BIOLOGIA PLANTARUM 53 (1): 100-104, 2009

Chromium increases photosystem 2 activity in *Brassica juncea*

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Abstract

In 7-d-old seedlings of *Brassica juncea* chromium (VI) promoted photosystem 2 (PS 2) mediated photoreactions. The increase in PS 2 activity in the thylakoids from Cr-treated seedlings, in the presence of uncoupler (5 mM NH_4Cl), was similar to that recorded with the control thylakoids. Thus Cr enhanced PS 2 activity was not due to uncoupling of electron transport from photophosphorylation. Photon saturation kinetics revealed that the PS 2 activity of thylakoids from Cr-treated seedlings was significantly higher at almost all irradiances in comparison to that of controls. PS 2 activity of thylakoids from Cr-treated plants at 25 % of the saturating irradiance was *in par* with the PS 2 activity of the thylakoids from control plants at saturating irradiance. Thylakoids from both control and Cr-treated seedlings exhibited maximum PS 2 activity at pH 7.5. The PS 2 activity of thylakoids from Cr-treated plants remained high even at pH 8.0 and 8.5, demonstrating Cr enhances tolerance of PS 2 to alkaline pH.

Additional key words: irradiance, NH_4Cl, pH curve, uncoupler.

Introduction

In nature chromium is largely found in either +3 or +6 oxidation states, as it is unstable/short-lived in other oxidation states (Arduini *et al.* 2006). Both hexavalent Cr(VI) as well as trivalent Cr(III) forms of Cr are phytotoxic and Cr(VI) is more toxic than Cr(III) (Nichols *et al.* 2000, Bal and Kasprzak 2002, Dixit *et al.* 2002, Mei *et al.* 2002). Cervantes *et al.* (2001) reported that uptake of Cr into plants depends on the carriers involved in the uptake of essential metals/anions. In general, plants exposed to toxic amounts of Cr exhibit overall reduction in growth by influencing essential metabolic events such as photosynthesis and respiration (Bishnoi *et al.* 1993a,b, Dixit *et al.* 2002, Shanker *et al.* 2005). Inhibition of seed germination, pigment degradation, nutrient imbalance, induction of oxidative stress, and disorganization of chloroplast and membrane ultrastructure are some examples of Cr phytotoxicity (Vajpayee *et al.* 1999, 2000, 2001, Zeid 2001, Samantary 2002, Han *et al.* 2004, Shanker *et al.* 2005, Pandey *et al.* 2005, Scoccianti *et al.* 2006, Sinha *et al.* 2006).

In order to identify/develop ideal plant system that, can be appropriately exploited to clean up the sites contaminated with Cr, it is vital to understand the basic mechanism(s) that promote uptake and detoxification/compartmentalization of Cr (Zayed and Terry 2003, Arduini *et al.* 2006). During the investigations carried out in this direction we had recorded an unusual increase in PS 2 activity in plants of *Brassica juncea* exposed to Cr(VI). This unique repeatable finding provoked us to characterize various processes linked to PS 2 activity in absence/presence of Cr(VI).

Materials and methods

Plants and growth conditions: Seeds of Indian mustard [*Brassica juncea* (L.) Czern. & Coss. cv. Varuna] were obtained from Dr. Katiyar, Indian Agricultural Research Institute, New Delhi, India. The seedlings were raised in glass bottles (18 × 8 cm) on cotton wetted with 100 cm^3 of Murashige and Skoog (1962; MS) basal liquid medium (Arora and Pardha Saradhi 1995) supplemented with various concentrations (0, 100, 200, 300, 400, and 500 µM)

Received 16 May 2007, *accepted* 8 November 2007.

Abbreviations: Chl - chlorophyll; Cr - chromium; DCIP - 2,6-dichlorophenol indophenol; DCMU - 3(3,4-dichlorophenyl)-1,1-dimethylurea; MES - 2-(N-morpholino)ethanesulfonic acid; MV - methyl viologen; pBQ - *p*-benzoquinone, PS 2 - photosystem 2.

