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Variation in Growth, Photosynthesis Functions and Yield of Five Mustard (*Brassica juncea* L.) Cultivars under High Cadmium Stress

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ABSTRACT

Five cultivars of mustard (*Brassica juncea* L. Czern and Coss.) namely 'Alankar', 'Varuna', 'Pusa Jai Kisan', 'SS2' and 'Dhanuka Bold' were tested for tolerance to cadmium (Cd). Plants were raised from seeds in earthen pots with treatment of 0, 25 and 50 μM Cd in nutrient solution. All levels of Cd decreased growth and yield of the tested cultivars with varying degrees. Cadmium tolerance (CdT), the ability of plant to maintain high yield at maximum level of Cd, was calculated as the ratio of yield at the untreated and Cd-treated soils. Among mustard cultivars 'Pusa Jai Kisan' was identified as the Cd tolerant, while 'SS2' as the Cd non-tolerant cultivar. To find out the physiological basis of these differences, we investigated the possible role of photosynthetic pigments [chlorophyll (Chl), carotenoid, pheophytin, anthocyanin], dry matter accumulation and leaf-Cd accumulation capacity of the cultivars. Among photosynthetic traits, Cd treatment decreased the content of Chl (Chl *a*, Chl *b*, total Chl), Chl fluorescence (F_v/F_m) and carotenoid. However, the content of pheophytin and anthocyanin increased significantly in all the cultivars. Cd accumulation in leaves also increased with increase in Cd level. However, the extent of Cd-induced decrease or increase characteristics was found greater in Cd-non-tolerant ('SS2') than Cd-tolerant ('Pusa Jai Kisan') cultivars. 'Pusa Jai Kisan' maintained a higher content of Chl, carotenoid and relative amount of anthocyanin although it had the least percent pheophytin and Cd-content in leaves and subsequently produced higher dry matter and seed yield than the other cultivars at all levels of Cd.

Keywords: anthocyanins, cadmium toxicity, carotenoids, Cd content, Chl fluorescence, photosynthetic pigments

Abbreviations: Cd, cadmium; **Chl**, chlorophyll; **DAS**, days after sowing; **DMSO**, dimethyl sulfoxide; **FW**, fresh weight; **LHCP**, light harvesting chlorophyll protein; **GST**, glutathione-*S*-transferase

INTRODUCTION

India is a major rapeseed mustard growing country of the world. It ranks first in the world in respect of acreage and second in production next to Canada. In India, mustard is the second most important edible oilseed crop after groundnut sharing 27.8% in India's economy (Kumar 1997). Its cultivation is mainly in Uttar Pradesh, Rajasthan, Madhya Pradesh, Haryana, Punjab, Assam, Bihar, Gujarat and West Bengal states of India. Plant growth and productivity of crops are limited in many areas of the world by a variety of environmental stresses. Stress, in fact, implies adverse affect on an organism, which invariably leads to reduced growth, metabolism and productivity. Among several abiotic stresses that plants encounter, heavy metal stress occupies a specific importance because they are added to the soil and affect plant right from juvenile stage to productivity. Cadmium (Cd) is one such heavy metal which has attracted the attention because of its potential toxicity to plant systems and relative high mobility, in soil plant system (Zhang *et al*. 2006).

Cd is a toxic heavy metal that enters the environment mainly through anthropogenic sources (Davis 1984; Guo 1994). The increasing Cd concentration in agricultural soils is mainly due to the application of phosphate fertilizers and sewage sludge as well as atmospheric deposition from industrial sources (McLaughlin *et al*. 1999; Nolan *et al*. 2003). Cd has high mobility in the soil-plant system and is easily taken up by plant roots and translocated to the aboveground tissues (Yang *et al*. 1998; Alpha *et al*. 2009), and causes toxicity even at lower concentration (Sanitá di Toppi and Gabbrielli 1999).

Cd is an active inhibitor of photosynthetic process and of chloroplast development (Padmaja *et al*. 1990; Rascio *et al*. 1993). It can cause disorganization of grana and influences chlorophyll (Chl) biosynthesis (Baszyñski *et al*. 1980). In addition, it disturbs the balance in the accumulation, distribution and correlation of photosynthetic pigments (Sanitá di Toppi and Gabbrielli 1999). In addition, Cd reduces plant growth and biomass (Sandalio *et al*. 2001; Khan *et al*. 2006, 2007; Anjum *et al*. 2008; Singh *et al*. 2008) and decreases seed yield (Khan *et al*. 2007; Ghani and Wahid 2007; Wahid and Ghani 2008).

Cd toxicity in plants results from a range of interactions at cellular level and/or imbalance between the production and elimination of reactive oxygen species (ROS). Plants possess a range of potential cellular mechanisms that may be involved in the diminution of ROS and tolerance to metal stress (Hall 2002). The antioxidative defense system in plants comprises enzymatic and non-enzymatic antioxidants. Photosynthetic pigments are of primary importance in augmenting growth under normal and environmental limited conditions. They harness solar radiation and play an important role in biomass and yield production. Any effect of Cd toxicity on growth and yield will be the result of its effect on photosynthetic pigments. The tolerance of plants to stress, therefore, may be the manifestation of the inherent contents of the photosynthetic pigments of a genotype/cultivar. Thus, five cultivars of mustard were tested for tolerance to Cd stress and to select tolerant and non-tolerant types.

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MATERIALS AND METHODS

Plant growth conditions

Seeds of mustard (*Brassica juncea* L. Czern and Coss.) cultivars namely 'Alankar', 'Varuna', 'Pusa Jai Kisan', 'SS2' and 'Dhanuka Bold' were sown in 23 cm-diameter earthen pots containing 4 kg reconstituted soil [soil composed of peat and compost, 4:1, v/v, mixed with sand, 3:1, v/v)] in the winter season under natural day/ night condition with average day and night temperature of 21 ± 2 and 12 ± 2°C, respectively. Relative humidity was 58 ± 6% and PAR 900 ± 25 µmol/m^2/s. The plants were treated with 0, 25 and 50 µM Cd as $CdCl_2$ given along with Hoagland nutrient solution. Three replicates for each treatment were maintained. After 30 days of emergence, photosynthetic characteristics (content of Chl, anthocyanin, carotenoid, pheophytin, Chl fluorescence), Cd-content in leaves and dry mass were recorded while seed yield was recorded at harvest.

