roots were observed as compared to the activities after 14 days, which can be interpreted as a sign of cytotoxicity due to overproduction of ROS. Another explanation of our results could be that SOD activity decreased because of the binding of Cr ions to the active center of the enzyme (Stroinski and Kozlowska, 1997).

Photosynthesis was reduced significantly by increasing Cr(VI) concentrations in the growth medium. We also noted modification of total chlorophyll content and the chl *a*/*b* ratio. Chl *a* and *b* content decreased significantly in the 100 μM and 10 μM Cr(VI) treatments, and the inhibition strengthened with the duration of stress (Fig. 4a,b), showing the negative effect of Cr(VI) on processes of chlorophyll biosynthesis. The increase in chl *a* and *b* content at the 1 μM Cr(VI) dose during the first 14 days suggested that low concentrations of Cr(VI) stimulate maize growth, in accord with the results for root length and plant height (Fig. 1a,b).The decrease in chlorophyll content with increasing Cr(VI) dose has been described in the literature repeatedly (Liu et al., 2008). It is thought to be related to direct inhibition of chlorophyll pigment synthesis. Other authors have reported a decrease in chlorophyll content correlated with metal concentration (Panda and Choudhury, 2005; Scoccianti et al., 2006). Chromium possesses the capacity to degrade δ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting δ-aminolevulinic acid (ALA) utilization; this results in the buildup of ALA and reduction of the level of chlorophyll (Vajpayee et al., 2000). Chromium, mostly in its hexavalent form, can replace Mg ions from the active sites of many enzymes. Cr(VI) also causes Fe deficiency in stressed plants, disrupting chlorophyll biosynthesis (Zou et al., 2006; Liu et al., 2008). In our investigation the chl *a*/*b* ratio decreased when the plants were exposed to 10 μM and 100 μM Cr(VI), perhaps due to faster breakdown or decreased synthesis of chl *a* as compared to chl *b*, although chl *b* also decreased (Vajpayee et al., 2000; Appenroth et al., 2003).

The chl *a* fluorescence emission technique has improved our understanding of photochemical and nonphotochemical processes occurring in thylakoid

membranes of chloroplasts. The F_v/F_m ratio, the maximum quantum yield of PSII photochemistry, is frequently used as an indicator of photoinhibition or of other kinds of stress to photosystem II (Calatayud et al., 2002; Calatayud and Barreno, 2004). In our experiment, F_v/F_m was variously reduced by the different concentrations of Cr(VI), strongly suggesting impairment of PSII. Reduction of PSII photochemical efficiency can be attributed in part to the destruction of antennae pigments (Calatayud and Barreno, 2004), as borne out by the results of chlorophyll content assays in our study (Fig. 4a,b). Similar changes in the F_v/F_m ratio were observed in crop varieties (Calatayud et al., 2002). The F_v/F_m ratio may decrease as a result of an increase in protective nonradiative energy dissipation (nonphotochemical quenching) associated with a regulated decrease in photochemistry and photosynthesis rates, photodamage of PSII centers, or both, manifest in the decrease of q_P values and increase of q_N values in Cr-treated plants. After steady-state photosynthetic induction there was a decrease in actual PSII photochemical efficiency (ΦPSII), which is closely correlated with the quantum yield of non-cyclic electron transport observed in plants (Subrahmanyam and Rathore, 2000). Reduction of ΦPSII might be explained by decreased carbon metabolism capacity and/or by low utilization of ATP and NADPH in the dark phase of photosynthesis (Subrahmanyam and Rathore, 2000). The reduced q_P values for the Cr(VI)-treated maize indicate that Cr(VI) decreased the capacity for reoxidizing QA during actinic illumination and increased the excitation pressure on PSII. A decrease in q_P and F_v/F_m is known to be closely associated with photoinhibition (Calatayud and Barreno, 2004). On the other hand, the decreases in photochemical quenching (q_P) were associated with increases in nonphotochemical quenching (q_N), which may reflect the capacity of plants to carry out non-radiative dissipation of excess energy.

In our experiments, q_N increased considerably with increasing Cr(VI) concentration (Fig. 5e). The mechanism of q_N in maize is still not clear. One possibility is that Cr(VI)-induced inhibition of PSI activity was higher than the inhibition of PSII activity (Murthy and Mohanty, 1991). In our study the energy distribution in PSII showed differences between the treatments and the controls. High Cr(VI) limited the fraction of light absorbed in the PSII antennae used in PSII photochemistry (%P). In our work, on the other hand, the %X fraction increased remarkably in maize leaves. This fraction is the least desirable pathway since it may lead to de-excitation of singlet chlorophyll (Demmig-Adams et al., 1996). This can be related to Calatayud and Barreno's (2004) findings of a lower fraction of excitation energy utilized for photochemistry, and a significant fraction accumulated in chl *a*, chl *b* and total carotenoids in lettuce in the PSII antennae. The accumulated energy in chlorophyll may subsequently be transported to O_2 and turn it into harmful singlet oxygen. Meanwhile the reduction of CO_2 assimilation reduces the need for ATP and NADPH, leading to accumulation of NADPH and to a shortage of $NADP^+$. Then the excess excited energy cannot be accepted by $NADP^+$, but accepted by O_2 to produce superoxide anion radicals, causing oxidative damage such as lipid peroxidation and thereby engaging the antioxidant systems.

CONCLUSION

Various physiological processes were affected in maize growing in Cr(VI)-spiked nutrient solution. As a result of chromium-induced oxidative stress, MDA content increased with the Cr(VI) concentration and duration of treatment, reflecting enhancement of lipid peroxidation. Plants have evolved antioxidant defense mechanisms to combat oxidative stress. The activities of SOD, CAT and POD were altered differently in the different Cr(VI) treatments for 7–21 days. On the other hand, chl *a* and *b* content and the chl *a*/*b* ratio significantly decreased in the 100 μM and 10 μM Cr(VI) treatments over time. Chromium(VI) had a significant effect on the primary photochemistry of PSII; photoinhibition occurred in leaves at high concentrations of Cr(VI). Alteration of PSII photochemistry regulated ΦPSII to match the decreased requirements for ATP and NADPH due to decreased CO_2 assimilation capacity induced by Cr(VI) excess. The results suggest that excess Cr(VI) was associated with high accumulation of inactivated PSII reaction centers and a higher fraction of the reduced state of QA. However, our results were laboratory-based; before exploiting the results in the field, a pilot field study is recommended. Natural variables (temperature, pH, light, soil quality, etc.) may affect the results.

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