Acknowledgements: We are grateful to the Department of Biotechnology (Govt. of India) for financial support. Shipra Gupta is also thankful to Council of Scientific and Industrial Research (Govt. of India) for Junior and Senior Research Fellowships.

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of Cr(VI) using potassium dichromate. Seedlings were grown in culture room at temperature of 25 ± 2 °C, 16-h photoperiod and irradiance of ~120 μmol m^{-2} s^{-1}. The cotyledonary leaves from 7-d-old seedlings were used for the isolation of thylakoids.

Isolation of thylakoids: After 40-min dark incubation in ice-cold isolation buffer (pH 7.6, consisting of 330 mM sorbitol, 5 mM NaCl, 1 mM $MgCl_2$, 2 mM EDTA, and 50 mM HEPES), the excised cotyledonary leaves were homogenized in the pre-chilled mortar and pestle in dark. The homogenate was filtered through 4 layers of muslin cloth and the filtrate was centrifuged at 500 *g* for 10 min at 4 °C. The pellet was washed and re-suspended in minimal volume of ice-cold suspension buffer (consisting of 330 mM sorbitol, 5 mM NaCl, 1 mM $MgCl_2$, 2 mM EDTA, 50 mM HEPES, and 1 mM sodium ascorbate, pH 7.5) (Alia *et al.* 1992). Chlorophyll (Chl) content of thylakoids was determined according to Arnon *et al.* (1974).

Estimation of photochemical activities: Photosystem(s) dependent O_2 evolution/consumption was assayed polarographically using Clark-type oxygen electrode (*Hansatech*, UK). The reaction mixture for *p*-benzoquinone (pBQ) supported PS 2 activity consisted of suspension buffer, 1 mM pBQ and 5 mM NH_4Cl used as uncoupler. The reaction mixture for 2,6-dichlorophenol indophenol (DCPIP) supported PS 1 activity consisted of suspension buffer, 0.5 mM DCPIP, 2 mM ascorbate, 0.4 mM 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2 mM sodium azide, and 0.1 mM methyl viologen (MV). Chloroplast equivalent to 10 μg of Chl cm^{-3} were used for each assay.

PS 2-dependent oxygen evolution was measured under different irradiances by using calibrated neutral density filters (*Blazers*, Berkhamsted, UK). The maximum irradiance used was 920 μmol(photon) m^{-2} s^{-1}. Variation in pBQ-supported PS 2 activity of isolated thylakoids in response to pH of the reaction mixture was also measured. For this experiment the reaction mixture consisted of 100 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 20 mM buffer [2-(N-morpholino)ethanesulfonic acid (MES) for pH 6.0 and 6.5, and HEPES for pH 7.0 and above].

Statistical analysis: All the experiments were independently carried out at least three times. For measuring growth (length, fresh mass and dry mass) 10 replicates were taken each time (making *n* = 30). Data shown in figures and tables represent means ± standard deviation (SD).

Results and discussion

Cr(VI) significantly curtailed growth of *Brassica juncea* cv. Varuna, and the reduction in height and fresh mass (FM) of the shoots intensified with increase in the Cr(VI) concentration (Fig. 1, Table 1). About 50 % reduction in height and shoot FM was recorded in the seedlings exposed to 200 μM Cr(VI) over control (*i.e.* in absence of Cr). Similar inhibitory effects of Cr on plant height and growth of shoots have been reported earlier (Bishnoi *et al.* 1993b, Rout *et al.* 1997, Barton *et al.* 2000, Mei *et al.* 2002). Han *et al.* (2004) found in *Brassica juncea* that Cr reduced number of cells belonging to palisade and spongy parenchyma in leaves. Shoot dry mass (DM) of seedlings exposed to Cr(VI) was significantly higher than that of control. For instance, the DM of shoots of seedlings exposed to 500 μM Cr(VI) was approximately 57 % higher than that of control. Presence of significantly higher dry matter in shoots of seedlings exposed to Cr(VI) could be due to reduction in utilization of stored organic skeletons in the cotyledonary leaves. This could be attributed to the negative impact of Cr(VI) on general metabolic events associated with breakdown of stored compounds in the cotyledons for plant growth and development. Cr(VI) has been well documented to inhibit cellular metabolism (Bishnoi *et al.* 1993a, Vajpayee *et al.* 2000, Zeid 2001, Shanker *et al.* 2005).