Measurements

1. Chlorophyll and carotenoid

Total Chl and carotenoid content was extracted using the method of Hiscox and Israelstam (1979) by using dimethyl sulphoxide (DMSO) as an extraction medium, and estimated and calculated by the method of Arnon (1949).

Fresh leaves (100 mg) were cut into small pieces and collected in test tubes containing 7 ml of DMSO. The test tubes were covered with black paper and incubated at 45°C for 40 min for the extraction. The content was transferred to a graduated tube and the final volume was made to 10 ml with DMSO. Extract measuring 3 ml was transferred to a cuvette and the absorbance was read at 645 and 663 nm for Chl content and at 480 and 510 nm for carotenoid content on UV-VIS spectrophotometer (SL164, Elico, Hyderabad, India). Total Chl and carotenoid content were calculated according to the following equations.

$$\text{Chl } a \text{ (mg g}^{-1}\text{ FW)} = [(12.7 \times OD_{663}) - (2.69 \times OD_{645})] \times (V/(1000 \times W))$$

$$\text{Chl } b \text{ (mg g}^{-1}\text{ FW)} = [(22.9 \times OD_{645}) - (4.68 \times OD_{663})] \times (V/(1000 \times W))$$

$$\text{Total Chl (mg g}^{-1}\text{ FW)} = [(20.2 \times OD_{645}) + (8.02 \times OD_{663})] \times (V/(1000 \times W))$$

$$\text{Carotenoid (mg g}^{-1}\text{ FW)} = [(7.6 \times OD_{480}) - (1.49 \times OD_{510})] \times (V/(1000 \times W))$$

where V = volume of the extract, W = mass of the leaf tissue taken.

2. Chlorophyll fluorescence

Chl fluorescence F_v/F_m was measured by chlorophyll fluorometer (OS-30p, USA).

3. Cadmium content in leaves

The leaf sample was dried for 48 h at 80°C and ground to a fine powder. Dried leaf tissue (200 mg) was transferred to digestion tubes. Four ml of acid mixture (HNO_3 and $HClO_4$ 3:1, v/v) were added to the tubes. Digestion tubes were heated in the digestion chamber. After heating for about 30-45 min, tubes were kept for cooling for 10 min. To get the extracts clear and almost colorless, 3-4 drops of hydrogen peroxide were added followed by heating for another 30 min. Cd concentration was determined by an atomic absorption spectrophotometer (GBC, 932 plus, Australia).

4. Pheophytin

The method of Bowler *et al*. (1991) was followed for the determination of pheophytin as percent degradation of Chl to pheophytin. Fresh leaf tissues (1 g) were ground with sufficient amount of 80% acetone and centrifuge at 10,000 rpm for 5 min and the increase in the absorbance was recorded at 553 and 665 nm.

5. Anthocyanin

The relative amount of anthocyanin was estimated following the method of Mancinelli (1984). Fresh leaf tissues (1 g) were grinded in acidified methanol (CH_3OH: H_2O: HCl, 79:20:1) and centrifuged at 10,000 rpm for 5 min. The supernatant was collected and read at 530 and 657 nm while the Chl and non-specific degradation products were corrected at 530 and 657 nm.

6. Dry mass

To obtain dry mass, plants were dried in an oven at 80°C until constant weight is obtained.

7. Seed yield

It was determined at harvest. Pods from each treatment were collected, sun-dried and thrashed to collect seed, and seeds per plant were recorded as seed yield.

8. Tolerance index

It was calculated using the data obtained on seed yield in 50 µM Cd treatment and control, and expressed in percentage.

$$\text{Tolerance index} = \frac{\text{seed yield of treated plants}}{\text{seed yield of control plants}} \times 100$$

Data analysis

Data were analyzed statistically using SPSS version 10.0; Inc., Chicago, IL, USA) and presented as mean ± SE. Analysis of variance was performed on the data and least significant difference (LSD) was calculated for the significant data to identify difference in the mean of the treatment. The treatment mean was separated by the LSD test.

RESULTS

As a consequence of Cd stress Chl *a*, Chl *b* and total Chl decreased in all cultivars with increasing Cd concentrations. The decrease was maximum with 50 µM Cd concentration (**Figs. 1, 2**). The decrease in Chl *a* was 26.3, 28.4, 31.3, 35.6 and 38.2% in 'Pusa Jai Kisan', 'Alankar', 'Varuna', 'Dhanuka Bold' and 'SS2', respectively with 50 µM Cd compared to control. Among cultivars maximum reduction in Chl *b* and total Chl of 50.0 and 42.1% occurred in 'SS2' with 50 µM Cd while 'Pusa Jai Kisan' showed least reduction of 32.3 and 28.9% with 50 µM Cd compared to control. Other cultivars lied in this range with a reduction in Chl *b* and total Chl of 37.7 and 31.8% in 'Alankar', 40.4 and 34.4% in 'Varuna', 42.5 and 37.8% in 'Dhanuka Bold', respectively with 50 µM Cd compared to their respective control. In all cultivars, Chl *b* was more affected than Chl *a* as shown by increasing Chl *a*/Chl *b* ratio with increase in Cd concentration (**Fig. 2**). In all cultivars, Chl fluorescence (F_v/F_m) decreased with increase in Cd level. A decrease of 6.4% in 'Pusa Jai Kisan', 11.8% in 'Alankar', 14.9% in 'Varuna', 20.1% in 'Dhanuka Bold' and 32.5% in 'SS2' occurred with 50 µM Cd treatment compared to control (**Fig. 3A**).

Cd-content in leaves increased with increasing Cd concentration. The cultivar 'Pusa Jai Kisan' accumulated minimum Cd while 'SS2' accumulated maximum Cd. The trend in the increase of Cd content among cultivars was 'SS2'> 'Dhanuka Bold'> 'Varuna'> 'Alankar'> 'Pusa Jai Kisan' (**Fig. 3B**).

