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Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant

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Abstract

Increasing human and industrial activities lead to heavy metal pollution. Heavy metal chromium (Cr) is considered to be a serious environmental contaminant for the biota. Phytotoxic effects of Cr were studied in wheat plants. Growth parameters were largely inhibited as a result of disturbances in the plant cell metabolism in response to Cr toxicity. Chromium toxicity led to decline in a number of active reaction centres of PSII, rate of electron transport, and change in PSII heterogeneity. Chromium did not cause any change in heterogeneity of the reducing side. A significant change in antenna size heterogeneity of PSII occurred in response to Cr toxicity. Chromium seems to have extensive effects on the light harvesting complex of PSII.

Additional key words: Chl *a* fluorescence; growth; photosystem II; PSII heterogeneity; wheat.

Introduction

Heavy metals (HM) are innately present as trace elements in environment and their presence is marked in soil, water *etc*. The term „heavy metals“ refers to any metallic element that has a relatively high density and is toxic or poisonous even at a low concentration. In general, the term applies to the group of metals and metalloids with atomic density greater than 4 g cm^{-3}, five times or more greater than water (Hawkes 1997). However, chemical property of HM is regarded as a more influencing factor in comparison to its density (Gill 2014). Their presence in soils is in the form of free metal ions, soluble metal complexes, organically bound metals, exchangeable metal ions, precipitated or insoluble compounds, such as carbonates and oxides (Shanker *et al.* 2005). The excessive augmentation of HM in nature is causing hazardous affects on plants, animals, and human. Once these HM cross the threshold and enter the food chain through any of sources, their mushrooming is impossible to end. HM stress triggers different responses in plants, ranging from biochemical responses to crop yield.

Amongst HM, the effects of Cr on living organisms have received highlighted attention due to strong toxicity and a relatively less known mode of action. Soil and ground water contamination due to excessive use of Cr by various anthropogenic activities has become a serious problem for plants and animals over the past few decades (Gill *et al.* 2015, Shanker *et al.* 2005). Cr is a transition element classified into the group VI–B of the periodic table with a ground-state electronic configuration of Ar $3d^5 4s^1$. The stable forms of Cr are the trivalent Cr(III) and the hexavalent Cr(VI) species, although there are various other valence states which are unstable and short lived in biological systems. Cr(VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. These compounds are widely engaged in leather processing and finishing, in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture, and in the production of chromic acid and special chemicals. Hexavalent Cr

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Abbreviations: ABS – absorption; Chl *a* – chlorophyll *a*; CS – cross section; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; Di_0 – dissipation; DM – dry mass; ET_0 – electron transport; F_0 – initial fluorescence; F_M – maximum fluorescence; FM – fresh mass; F_V – variable fluorescence; HM – heavy metal; OJ, JI, IP – phases of Chl *a* fluorescence induction curve; PEA – plant efficiency analyser; PQ – plastoquinone; RC – reaction centre; TR_0 – trapping.
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compounds are mainly used in various industries for metal plating, cooling tower water treatment, hide tanning, and wood preservation. The toxic properties of Cr(VI) originate 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) occurring inside the cell.

Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Presence of Cr in the external environment influences growth and development of plants. It has been found that Cr caused stunting plant growth, chlorosis in new leaves, wilting of tops, impaired photosynthesis, damage of roots, and finally plant death (Sharma *et al.* 2003, Scoccianti *et al.* 2006). The overall adverse effect of Cr on growth and development of plants could be serious impairment in uptake of mineral nutrients and water leading to deficiency in shoots. In addition, the normal mechanism of selective inorganic nutrient uptake might be destroyed by oxidative damage, thus permitting larger quantities of Cr(VI) to enter roots passively and further translocation of Cr(VI) to shoots causing oxidative damage to the photosynthetic and mitochondrial apparatus eventually reflected in poor growth. Furthermore, plants growing in Cr-stressed environments also stimulate the formation of reactive oxygen species (ROS), which can harm the production of biomolecules, such as lipids, proteins, and nucleic acids, thereby, interrupting both mitochondrial respiration and carbohydrate metabolism (Gill and Tuteja 2010, Gill *et al.* 2015). Cr stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation, and enzyme activities. However, it is not well understood to what extent Cr-induced inhibition of photosynthesis occurs due to disorganization of chloroplasts ultrastructure (Vázques *et al.* 1987), inhibition of electron transport or the influence of Cr on enzymes of the Calvin cycle. It was investigated that Cr-toxicity caused ultrastructural changes apparent as poorly developed lamellar system with widely spaced thylakoids and fewer grana (Ali *et al.* 2013a). This aberration in thylakoid membrane might have some negative impact on photosynthesis and excitation energy transfer imbalance may also occur (Ali *et al.* 2013a). Nevertheless, it is not clear at what concentration Cr induces the ultrastructural changes in chloroplasts and causes the inhibition of photosynthesis (Ali *et al.* 2013a,b; Gill *et al.* 2015).