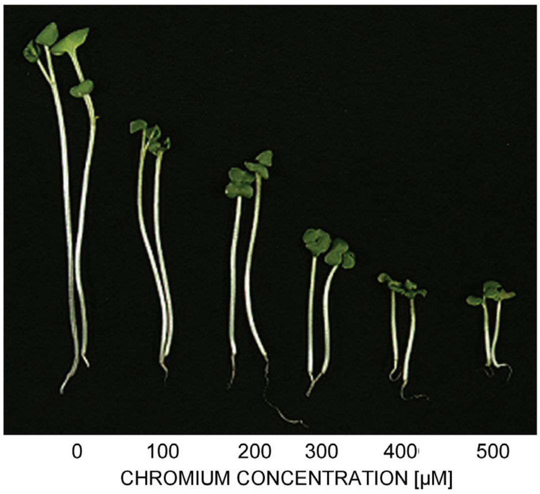

Fig. 1. Effect of various Cr(VI) concentrations (0, 100, 200, 300, 400 and 500 μM) on growth of seedlings of *Brassica juncea* cv. Varuna.

Few papers mentioned decline in Chl or carotenoid content and inhibition of enzymes involved in Chl biosynthesis as Cr-induced primary phytotoxic effects (Bishnoi *et al.* 1993a, Vajpayee *et al.* 1999, 2001, Panda and Patra 2000). In contrast, during present investigations the Chl content in the cotyledonary leaves of seedlings exposed to Cr(VI) was significantly higher than that of controls (Table 1). The higher content of Chl per fresh mass unit in the cotyledonary leaves of Cr(VI)-treated seedlings in comparison to that of controls was due to a significant reduction in their expansion. Carotenoids

Table 1. Effect of Cr(VI) on growth and pigment contents of *Brassica juncea* (means ± SD, *n* = 30 for growth parameters and 3 for pigments).

Cr (VI) [μM]	Shoot height [cm]	Fresh mass [mg plant^{-1}]	Dry mass [mg plant^{-1}]	Chl *a* [mg kg^{-1}(FM)]	Chl *b* [mg kg^{-1}(FM)]	Carotenoids [mg kg^{-1}(FM)]
0	9.0 ± 0.5	68.7 ± 5.7	13.5 ± 1.2	836.5 ± 77.0	371.9 ± 80.2	184.8 ± 50.1
100	7.0 ± 0.6	46.0 ± 5.8	13.8 ± 1.2	1020.7 ± 56.6	492.9 ± 19.2	228.5 ± 7.0
200	4.5 ± 0.5	36.5 ± 4.4	15.9 ± 1.3	1135.7 ± 104.7	526.2 ± 19.0	264.8 ± 34.8
300	3.5 ± 0.5	29.1 ± 4.2	18.9 ± 2.4	1251.0 ± 161.4	518.5 ± 66.5	222.3 ± 52.6
400	2.6 ± 0.4	25.3 ± 3.5	20.2 ± 1.3	1151.0 ± 200.5	494.3 ± 58.8	319.7 ± 61.9
500	1.8 ± 0.2	23.2 ± 2.3	21.2 ± 2.5	1118.7 ± 176.8	532.4 ± 40.8	248.7 ± 18.3