In all the cultivars, percent content of pheophytin showed an increasing trend with increase in Cd concentration. An increase of 14.9, 16.2, 16.9, 20.7 and 19.3% pheophytin was found in 'Pusa Jai Kisan', 'Alankar', 'Varuna', 'Dhanuka Bold' and 'SS2', respectively at 50 µM Cd treat-

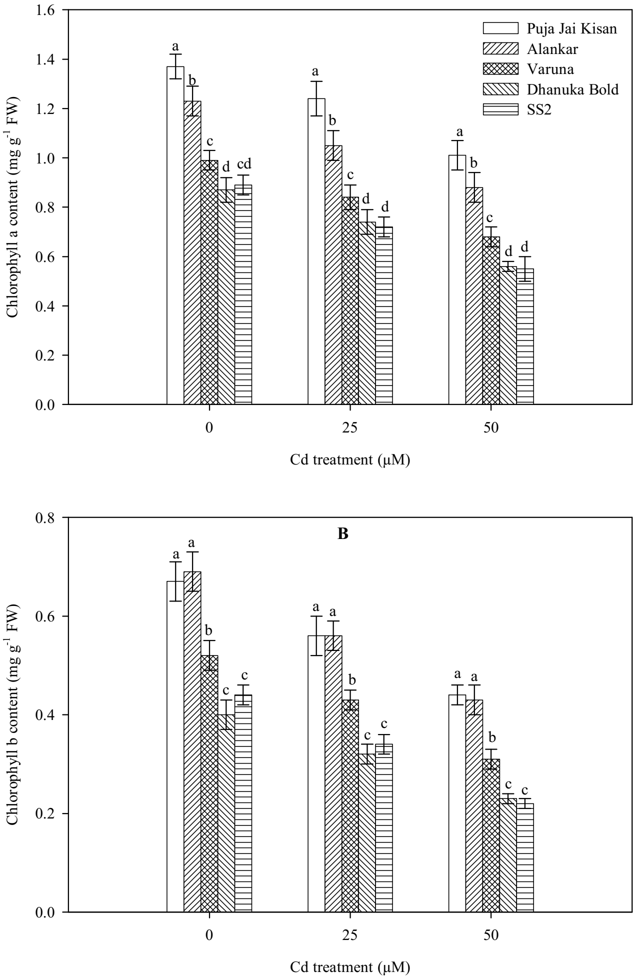

Fig. 1 Effect of 0, 25 and 50 µM Cd on Chl *a* (A) and *b* (B) contents of mustard (*Brassica juncea* L.) cultivars 30 d after emergence. Values are means ± SE (*n* = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by the LSD test.

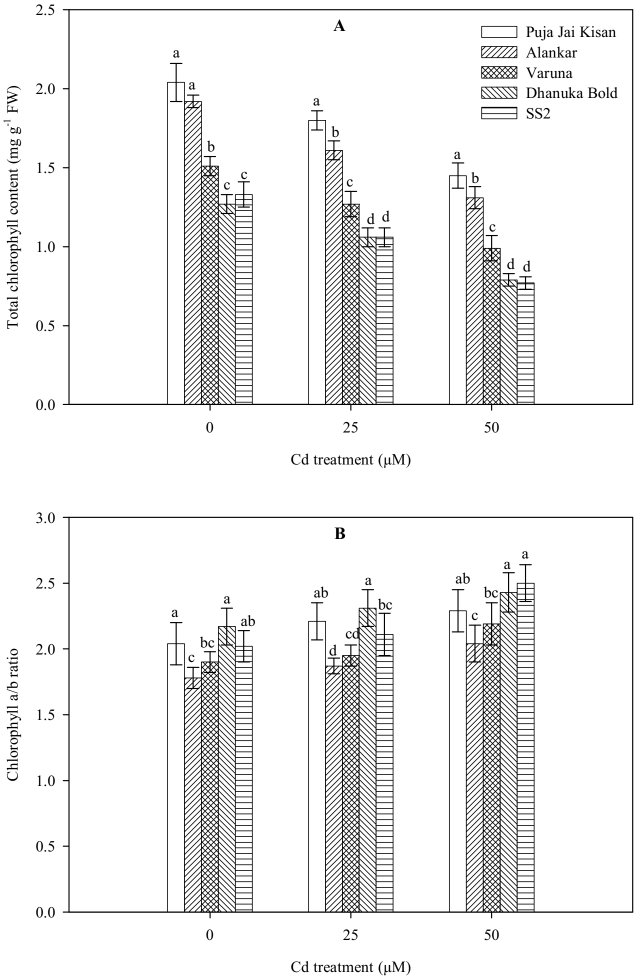

Fig. 2 Effect of 0, 25 and 50 µM Cd on total Chl content (A) and Chl *a* to Chl *b* ratio (B) of mustard (*Brassica juncea* L.) cultivars 30 d after emergence. Values are means ± SE (*n* = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by LSD test.

ment compared to control (**Fig. 4A**).

Carotenoid content was reduced in all the cultivars. ‘Alankar’, ‘Varuna’ and ‘Dhanuka Bold’ showed a reduction of 33.3, 35.3 and 31.4%, respectively with 50 µM Cd treatment compared to control. However, a maximum reduction of 47.3% occurred in ‘SS2’ and the least reduction of 31.2% in ‘Pusa Jai Kisan’ with 50 µM Cd treatments compared to control (**Fig. 4B**). In all cultivars, relative amount of anthocyanin increased (**Fig. 5A**). Maximum increase of 170.0% was found in ‘Pusa Jai Kisan’ followed by 150.9, 108.8, 110.2 and 105.0% in ‘Alankar’, ‘Varuna’, ‘Dhanuka Bold’ and ‘SS2’, respectively with 50 µM Cd treatment compared to control.