In this study, we estimated photosynthetic efficiency of Cr-stressed plants by the technique of fast chlorophyll (Chl) *a* fluorescence induction measurement which were analysed to obtain comprehensive information on effects of Cr on the primary events and electron transport of photosynthesis. Fast Chl *a* fluorescence induction is an informative tool for studying the effects of various environmental stresses on the process of photosynthesis, particularly on the function of PSII and its reactions (Lazár 2006, Tomar and Jajoo 2013, Kalaji *et al.* 2014, Brestič *et al.* 2015). The first second of the fast induction curve is characterized by three apparent kinetic steps denoted OJ, JI, and IP. These phases of fluorescence induction have been correlated with peak accumulation of different reduced species on the acceptor side of PSII. Due to potential destructive capability of HM on biological membranes, photosynthetic pigments, and proteins at cellular level, effects of Cr(VI) could be assessed by means of Chl fluorescence characterizing alterations in the functioning of PSII. Aim of this study was to understand more precisely the role and target of chromate on the photosynthetic apparatus. This study combined growth parameters along with changes occurring at PSII level, especially PSII heterogeneity, which has received strong attention recently because of its role in adaptation to stress conditions.

Materials and methods

Plant material: Wheat (*Triticum aestivum;* Purna HI 1544) cultivar was used as plant material. Uniform seeds were selected for sowing.

Plant cultivation/growth: Wheat seeds were sown in soil and allowed to germinate in 15 cm (length) plastic pots containing 700 g of soil. Ten seeds were sown in each pot. After seedling emergence, ten seedlings were reduced/thinned to six seedlings per pot. The plantlets were allowed to grow under control conditions in a culture room. The plants were grown under 8/16 h of light/dark regime. The plants were allowed to grow till 3–4 mature leaf stage. Cultivation was done under PPFD of 300 μM m^{-2} s^{-1} at 22–25°C ± 2°C. The plants were daily replenished with normal tap water (control) and with different concentrations of Cr until 40 d. All the measurements were performed with different concentrations of Cr on 25, 35, and 40 d (DAT). Since maximum changes were observed after 25 DAT and leaves started to become yellow after 30 DAT, the data obtained after 25 DAT were discussed in this paper.

Chromium treatment: Cr was used in dichromate form to detect toxicity in plants. It was prepared from potassium dichromate (*Fisher Scientific Qualigens, Thermo Fisher Scientific;* India). The stock solution (20 mM $K_2Cr_2O_7$) was prepared. For the treatment, we used 100 μM, 200 μM, and 300 μM concentrations of Cr. The plants without any Cr were considered as control.

Growth and germination: To measure a rate of germination in plantlets, 50 seeds were used per one Petri plate. The germination rate was expressed as the percentage of plantlets germinated from the total number of seeds (100%). Fresh and dry mass (FM and DM) of shoots was determined in five replicates (three plants of each set

randomly selected) of the control and treated plants. For DM estimation, the individual plants were oven-dried at 80ºC for 4 h.