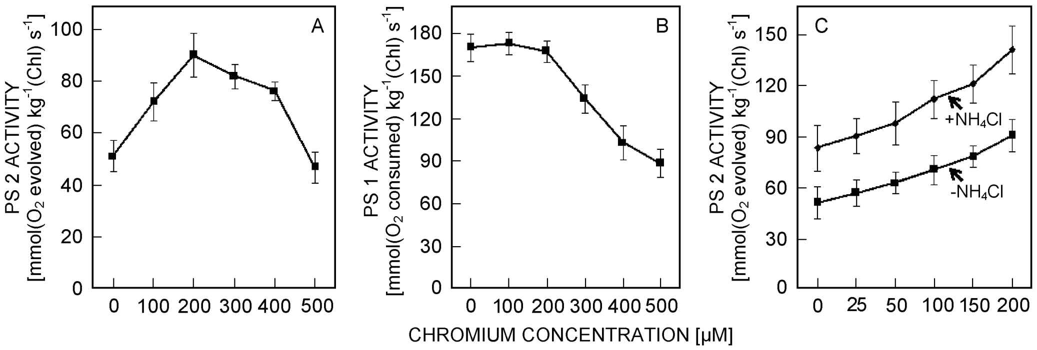

Fig. 2. Effect of various Cr(VI) concentrations (0, 100, 200, 300, 400 and 500 μM) on PS 2 activity [mmol O_2 (evolved) kg^{-1}Chl s^{-1}] (*A*) and PS 1 activity [mmolO_2 (consumed) kg^{-1}Chl s^{-1}} (*B*) of the thylakoids from control and Cr stressed seedlings. Alteration in the PS 2 activity in presence and absence of an uncoupler (5 mM NH_4Cl) at various concentrations of Cr(VI) (*C*). Means ± SD of three independent experiments. .

act as antioxidants and can scavenge reactive oxygen species generated under heavy metal stress (Panda and Choudhury 2005). Accordingly, the overall carotenoid content per unit FM in cotyledonary leaves of Cr(VI) treated seedlings was significantly higher than that of controls. These findings are in accordance to those reported by Tripati and Smith (2000) and Vajpayee *et al.* (2001).

Thylakoids isolated from seedlings exposed to Cr(VI) up to the concentration of 400 μM showed significantly higher PS 2 activity in comparison to those from controls. Thylakoids from seedlings exposed to 200 μM Cr(VI) showed highest PS 2 activity in comparison to other treatments (Fig. 2*A*). On the contrary, PS 1 activity of the thylakoids from seedlings exposed to Cr(VI) up to a concentration of 200 μM remained similar to that of control thylakoids, but the thylakoids from seedlings exposed to Cr(VI) at 300 μM and above showed significantly lower PS 1 activity in comparison to control (Fig. 2*B*).

PS 2 activity of the thylakoids from plants exposed to 100 and 200 μM Cr(VI) was approximately 1.3 and 1.7 folds higher than that of controls, respectively (Fig. 2*C*). To validate whether observed Cr(VI)-induced increase in PS 2 activity was due to uncoupling of electron transport chain (ETC) from photophosphorylation, PS 2 activity was measured in presence of NH_4Cl, a well known uncoupler (Alia *et al.* 1992). In general, a significant and similar enhancement (~1.5 folds) in PS 2 activity of thylakoids from both control as well as Cr(VI)-stressed seedlings was observed in presence of NH_4Cl (Fig. 2*C*). Thus Cr(VI)-stimulated PS 2 activity was not due to uncoupling of ETC from photophosphorylation.

Thylakoids from 200 μM Cr(VI) treated seedlings which exhibited peak PS 2 activity were further characterized in order to evaluate if there is any alteration in pH optimum and irradiance requirements. PS 2 activity in the thylakoids from both Cr(VI) stressed as well as control seedlings was highest at pH 7.5 with a decline at acidic as well as alkaline pH. However, irrespective of pH ranging from 6.0 to 8.5, PS 2 activity of the thylakoids from Cr(VI) exposed seedlings was higher than that from control. Alkaline pH inhibits electron donation reactions in the oxygen-evolving complex. This is accredited either to the loss of 23 kDa extrinsic polypeptide or the competition between OH^- and Cl^- for anion binding sites on PS 2 (Alia *et al.* 1992). Thus, high PS 2 activity of thylakoids from seedlings exposed to Cr(VI) especially under alkaline pH can be attributed to Cr(VI)-induced stabilization of oxygen evolving complex (Fig. 3*A*).