Plant dry mass and seed yield decreased in all the cultivars with maximum decrease of 38.5 and 69.0%, respectively in ‘SS2’ and minimum decrease of 24.3 and 26%, respectively in ‘Pusa Jai Kisan’ with 50 µM Cd treatment compared to their respective control (**Figs. 5B, 6A**). The trend in the decrease of plant dry mass and seed yield was ‘SS2’> ‘Dhanuka Bold’> ‘Varuna’> ‘Alankar’> ‘Pusa Jai Kisan’.

Pusa Jai Kisan has highest tolerance index followed by ‘Alankar’, ‘Varuna’, ‘Dhanuka Bold’ and ‘SS2’ (**Fig. 6B**).

DISCUSSION

In the present study, variation in response of five mustard cultivars were studied for Cd accumulation in leaves and concurrent changes in photosynthetic traits, dry mass and seed yield under Cd stress, to select tolerant and non-tolerant cultivars. The addition of Cd to the soil resulted in reduction in growth of mustard plants in terms of plant dry mass and seed yield. This reduction occurred as a result of Cd stress-induced generation of reactive oxygen species (ROS) which lead to oxidative stress in plants (Skórzyńska-Polit *et al.* 2003/04; Mobin and Khan 2007; Anjum *et al.* 2008; Rodríguez-Serrano *et al.* 2009). ROS causes oxidative stress resulting in damage to photosynthetic pigments, biomolecules such as lipid, proteins and nucleic acid leading to reduction in growth and productivity (Foyer *et al.* 1994). Plant species and genotypes differ significantly in the uptake of Cd and its subsequent translocation from roots into shoots (Metwally *et al.* 2005; Khan *et al.* 2006). In our study, Cd treatment caused an increase in Cd-accumulation in leaves in all the cultivars. Cd accumulation in all tissues of Cd treated plant agrees with the results of Dixit *et al.* (2001), Arao *et al.* (2003), Metwally *et al.* (2005), Mobin and Khan (2007), Anjum *et al.* (2008), Singh *et al.* (2008) and Markovska *et al.* (2009). Among cultivars, ‘SS2’ accumulated maximum Cd in leaves and ‘Pusa Jai Kisan’ accumulated least Cd. This variation in accumulation of Cd depends on the binding to extracellular matrix (Horst 1995), complexing inside the cell (Cobbett *et al.* 1998) and on the transport efficiency (Marchiol *et al.* 1996). Low Cd content in leaves of ‘Pusa Jai Kisan’ may be due to retention of Cd in roots which occurs due to cross-linking of Cd to carboxyl group of cell wall proteins (Barceló and Poschenrieder 1990) and this can be regarded as an important protection mechanism against diffusion of metal in plants. Low Cd content in leaves protects the photosynthetic function in

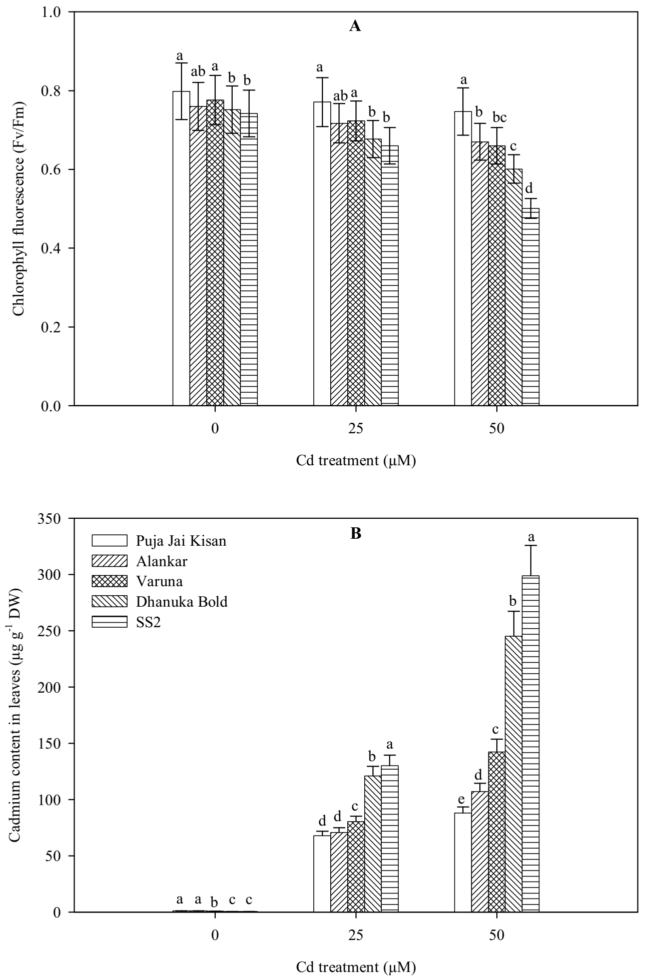

Fig. 3 Effect of 0, 25 and 50 μM Cd on Chl fluorescence (A) and Cd-content (B) in leaves of mustard (*Brassica juncea* L.) cultivars 30 d after emergence. Values are means ± SE (*n* = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by LSD test.