Fast Chl *a* fluorescence induction kinetics was measured at room temperature using a plant efficiency analyzer (*PEA*, *Hansatech*, England). The light was provided by an LED array of 650 nm focused onto the sample to provide homogeneous irradiance over the exposed area (d = 4 mm). Light intensity reaching the leaf was 3,000 μmol(photon) m^{-1} s^{-1} which was sufficient to generate maximal fluorescence (F_M) for all the treatments. All samples were dark-adapted for 30 min prior to the measurements. The JIP-test is named after the basic steps in the fluorescence transient when plotted on a logarithmic time scale. The shape of the O–J–I–P fluorescence rise has been related to a major change in the photosynthetic electron transport (Joly and Carpentier 2009, Papageorgiou and Govindjee 2011). Control leaves exhibited a polyphasic rise called O–J–I–P Chl *a* fluorescence transient. The fluorescence intensities at 50 μs (O-step), 2 ms (J-step), and 30 ms (I-step) and 500 ms (P-step) were denoted as as F_0, F_J, F_I, and F_M respectively [Strasser *et al.* 2004 (Fig. 1), Chen and Cheng 2009], as described in Appendix. Measurements were done 5 cm away from tip and base of the plants, *i.e*., in the middle part of the fully developed leaves. Fifteen to twenty measurements were done for each replicate.

Energy pipeline leaf model and flux ratios: *Biolyzer HP-3* software was used to deduce energy pipeline leaf model [*i.e*., activities expressed per excited cross section (CS) and flux ratios]. Yield or flux ratios were calculated according to Mathur *et al.* (2013), Mathur and Jajoo (2015), and Strasser *et al.* (2004) and are described in Appendix.

PSII heterogeneity

Antenna heterogeneity: For determination of antenna heterogeneity, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) poisoning method was used (Hsu *et al.* 1989). The method was previously described in Tóth *et al.* (2005) and Mathur *et al.* (2011). Alpha (α), beta (β), and gamma (γ) centers were calculated from the complementary area of the growth curve. The antenna heterogeneity was also calculated on the basis of connectivity by the method described in Tóth *et al.* (2005) and Mathur *et al.* (2011).

Heterogeneity of the reducing side: Double hit (pulse) method was followed for the calculation of Q_B reducing and Q_B-nonreducing centres (Strasser and Tsimilli-Michael 1998). In this method, two fluorescence transients were induced by two subsequent pulses each of 1-s duration. Q_B-reducing and Q_B-nonreducing centres were calculated as described in Mathur *et al.* (2011).

Statistical analysis: Data was analyzed by using *Graph pad Prism 5.01* software (*La Jolla*, CA, USA). Results were analyzed using one way analysis of variance (*ANOVA*) followed by *Newman-Keul*'s multiple comparison test. Significance was determined at $p<0.001$ ($^*p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$) and the results are expressed as mean values and standard deviation (SD) and all assays were carried out in replicates (six sets of each analysis).

Results and discussion

Growth parameters: HM, especially Cr, phytotoxicity has negative effects on the crop yield. Plant morphology in terms of leaf length, number of leaves, and leaf area per plant, FM, DM, and percentage of the plants germinated per pot were studied in response to various Cr concentrations (Table 1). Cr interferred with various metabolic processes and was toxic to the plants; it was exhibited by stunted growth, reduced root growth, phytomass chlorosis, photosynthetic impairing, stunting, and finally by plant death (Table 1). In comparison with control (100% plantlet germination), the rate of germination remained only 30% at higher Cr concentrations (Table 1). The reduced germination of plantlets could represent a depressive effect of Cr on the activity of amylases (Shanker *et al.* 2005).

It is established that the leaf number, leaf growth area, *etc*. determine the crop yield. Table 1 clearly indicates that the leaf numbers decreased drastically after the treatment with 200 and 300 μM Cr. Deleterious effects of Cr was maximal on shoot and leaf growth and development. The reduced plant height at higher Cr concentrations may be due to reduced root growth, as reported earlier and results in lesser nutrients and water uptake in plants (Shanker *et al.* 2005). Wilting, yellowing of the whole plant, and necrosis followed by early senescence was the main physiological change observed in our study in wheat plants in response to enhanced Cr concentration.

Primary condition for the higher crop yield and production in plants is estimated in terms of enhancement in biomass production. Biomass production in terms of FM and DM showed also a negative trend in response to the increased Cr concentrations indicating that Cr had phytotoxic effects at the higher concentrations. DM was severely affected by the increasing Cr concentrations (Table 1). The overall adverse effect of Cr on growth and development of wheat could be due to impairment of uptake of mineral nutrients leading to retarded plant growth. This reduction in plant growth in response to Cr during early stages affects the crop yield of wheat in later stages.