Irradiance saturation kinetics of PS 2 activity in the thylakoids from 200 μM Cr(VI)-stressed and control seedlings was carried out to ascertain whether the Cr(VI) induced enhancement in activity of PS 2 was due to any alteration in its structural components. A typical irradiance saturation curve of PS 2 activity with maximum activity (V_{max}) at 80 % saturating irradiance was obtained using thylakoids isolated from both control and Cr(VI)-treated seedlings. However, thylakoids from seedlings exposed to 200 μM Cr(VI) showed significantly higher PS 2 activity at all irradiances in comparison to that of control. The photochemical activity of PS 2 of thylakoid from the Cr(VI) treated seedlings at 25 % of the saturating irradiance was *in par* with the PS 2 photochemical activity from controls at saturating irradiance (80 %) indicating that the thylakoids from Cr(VI)-treated seedlings possessed higher potential to utilize the trapped photon energy. This might be related to an increase in the reaction centers and/or an increase in the antenna size of PS 2 in the thylakoids from Cr(VI) exposed seedlings in comparison to that of control (Fig. 3*B*).

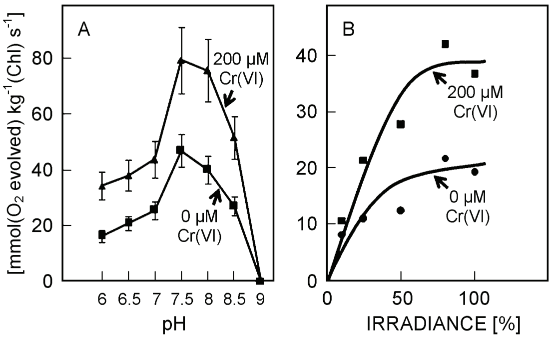

Fig. 3. Effect of various Cr(VI) concentrations (0 and 200 μM) on PS 2 activity [mmol(O_2 evolved) kg^{-1}(Chl) s^{-1}] at different pH (*A*) and different irradiances (*B*) expressed as a percentage of maximum irradiance 920 μmol m^{-2} s^{-1}. Means ± SD of three independent experiments.

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Koopowitz, H.: **Tropical Slipper Orchids. *Paphiopedilum* and *Phragmipedium* species and hybrids.** - Timber Press, Portland 2008. 411 pp. USD 59.95. ISBN-13: 978-0-88192-864-8.

The author of the book is professor emeritus of ecology at the University of California, Irvine, and editor-in-chief of Orchid Digest. The book is a complete guide to 482 tropical slipper orchids in cultivation. It is not a taxonomic revision but a horticultural treatise concerning the cultivation, history, and hybridization of tropical slipper orchids as hobby plants. The text of the book is divided into 23 chapters. In the first ones the history of slipper orchids in cultivation, their biology and classification, the connections to CITES (Convention on International Trade in Endangered Species), the methods of cultivation, the pests and diseases are handled in detail. Furthermore, the reader is advised how to select plants for a collection (species or hybrids?) and gets instructions for hybridization and breeding of slipper orchids. Some species of *Paphiopedilum* are especially important to hobbyists and growers, therefore they are organized into horticulturally important alliances. The members of these alliances share a close affinity with each other and it is relatively easy to make hybrids between species within an alliance. The main *Paphiopedilum* alliances are as follows: Barbatum alliance, Insigne a., Parvisepalum a., Cochlopetalum a., Coryopedilum a., Pardalopetalum a. and Brachypetalum a. The major species of the alliances were described based on their flower characteristics. The following chapters deal with several different strategies which have followed the breeding of slipper orchids (*e.g.* production of ever-bigger flowers). Five favoured species of *Paphiopedilum* were used in another type of breeding. „What is a good standard-complex flower in standard-complex hybrids“ was the question which the author asked himself when grouping the plants in groups with green, yellow and fall tones, in red tone and flowers with spots, and in white and pink flowers. A new trend was also selection of small species, called mini paphs. One of the most exciting developments of the 1990s was the production of primary hybrids between the various *Parvisepalum* species: they were distinctive, easy to grow, colourful and manyflowered during summer. Breeding with the *Brachypetalum* species and hybrids is one of the hottest areas of interest in the slipped orchid world now. The Volume is illustrated with 359 perfect special photographies made by James Comstock and watercolors by Carol Woodin. At the end the book contains an index of resources, a glossary of the most important botanical terms, References, a general index including authors and a voluminous index of plant names.

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