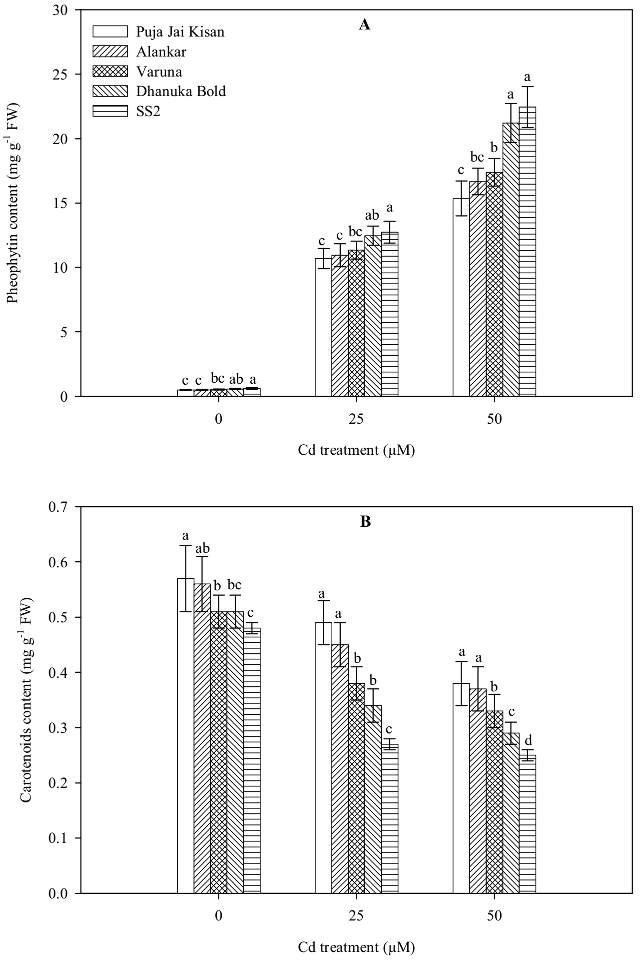

Fig. 4 Effect of 0, 25 and 50 μM Cd on pheophytin (A) and carotenoid (B) contents of mustard (*Brassica juncea* L.) cultivars 30 d after emergence. Values are means ± SE (*n* = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by LSD test.

plants against the Cd-induced oxidative stress (Dixit *et al.* 2001; Anjum *et al.* 2008; Singh *et al.* 2008). Cd causes decrease in the photosynthesis (Mobin and Khan 2007; Anjum *et al.* 2008; Shi *et al.* 2009; Khan *et al.* 2009) which may occur due to decrease in the level of photosynthetic pigments (Padmaja *et al.* 1990; Mobin and Khan 2007). Photosynthetic pigments have been shown as one of the main target of Cd toxicity (Vassilev *et al.* 2002a, 2002b) resulting in reduced photosynthesis (Somashekaraiah *et al.* 1992; Draz˙kiewicz *et al.* 2003; Mobin and Khan 2007) and dry matter production. 'Pusa Jai Kisan' accumulated least Cd in leaves and thus protected its photosynthetic pigments than the other cultivars. Among photosynthetic pigments Chl, carotenoid, pheophytin and anthocyanin are of significant importance. A Cd-induced decrease in Chl content has been extensively studied in various crops (Chettri *et al.* 1998; Öncel *et al.* 2000; Monni *et al.* 2001; Anjum *et al.* 2008; Singh *et al.* 2008). In the present study, 'Pusa Jai Kisan' exhibited lesser decrease in Chl content compared to control followed by 'Alankar', 'Varuna', 'Dhanuka Bold' and 'SS2'. This indicated the higher tolerance of 'Pusa Jai Kisan' to Cd stress. Higher decrease in Cd-induced reduction in Chl content in 'SS2' may be associated with inhibition of protochlorophyll reductase, synthesis of amino levulinic acid and/or Cd-induced production of free radicals (Stobart *et al.* 1985; Radotic *et al.* 2000; Zengin and Munzuroglu 2006). The report on the decrease in tetrapyrrol intermediates in Chl biosynthetic pathway, protoporphyrin IX, Mg-porphyrin IX and protochlorophyllide has been found under chill and heat (Tewari and Tripathy 1998) and salinity stress (Khan 2003). In addition, a greater reduction in Chl *b* content, evident by higher Chl *a*/Chl *b* ratio was found in all the cultivars. Environmental stress-induced increase in Chl *a*/ Chl *b* ratio has been studied by many researchers (Delfine *et al.* 1999; Hammani *et al.* 2004; Mobin and Khan 2007). In fact, the ratio of Chl *a* to Chl *b* is an indicator of stress-effects (Zengin and Munzuroglu 2006) which is linked with the reduction in light harvesting Chl protein (LHCP) (Loggini *et al.* 1999). This reduction is an adaptive defense mechanism of chloroplast which allows them to reduce the adverse condition (Asada *et al.* 1998). In the present study, Chl *a* to Chl *b* ratio increased in all cultivars but in 'Pusa Jai Kisan' this ratio showed least reduction and thus protected its chloroplast from Cd stress to the maximum extent. The maximum protection of Chl content in 'Pusa Jai Kisan' is supported by the lowest content of percent pheophytin. Percent pheophytin represents the degradation of Chl and its conversion to pheophytin. The conversion occurs by substitution of Mg in Chl by heavy metal and that this subsitution in vivo makes plant incapable of photosynthesis (Küpper *et al.* 1996).

Cd treatments also caused reduction in carotenoid content in all the cultivars. The reduction was greatest in 'SS2' and least in 'Pusa Jai Kisan' (**Fig. 3B**). Carotenoids are involved in the protection of photosynthetic apparatus against photoinhibitory damage caused by singlet $^1O_2$ which is produced by the excited triplet state of Chl under stress. Carotenoids act as quenchers deactivating singlet $^1O_2$ (Foyer and Harbinson 1984; Sieferman-Harms 1987). In 'Pusa Jai Kisan', higher amount of carotenoids than the other cultivars helped to decrease the Cd-induced singlet $^1O_2$ effects.