Table 1. Effect of various Cr concentrations on different growth parameters in wheat plants. Each experiment is a repetition of six replicates with five plants each. Values are given as mean ± SD. Significant differences were calculated according to *Newman-Keuls*' multiple comparison test at $p<0.001$.

Cr treatment [μM]	Leaf length [cm]	Fresh mass [%]	Dry mass [%]	Plant germinated per pot [%]
Control	27.5 ± 2	100 ± 1	100 ± 1	100 ± 1
100	18.3 ± 2***	57 ± 1***	50 ± 1***	80 ± 2***
200	12.5 ± 2***	80 ± 1***	55 ± 2***	50 ± 2***
300	7.5 ± 3***	87 ± 2***	75 ± 2***	30 ± 1***

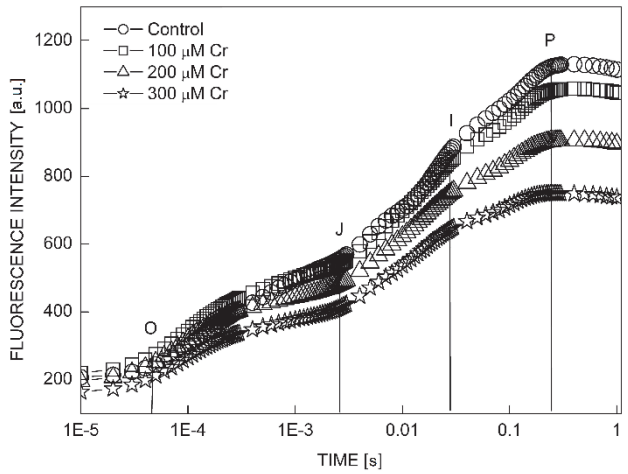

Fig. 1. Change in Chl *a* fluorescence induction curves (OJIP) plotted on logarithmic scale in control and various Cr concentration in wheat plants.

Effect of Cr on Chl *a* fluorescence parameters: Inhibition in the plant growth parameters was a result of disturbances in the plant cell metabolism in response to Cr toxicity. Cr interferes with photosynthetic processes and thus Chl *a* fluorescence measurement can reveal the inhibition caused by Cr at different levels in the photosynthetic apparatus. The control plants exhibited a polyphasic rise called O–J–I–P transient (Fig. 1). The increase in the Cr concentration caused significant inhibition of F_M while no change was observed in F_0 (Fig. 1). The J, I, and P phase was inhibited with the increasing Cr concentration which might occur due to two reasons: first, by inhibition of electron transport at the donor side of PSII which results in the accumulation of $P680^+$; second, due to a decrease in the pool size of Q_A^- (Govindjee 1995, Neubauer and Schreiber 1987) which was also evident by a decrease in area (Table 2). Changes in various fluorescence parameters were observed in response to the increasing Cr concentration (Table 2). The ratio of F_V/F_M represents the conversion and capture efficiency of primary light energy and is an excellent measure for the evaluation of quantum yield of primary photochemistry of PSII (Butler and Kitajima 1975, Strasser 2004, Subrahmanyam 2008, Mathur and Jajoo 2015) (Appendix). A gradual decline in F_V/F_M ratio (Table 2) suggested that Cr decreased the quantum efficiency of PSII photochemistry either by causing a decrease in the rate of primary charge separation or by disconnection of some minor antenna from PSII. Area represents the area over the fluorescence induction curve between F_0 and F_M and is proportional to the pool size of the electron acceptor Q_A on the reducing side of PSII and also Q_B, PQ, and PSI acceptors (Strasser *et al.* 2004). Linear drop in the area with the enhanced Cr concentration indicated that the electron transfer from the reaction center to quinone pool was blocked (Table 2, Fig. 1). Nearly 61% decrease was obtained in the area at 300 μM Cr. The density of active PSII reaction centers per Chl and the antenna size of Chl molecules are represented by the ratio of RC/ABS (Christen *et al.* 2007, Mathur and Jajoo 2015). The Cr treatment decreased the number of active reaction centres per Chl (Table 2). As compared with the control, the ratio of F_V/F_0 decreased by 20, 27, and 30% in 100, 200, and 300 μM Cr, respectively. This shows that maximum damage to water splitting complex of PSII occurred at 300 μM (Kalaji *et al.* 2012). This decrease also indicated an impairment of PSII photochemistry (Chen and Cheng 2009, Essemine *et al.* 2012, Kalaji *et al.* 2012).

Table 2. Effect of various Cr concentrations on different chlorophyll (Chl) *a* transients in wheat plants. Each experiment is a repetition of six replicates with five plants each. Values are given as mean ± SD. Significant differences were calculated according to *Newman-Keuls*' multiple comparison test at $p<0.001$. F_V/F_M – quantum yield of primary photochemistry of PSII; F_V/F_0 – conformation term for primary photochemistry; $PI_{(ABS)}$ – performance index on the basis of absorbance; RC/ABS – density of active PSII reaction centers per Chl and the antenna size of Chl molecules.

Cr treatment [μM]	F_V/F_M	Area	RC/ABS	F_V/F_0	$PI_{(ABS)}$
Control	0.823 ± 0.03	29620 ± 74	0.939 ± 0.01	4.655 ± 1	26 ± 1
100	0.788 ± 0.02**	24778 ± 87***	0.764 ± 0.02***	3.707 ± 1**	17 ± 1***
200	0.773 ± 0.01**	17626 ± 75***	0.686 ± 0.04***	3.398 ± 1**	13 ± 2***
300	0.768 ± 0.02**	11548 ± 58***	0.678 ± 0.05***	3.31 ± 1**	12 ± 1***

$PI_{(ABS)}$ represent the performance index on absorbance basis. $PI_{(ABS)}$ is a very sensitive index for stress and is used widely to compare the whole primary photochemical reactions (Chen and Cheng 2009) (Appendix) because it combines three main structural and functional characteristics of PSII, (*1*) a component referring to the density of active PSII reaction centres per Chl (RC/ABS), (*2*) a component that describes the performance of the light reactions as $\Phi_{PO}/(1 - \Phi_{PO})$, and (*3*) a component that describes performance of the dark redox reactions defined as $\psi_O/(1 - \psi_O)$. The performance index $PI_{(ABS)}$ is derived according to the principles of the Nernst equation for redox reactions (Christen *et al.* 2007). $PI_{(ABS)}$ decreased around 35, 50, and 54% with 100, 200, and 300 μM of Cr (Table 2), respectively. This shows that the primary photochemical reactions were affected with the higher Cr content.

An alteration of PSII energy fluxes in response to Cr phytotoxicity was also visualized by energy pipeline models of photosynthetic apparatus. Fig. 2 depicts energy pipeline leaf model that refers to the leaf cross-section (CS). This model deals with the phenomenological fluxes (Appendix). Leaf models were deduced using *Biolyzer HP-3* software. This is a dynamic model, where the energy fluxes in the control and Cr-treated plants were expressed by the width of the corresponding arrows. The model also shows the changes in the active and inactive PSII reaction centres per cross-section (RC/CS), as well as the flux of dissipated excitation energy at time zero (DI_0). Various parameters, such as the efficiency of light absorption, trapping, and electron transport and dissipation per cross section of PSII, are indicated by ABS/CS_m, TR/CS_m, ET/CS_m and DI_0/CS_m, respectively (where CS_m corresponds to F_m). It is evident that the increasing Cr concentrations declined ABS/CS_m, ET_0/CS_m, TR_0/CS_m ratios (Fig. 2). The decline in ABS/CS_m indicated a decrease of the energy absorbed per excited cross-section (Tsimilli-Micheal and Strasser 2008). Electron transport decreased after the Cr treatment as compared with the control plants indicating lower energy absorption by antenna pigments (ABS/CS_m) and inactivation of reaction centre complexes. Cr acted on active reaction centers producing dissipative sinks for excitation energy (Appenroth *et al.* 2001). The ratio of TR_0/CS_m gradually decreased with the increasing Cr concentration indicating that trapping of reaction centers was affected significantly. The decrease in the density of active reaction centers and the increase in the density of closed reaction centers (also known as inactive centers) was observed as a negative effect of Cr. The change in trapping could be a result of modified ABS (Fig. 2).