However, anthocyanin showed an increasing trend with

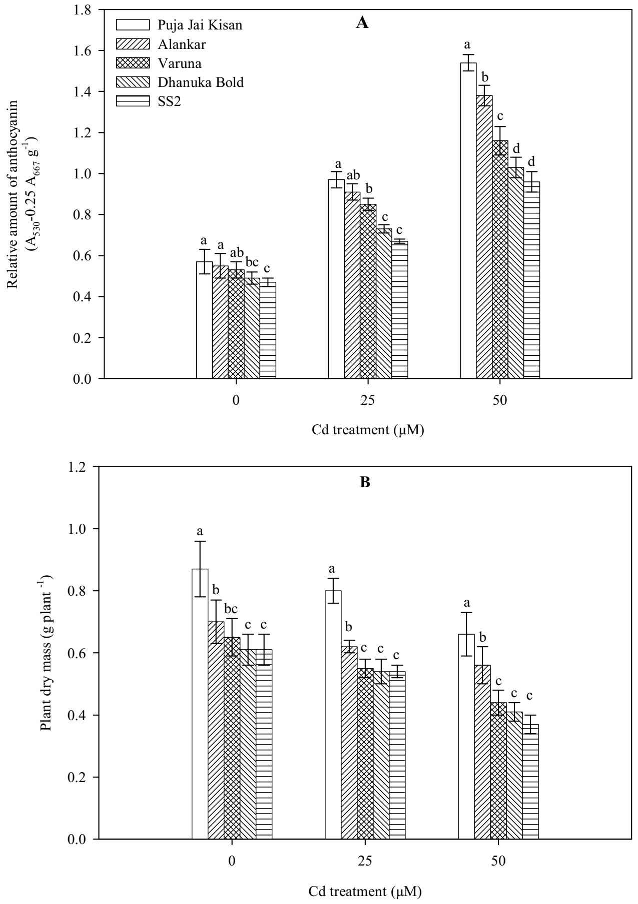

Fig. 5 Effect of 0, 25 and 50 μM Cd on relative amount of anthocyanin (A) and plant dry mass (B) of mustard (*Brassica juncea* L.) cultivars 30 d after emergence. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by LSD test.

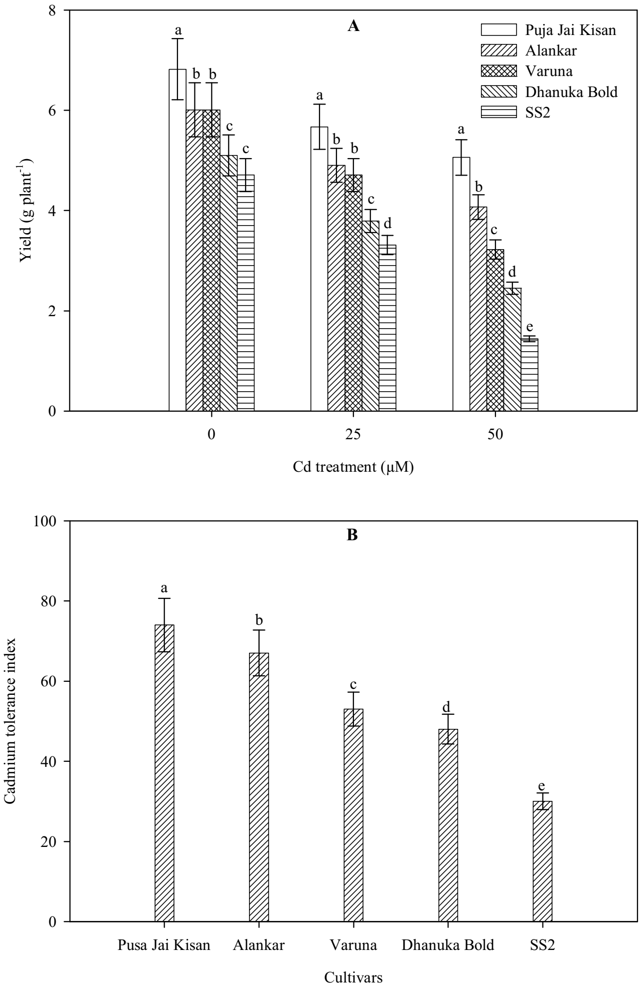

Fig. 6 Effect of 0, 25 and 50 μM Cd on seed yield (A) and tolerance index (B) of mustard (*Brassica juncea* L.) cultivars at harvest. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at P≤0.05 as determined by LSD test.

increase in Cd concentration. Anthocyanin has been associated with the quenching of oxygen free radicals and reducing Cd effects (Hale *et al.* 2002; Kalantari and Oloumi 2005). Stress-induced increase in the content of anthocyanin has been extensively found by different workers (Christie *et al.* 1994; Hale *et al.* 2002; Rivera-Becerril *et al.* 2002; Hasegawa *et al.* 2004; Erylmaz 2006). 'Pusa Jai Kisan' exhibited maximum anthocyanin which might be due to greater synthesis of glutathione-*S*-transferase (GST) enzyme as this enzyme is responsible for catalyzing last step of anthocyanin synthesis (Marrs and Walbot 1997; Schreder *et al.* 2003). Although the accumulation of anthocyanin may be only of secondary importance in living cell but its accumulation might help in protection of the photosynthetic apparatus from abiotic stress-generated superoxide radicals without limiting photosynthesis (Gould *et al.* 1995, 2002; Krupa *et al.* 1996; Mobin and Khan 2007).

Thus, the lower content of Cd in leaves of 'Pusa Jai Kisan' subsequently helped this cultivar to increase its Chl, carotenoid and anthocyanin content. The maintenance of higher Chl content and other accessory pigments are strongly related with photosynthesis and dry matter production (Mobin and Khan 2007).

Chl fluorescence was also studied under Cd stress and it was found to decrease with the increase in Cd concentration. Fluorescence is an indicator of stress and is used for screening of plants for tolerance to environmental stresses (Baker and Rosenquist 2004). In 'Pusa Jai Kisan', the lowest reduction in Chl fluorescence was found while in 'SS2' highest reduction occurred.

In all cultivars, Cd caused decrease in yield. This reduction in yield under Cd stress is also supported by Mahajan and Tuteja (2005), Ghani and Wahid (2007), Khan *et al.* (2007) and Wahid and Ghani (2008). Among cultivars, Pusa Jai Kisan showed least reduction in yield and 'SS2' showed maximum reduction in yield. This shows the tolerant nature of 'Pusa Jai Kisan' which is also indicated by its tolerance index (**Fig. 6B**).

In conclusion, the present study revealed that all cultivars of mustard responded differentially to Cd stress and the toxicity of Cd is obvious in terms of decrease in growth and yield. The higher capacity of 'Pusa Jai Kisan' to produce greater dry mass and yield than the other cultivars under Cd stress was the result of lower Cd content in leaves thereby maintaining higher contents of Chl, carotenoids, and greater relative amount of anthocyanin. Thus, photosynthetic pigments may be used as physiological trait for screening mustard cultivars for Cd tolerance.