Further analysis of the Cr treatment was done on the basis of parameters, such as δ_{R0}, φ_{R0}, $SF_{(ABS)}$, DF, φ_{E0} (Appendix), which are considered as electron transport parameters since these parameters furnish actual status of the electron transport rate in PSII. These parameters decreased with the increasing Cr concentrations. δ_{RO}

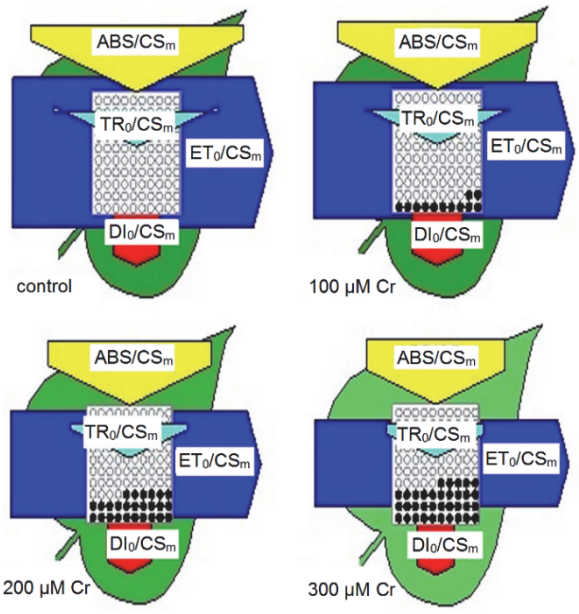

Fig. 2. Energy pipeline leaf model showing changes in phenomenological fluxes or apparent activities per cross section (CS) for control and different Cr concentrations. The fluxes used represent light absorbance (ABS), excitation energy trapping (TR), electron transport (ET), and energy dissipation (DI_0) beyond Q_A^-. *Open circles* (active) and *closed circles* (inactive) – PSII reaction centres per cross-section (RC/CS). For description of parameters, *see* text and Appendix.

(RE_0/ET_0) indicates efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (Chen and Cheng 2009). δ_{RO} is dependent on electrons transferred from PQH_2 to PSI. δ_{RO} decreased slightly after enhanced Cr exposure suggesting that Cr reduced the efficiency of electrons to reach PSI end electron acceptors. φ_{RO} represents quantum yield of the electron transport from Q_A- to the PSI end electron acceptors (Chen and Cheng 2009). The value of φ_{R0} did not decrease significantly in both 200 and 300 μM Cr (Fig. 3) indicating the quantum yield of electron transport from Q_A- to PSI end electron acceptor decreased. It is also supported by the fact that Q_A- was unable to reduce back due to Cr stress. $SFI_{(ABS)}$ is the product of three independent parameters, RC/ABS, φ_{PO}, Ψ_0. These three have been used as an expression, which represents an index combining functional and structural criteria of PSII, denoted as the structure function index for photosynthetic system (Srivastava *et al.* 1999, Appenroth *et al.* 2001). It is represented as:

$$SFI_{(ABS)} = (Chl_{RC}/Chl_{tot}) \times \varphi_{PO} \times \Psi_O$$

A decrease in $SFI_{(ABS)}$ was observed with the increasing Cr concentration. This indicated that Cr caused toxic effects and plants were unable to cope with stress conditions. The driving force (DF) (Srivastava *et al.* 1999), which quantifies the potential of plant photosynthesis, decreased with the enhanced Cr concentration (data not

shown) and indicated that plants were under stress. φ_{EO} has direct impact on the electron transport and represents the quantum yield for the electron transport at t = 0. Cr decreased the linear electron transport rate (also expressed as probability that an absorbed photon moves an electron further than to Q_A-) which was depicted by a decreased φ_{EO} value (Fig. 3). Retardation in electron transport processes due to Cr and a diversion of electrons from the electron-donating side of PSI to Cr is a probable justification for Cr induced decline in the photosynthetic rate. It is likely that electrons produced by the photochemical process were not essentially used for carbon fixation as supported by the decreased photosynthetic rate in the Cr-treated wheat plants. Due to the known oxidative potential of Cr, it is possible that alternative sinks for electrons possibly were enhanced by reduction of molecular oxygen (part of Mehler reaction) which to some extent explains the oxidative stress caused by Cr (Shanker *et al.* 2005).

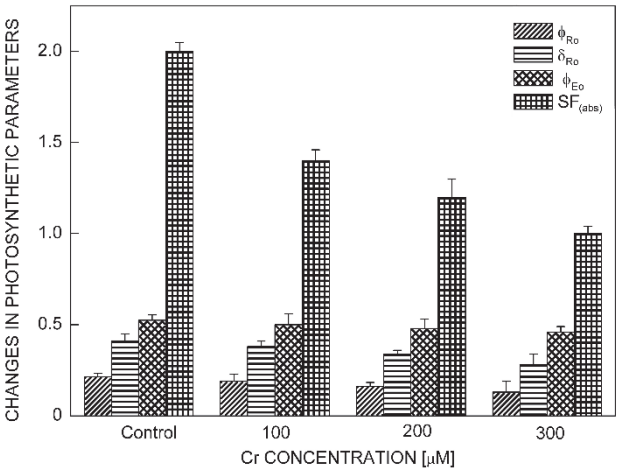

Fig. 3. Changes in various ratios of dark-adapted wheat plants derived from OJIP curves in response to enhanced Cr concentrations. All the values are relative to the control. For description of the parameters *see* text and Appendix.

Effect of Cr toxicity on PSII heterogeneity: Different from other pigment protein complexes participating in photosynthetic light reaction, PSII shows diversity in its nature in functional and structural aspects known as PSII heterogeneity. Investigations of PSII heterogeneity in early stages of plant's life cycle is a promising measure to detect stress conditions and tolerance in plants. PSII has been found to undergo changes in its structural and functional heterogeneity in response to different abiotic stresses, such as salinity (Tomar *et al.* 2012), organic pollutants (Tomar and Jajoo 2015), and high temperature stress (Mathur *et al*. 2011).

By using kinetic analysis of fluorescence induction curve with DCMU-poisoned chloroplasts (Tóth *et al*. 2005, Mathur *et al*. 2011), PSII has been distinguished into three components on the basis of antenna size, *i.e.*, $PSII_\alpha$, $PSII_\beta$, and $PSII_\gamma$. This method can provide direct information about the lifetime and the relative proportions of PSII(α), PSII(β), and PSII(γ) under the increasing Cr concentrations in wheat plants. In case of antenna heterogeneity, increased Cr caused changes in the relative amount of α, β, and γ centers (Table 3). The proportion (in terms of percentage) of α, β, and γ centers was found to be 64:30:6 in control, 61:33:6 at 100 μM, 57:37:6 at 200 μM, and 50:40:10 at 300 μM, respectively. At 100 and 200 μM Cr concentration, the active α centers were converted into inactive β centers, while at 300 μM, the active α centers were converted into both β and γ centers. The maximum active α centers were converted to β centers. Alteration in the α, β, and γ center is associated with downregulation of photochemical activity, damage of photosynthetic apparatus, and changes in the antenna organization. Changes in antenna organization following the Cr treatment involved the dissociation of $PSII_\alpha$ into free LHCII and $PSII_\beta$ and the latter migrated from the grana to the nonappressed thylakoid membranes. The antenna heterogeneity was also calculated on the basis of connectivity (data not shown). According to the concept of connectivity (also called grouping), closed PSII reaction centers (RC) may transfer their excitation energy to the open neighbouring PSII units that results in sigmoidal fluorescence rise instead of exponential rise (Strasser *et al.* 2004, Tóth *et al.* 2005). Loosing connectivity (ungrouping) between two PSII indicated that the PSII units of treated plants lost connection and became unstable under the increased Cr concentrations. Complete loss in connectivity was observed at the higher Cr concentrations.