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REFERENCES

Alpha JM, Chen J, Zhang G (2009) Effect of nitrogen fertilizer forms on growth, photosynthesis and yield of rice under cadmium stress. *Journal of Plant Nutrition* **32**, 306-317

Anjum NA, Umar S, Ahmad A, Iqbal M, Khan NA (2008) Sulphur protects mustard (*Brassica campestris* L.) from cadmium toxicity by improving leaf ascorbate and glutathione. *Plant Growth Regulation* **54**, 271-279

Arao T, Ae N, Sugiyama M, Takahashi M (2003) Genotypic differences in cadmium uptake and distribution in soybeans. *Plant and Soil* **251**, 247-253

Arnon DI (1949) Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24**, 1-5

Asada K, Endo T, Mano J, Miyake C (1998) Molecular mechanism for relaxation of and protection from light stress. In: Saton K, Murata N (Eds) *Stress Responses of Photosynthetic Organisms*, Elsevier, Amsterdam, pp 37-52

Baker NR, Rosenquist E (2004) Applications of chlorophyll fluroescence can improve crop production strategies: An examination of future possibilities. *Journal of Experimental Botany* **55**, 1607-1621

Barceló J, Poschenrieder CH (1990) Plant water relations as affected by heavy metal stress: A review. *Journal of Plant Nutrition* **13**, 1-37

Baszyñski T, Wajda L, Kroll M, Wolinka D, Krupa Z, Tukendorf A (1980) Photosynthetic activities of cadmium treated tomato plants. *Physiologia Plantarum* **48**, 365-370

Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J, Sybesma C, Montagu M, Inzé D (1991) Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *The EMBO Journal* **10**, 1723-1732

Chettri MK, Cook CM, Vardaka E, Sawidis T, Lanaras T (1998) The effect of Cu, Zn and Pb on the Chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. *Environmental and Experimental Botany* **39**, 1-10

Christie PJ, Alfenito MR, Walbot V (1994) Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **194**, 541-549

Cobbett CS, May MJ, Howden R, Rolls B (1998) The glutathione-deficient, cadmium-sensitive mutant, *cad2-1*, of *Arabidopsis thaliana* is deficient in gamma-glutamylcysteine synthetase. *The Plant Journal* **16**, 73-78

Dai L-P, Xiong Z-T, Huang Y, Li M-J (2006) Cadmium induced changes in pigments, total phenolics and phenylalanine ammonia lyase activity in fronds of *Azolla imbricate. Environmental Toxicology* **21**, 505-512

Davis RD (1984) Cadmium – a complex environmental problem: Cadmium in sludge used as fertilizer. *Experentia* **40**, 117-126

Delfine S, Alvino A, Villiani MC, Loreto F (1999) Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiology* **119**, 1101-1106

Dixit V, Pandey V, Shyam R (2001) Differential oxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany* **52**, 1101-1109

Draz˙kiewicz A, Tukendorf A, Baszyñski T (2003) Age-dependent response of maize leaf segments to cadmium treatment: Effect on chlorophyll fluorescence and phytochelatin accumulation. *Journal of Plant Physiology* **160**, 247-254

Eryılmaz F (2006) The relationships between salt stress and anthocyanin content in higher plants. *Biotechnology and Biotechnology Equipment* **1**, 47-52

Foyer CH, Harbinson J (1984) Oxygen metabolism and the regulation of photosynthetic electron transport. In: Foyer CH, Mullineaux PM (Eds) *Causes of Photooxidative Stress and Amelioration of Defense System in Plants*, CRC Press, Florida, pp 1-42

Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. *Physiologia Plantarum* **92**, 696-671

Ghani A, Wahid A (2007) Varietal difference for cadmium induced seedling mortality and foliar-toxicity symptoms in mungbean (*Vigna radiata*). *International Journal of Agricultural Biology* **9**, 555-558

Gould KS, Kuhn DN, Lee DW, Oberbauer SF (1995) Why leaves are sometime red. *Nature* **378**, 241-242

Gould KS, Mckelvie J, Markham KR (2002) Do anthocyanins function as antioxidant in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. *Plant Cell and Environment* **25**, 1261-1269

Guo DF (1994) Environmental sources of Pb and Cd and their toxicity to man and animals. *Advances in Environmental Science* **2**, 71-76

Hale KL, Tufan HA, Pickering IJ, George GN, Terry N, Pilon M, Pilon-Smits EAH (2002) Anthocyanins facilitate tungsten accumulation in *Brassica*. *Physiologia Plantarum* **116**, 351-358

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53**, 1-11

Hammami SS, Chaffai R, Ferjani EEL (2004) Effects of cadmium on sunflower growth, leaf pigment and photosynthetic enzymes. *Pakistan Journal of Biological Science* **7**, 1419-1426

Hasegawa H, Fukasawa-Akada T, Okuno T, Niizeki M, Suzuki M (2004) Anthocyanin accumulation and related gene expression in Japanese parsly (*Oenanthe stolonifera,* DC.) induced by low temperature. *Journal of Plant Physiology* **158**, 71-78

Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**, 1332-1334

Horst WJ (1995) The role of apoplast in aluminium toxicity and resistance of higher plants: A review. *Zeitschrift für Pflanzenernährung und Bodenkunde* **58**, 419-428

Kalantari M-Kh, Oloumi H (2005) Study the effects of $CdCl_2$ on lipid peroxidation and antioxidant compounds content in *Brassica napus*. *Iranian Journal of Science and Technology* **29**, 201-208

Khan NA, Ahmad I, Singh S, Nazar R (2006) Variation in growth, photosynthesis and yield of five wheat cultivars exposed to cadmium stress. *World Journal of Agricultural Science* **2**, 223-226

Khan NA, Anjum NA, Nazar R, Iqbal N (2009) Increased activity of ATP sulfurylase and increased contents of cysteine and glutathione reduce high cadmium-induced oxidative stress in mustard cultivar with high photosynthetic potential. *Russian Journal of Plant Physiology* **56**, 743-750

Khan NA, Samiullah, Singh S, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. *Journal of Agronomy and Crop Science* **193**, 435-444

Khan NA (2003) NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzymes in wheat. *Biologia Plantarum* **47**, 437-440