Table 3. Changes in PSII heterogeneity in terms of antenna size heterogeneity (showing percentage of α, β and γ centers) and amount of Q_B-reducing and nonreducing centers in response to different Cr concentrations. Each experiment is a repetition of six replicates with five plants each. Values are given as mean ± SD. Significant differences were calculated according to *Newman-Keuls*' multiple comparison test on $p<0.001$.

Cr treatment [μM]	α	β	γ	Q_B reducing centers [%]	Q_B nonreducing centers [%]
Control	64 ± 1	30 ± 1	6 ± 1	81 ± 1	19 ± 1
100	61 ± 2***	33 ± 1***	6 ± 1	80 ± 1***	20 ± 2
200	57 ± 2***	37 ± 2***	6 ± 2	80 ± 2**	20 ± 1
300	50 ± 1***	40 ± 2**	10 ± 1***	77 ± 1***	23 ± 2**

This indicated that all the PSII units were dissociated from each other due to the Cr treatment. A number of PSII centers, though photochemically competent, are unable to transfer electrons from electron acceptor Q_A^- to secondary electron acceptor Q_B. These centers are termed as Q_B-nonreducing centers. Reducing side heterogeneity was estimated by measuring relative amounts of Q_B-reducing and Q_B-nonreducing centers as described in Materials and methods. The population of Q_B-nonreducing centers was quantified. In this study, no significant change was observed in the number of Q_B-nonreducing centers (Table 3) in response to Cr stress.

Conclusion: We concluded that Cr is toxic at different stages of plant growth and development in wheat. It mainly interfered with the photosynthetic machinery. Chromium toxicity led to the decline in the number of active reaction centres of PSII, rate of electron transport, and change in PSII heterogeneity. Chromium did not cause any change in PSII heterogeneity of the reducing side while a significant change in the antenna size heterogeneity of PSII occurred in response to Cr toxicity. Chromium seemed to have extensive effects on the light harvesting complex of PSII. We conclude that Chl *a* fluorescence and PSII heterogeneity can be used as a tool for studying photochemical pathways of photosynthesis.

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Appendix

Derivation of the JIP-test parameters directly obtained from the recorded fluorescence transients [after Chen and Cheng (2009), Strasser *et al.* (2004)].

Fluorescence parameters	Description
F_0	Minimum reliable recorded fluorescence at 50 μs with the PEA
F_M	Maximum fluorescence
F_V	Variable fluorescence
Yields or flux ratios	
$\varphi_{P0} = TR_0/ABS = 1 - F_0/F_m = F_v/F_m$	Maximum quantum yield of primary photochemistry at t = 0
$\varphi_{E0} = TR_0/ABS = (F_v/F_m) \times (1 - V_J)$	Quantum yield for electron transport at t = 0
$\psi_0 = ET_0/TR_0 = 1 - V_J$	Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta_{R0} = RE_0/ET_0 = (1 - V_I)/(1 - V_J)$	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
$\Phi_{R0} = RE_0/ABS = \Phi_{P0} \times \psi_0 \times \delta_{R0}$	Quantum yield of electron transport from Q_A^- to the PSI end electron acceptors
$\Phi_{P0}/(1 - \Phi_{P0}) = TR_0/DI_0 = k_P/k_N = F_V/F_0$	Conformation term for primary photochemistry
Phenomenological fluxes or activities expressed per excited cross section (CS)	
$ABS/CS_m = F_m$	Absorption flux per CS at $t = tF_m$
$TR_0/CS = (TR_0/ABS)/(ABS/CS)$	Trapping at time zero, per CS
$ET_0/CS = (ET_0/RC)(RC/CS)$	Electron transport at time zero, per CS
$DI_0/CS = (ABS/CS) - (TR_0/CS)$	Dissipation at time zero, per CS
Vitality indexes	
$PI_{(ABS)} = [RC/ABS][\Phi_{P0}/(1 - \Phi_{P0})][\psi_0/(1 - \psi_0)]$	Performance Index on the basis of absorbance
$SFI_{(ABS)} = (Chl_{RC}/Chl_{tot})^* \varphi_{P0}^* \Psi_0$	Structure function index for photosynthetic system
$DF = \log PI_{(X)}$	Driving force for photosynthesis