Krupa Z, Baranowska M, Orze D (1996) Can anthocyanin be considered as heavy metal stress indicator in higher plants? *Acta Physiolgia Plantarum* **18**, 147-151

Kumar PR (1997) *Rapeseed Mustard Research in India. 21st Century Strategies*, National Research Centre Rapeseed-Mustard, Bharatpur, India, pp 1-50

Küpper H, Küpper F, Spiller M (1996) Environmental relevance of heavy metal-substituted chlorophylls using the example of water plant. *Journal of Experimental Botany* **47**, 259-266

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidant defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Journal of Plant Physiology* **119**, 1091-1099

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics* **444**, 139-158

Mancinelli A (1984) Photoregulation of anthocyanin synthesis. VIII. Effects of light pre-treatments. *Plant Physiology* **75**, 447-453

Marchiol L, Leita L, Martin M, Peressotti ZG (1996) Physiological responses of two soybean cultivars to cadmium. *Journal of Environmental Quality* **25**, 562-566

Markovska YK, Gorinova NI, Nedkovska MP, Miteva KM (2009) Cadmium-induced oxidative damage and antioxidant responses in *Brassica juncea* plants. *Biologia Plantarum* **53**, 151-154

Marrs KA, Walbot V (1997) Expression and RNA splicing of the maize glutathione-*S*-transferase Bronze 2 gene is regulated by cadmium and other stresses. *Plant Physiology* **113**, 93-102

McLaughlin MJ, Parker DR, Clarke JM (1999) Metals and micronutrients: Food safety issues. *Field Crop Research* **60**, 143-163

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *Journal of Experimental Botany* **56**, 167-178

Mobin M, Khan NA (2007) Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *Journal of Plant Physiology* **164**, 601-610

Monni S, Uhlig C, Hansen E, Magel E (2001) Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. *Environmental Pollution* **112**, 121-129

Nolan AL, McLaughlin MJ, Mason SD (2003) Chemical speciation of Zn, Cd, Cu, and Pb in pore waters of agricultural and contaminated soil using Donnan dialysis. *Environment Science Technology* **37**, 90-98

Öncel I, Keleş Y, Üstun AS (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environmental Pollution* **107**, 315-320

Padmaja K, Prasad DDK, Prasad ARK (1990) Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. seedlings by Cd acetate. *Photosynthetica* **24**, 399-405

Radotic K, Ducic T, Mutavdzic D (2000) Changes in peroxidase activity and isoenzyme in spruce needles after exposure to different concentration of cadmium. *Journal of Environmental and Experimental Botany* **44**, 105-113

Rascio N, Vecchia FD, Ferreti M, Merlo L, Ghisi R (1993) Some effects of cadmium on maize plants. *Archives of Environmental Contamination and Toxicology* **25**, 244-249

Rivera-Becerril F, Calantzis C, Turnau K, Caussanel J-P, Belimov AA, Gianinazzi S, Strasser RJ, Gianinazzi-Pearson V (2002) Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. *Journal of Experimental Botany* **53**, 1171-1185

Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, Corpas FJ, Gómez M, del Río LA, Sandalio LM (2006) Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation *in vivo*. *Plant Cell Environment* **29**, 1532-1544

Sandalio LM, Dalurzo HC, Gómez M, Romero-Puertas MC, del Río LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plant. *Journal of Experimental Botany* **52**, 2115-2126

Sanitá di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. *Environmental and Experimental Botany* **41**, 105-130

Schröder P, Fischer C, Debus R, Wenzel A (2003) Reaction and detoxification mechanism in suspension cultured spruce cells (*Picea abies* L. Karst) to heavy metals in pure mixture and in soil elutes. *Environmental Science Pollution Research* **10**, 225-234

Shi GR, Cai QS, Liu QQ, Wu L (2009) Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes. *Acta Physiologia Plantarum* **31**, 969-977

Sieferman-Harms D (1987) The light harvesting function of carotenoids in photosynthetic membrane. *Plant Physiology* **69**, 561-568

Singh S, Khan NA, Nazar R, Anjum NA (2008) Photosynthetic traits and activities of antioxidant enzymes in black gram (*Vigna mungo* L. Hepper) under cadmium stress. *American Journal of Plant Physiology* **3**, 25-32

Skórzyńska-Polit E, Drażkiewicz M, Krupa Z (2003/04) The activity of the antioxidative system in cadmium-treated *Arabidopsis thaliana*. *Biologia Plantarum* **47**, 71-78

Somashekaraiah BV, Padmaja K, Prasad ARK (1992) Phytotoxicity of cadmium ions on germinating seedlings of mungbean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. *Physiologia Plantarum* **85**, 85-89

Stobart AK, Griffiths WT, Ameen-Bukhari I, Sherwood RP (1985) The Effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum* **63**, 293-298

Tewari AK, Tripathy BC (1998) Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant Physiology* **117**, 851-858

Vassilev A, Lidon FC, Metos M, Ramalho JC, Yordanov I (2002a) Photosynthetic performance and content of some nutrients in cadmium and copper treated barley plants. *Journal of Plant Nutrition* **25**, 2343-2360

Vassilev A, Vangronsveld J, Yordanov I (2002b) Cadmium phytoextraction: Present state, biological backgrounds and research needs. *Bulgarian Journal of Plant Physiology* **28**, 68-95

Wahid A, Ghani A (2008) Varietal differences in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation. *Annals of Applied Biology* **152**, 59-69

Yang MG, Lin XY, Yang XE (1998) Impact of Cd on growth and nutrient accumulation of different plant species. *Journal of Applied Ecology* **19**, 89-94

Zengin FK, Munzuroglu O (2006) Toxic effects of cadmium (Cd^{2+}) on metabolism of sunflower (*Helianthus annuus* L.) seedlings. *Acta Agriculturae Scandinavica – Section B – Soil Plant Sciences* **56**, 224-229

Zhang G, Wu F, Wei K, Dong Q, Dai F, Chan F (2006) Cadmium stress in higher plants. In: Khan NA, Samiullah (Eds) *Cadmium Toxicity and Tolerance in Plants*, Alpha Science International Ltd., Oxford, UK, pp 87-102