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Role of nitric oxide in cadmium-induced stress on growth, photosynthetic components and yield of *Brassica napus* L.

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Abstract

Experiments were carried out to study the effect of cadmium (Cd) and exogenous nitric oxide (NO) on growth, photosynthetic attributes, yield components and structural features of *Brassica napus* L. (cv. GSL 1). Cadmium in the growth medium at different levels (1, 2 and 4 Mm) retarded plant growth viz. shoot (27%) and root (51%) length as compared to control. The accumulation of total dry matter and its partitioning to different plant parts was also reduced by 31% due to Cd toxicity. Photosynthetic parameters viz., leaf area plant^{-1} (51%), total Chl (27%), Chl *a* / Chl b ratio (22%) and Hill reaction activity of chloroplasts (42%) were greatly reduced in Cd-treated plants. Cd treatments adversely affected various yield parameters viz., number of branches (23) and siliquae plant $^{-1}$ (246), seed number siliqua $^{-1}$ (10.3), 1000-seed weight (2.30g) and seed yield plant^{-1} (7.09g). Different Cd treatments also suppressed the differentiation of various tissues like vessels in the root with a maximum inhibition caused by 4mM Cd. Exogenous application of nitric oxide (NO) improved the various morpho-physiological and photosynthetic parameters in control as well as Cd- treated plants.

Key words

Cadmium, Sodium nitroprusside, Nitric oxide, *Brassica napus*, Growth

Introduction

Heavy metals are important environmental pollutants and their toxicity poses great health hazards to plants and animals. Among an array of heavy metals, cadmium (Cd) is a widespread pollutant with long biological half life (Wagner, 1993). It is released into the environment by traffic, metal working industries, mining and as a byproduct of mineral fertilizers and from other sources. It is easily absorbed by roots and translocated into different plant parts. Excess of Cd has been reported to impair several physiological and biochemical processes in plants such as photosynthesis, respiration, uptake of nutrient elements, cell elongation, nitrogen and carbohydrate metabolism that further impairs the normal growth and development (Artexe *et al.*, 2002; Dong and Zhang, 2005). Cd also promotes generation of active oxygen species (AOS) and lipid peroxidation thus leading to oxidative stress in plant tissues (Hsu and Kao, 2004). The most apparent symptom of Cd toxicity is chlorosis of leaves which is closely related to inhibition of chlorophyll synthesis and light harvesting Chl a/Chl b protein complex. The determination of chlorophyll characters can indicate the severity of Cd toxicity, and is used as senescence marker in selecting plants tolerant/susceptible to this metal (Zhang *et al.*, 2003). In recent years, nitric oxide (NO), a bioactive free radical has been shown to be involved in many developmental and physiological processes in plants including pathogen defense, programmed cell death, flowering and stomatal closure etc (Setia and Setia, 2006). Further, there are several reports on the alleviation of toxic effects of heavy metals by exogenous application of NO (Tian *et al.*, 2007). NO is able to counteract toxicity of AOS and H_2O_2 in plant tissues (Hsu and Kao, 2004). Among oleiferous *Brassica* species, oilseed rape (*Brassica napus*) is an important oilseed crop of India and can tolerate heavy metal toxicity to some extent (Xiong, 2006). *Brassica napus* can be grown for phytoremedation of heavy metal contaminated lands because of its prolific growth (Meng *et al.*, 1993) .Therefore, the toxic effect of Cd on growth and some physiological parameters of oilseed rape, and the effect of exogenous application of NO using SNP as donor to mitigate phytotoxicity of Cd were investigated.

Materials and Methods

The seeds of *Brassica napus* L. (cv. GSL 1) were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Germination tests were carried out in Petri plates using different concentrations of $Cd(NO_3)_2$ in the growth medium. Based on germination results, the three concentrations of Cd (1, 2 and 4 mM) were selected. In the first set, plants were raised in earthen pots containing reconstituted soil supplied with different levels of $Cd(NO_3)_2$ to create 1, 2 and 4 mM Cd concentrations, and irrigated with half strength Hoagland solution throughout the experiment. In the second set, 20 days old plants raised under Cd toxicity were sprayed with sodium nitroprusside (SNP, donor of NO, at the concentration of 200 μg ml^{-1}). All the spray treatments contained 0.5 % (v/v) Triton- X as surfactant. The spray treatments were repeated after one week interval. The plants grown in unamended soil and sprayed with water containing 0.5 % Triton- X served as control. At each harvest, 3 pots from each treatment were harvested and thus 24 samples were analysed for each parameter. The data on plant height (cm), root length (cm) and dry matter accumulation (mg) were recorded at 90 days after sowing (DAS). Leaf number plant^{-1} was recorded in control and treated plants at various stages of development viz. 30, 60, 90 and 120 DAS. Photosynthetic parameters viz. leaf area plant^{-1} (cm^2), total chlorophyll content (Anderson and Boardman, 1964) and Hill reaction activity (Cherry, 1973) in leaves were measured at 90 DAS in control and treated plants. At final harvest (150 DAS), data were recorded on various yield parameters. For comparing the root structure of control and Cd-treated plants, roots were fixed in formalin acetic acid (FAA) at 120 DAS. The fixed materials were processed for microtomy, sectioned at 10 μm and stained with Safranin and Fast Green (Jensen, 1962). Photomicrographs were taken on bright field research microscope fitted with digital camera and computer imaging systems (Leica Bright field research microscope, Germany). The transectional area of cells and tissues was calculated from Camera Lucida diagrams drawn on graph paper. The data on various parameters were subjected to statistical analysis. Critical difference values were calculated by doing analysis of variance (Singh *et al.*, 1991).

Results and Discussion

Brassica napus L. plants grown under Cd toxicity exhibited general reduction in growth and yield attributes. Increasing Cd concentrations from 1 to 4 mM progressively and significantly reduced plant height, root length, and root and shoot dry weights (Table 1). The decrease in plant height in response to 1, 2 and 4 mM Cd treatments was about 10,15 and 27 %, respectively, as compared to control. Similarly, the root length also showed decline about 15, 34 and 52 %, respectively, as compared to control following 1, 2 and 4 mM Cd treatments. Dry matter accumulation in roots and shoots also decreased consistently with increasing Cd concentrations and at 4mM Cd level the decrease in root and shoot dry weight was by about 36 and 29 %, respectively, over controls. The reduction of plant growth induced by Cd is in accordance with the effects of Cd in other plant species such as cucumber (Zhang *et al.*, 2003) and bean (Ismail, 2008). The growth inhibition at different Cd levels may be linked either to a lower mitotic activity in the root meristematic zone and/or to an inhibition of cell enlargement in the elongation zone as a consequence of decreased cellular turgor (Gabbrielli *et al.*, 1990) or due to the reduced extensibility of the cell wall (Pandolfini *et al.*, 1992).

Foliar application of 200 μg ml^{-1} SNP caused significant enhancement in the growth of control as well as Cd-treated plants. The increase caused by SNP treatments in plant height, root dry matter and shoot dry matter for control plants at 90 DAS was about 7, 12 and 5 %, respectively, whereas this increase was about 5, 19 and 17 %, respectively, for 4mM Cd treated plants supplemented with SNP. The enhancements obtained with SNP for various plant growth parameters varied considerably in plants raised under different levels of Cd concentrations (Table 1). A similar protective role of exogenous NO using SNP in reducing the Cd induced stresses has been reported for rice leaves (Hsu and Kao, 2004), sunflower leaves (Laspina *et al.*, 2005) and roots of *Cassia tora* (Wang and Zang, 2005), *Hibiscus moschetuos* (Tian *et al.*, 2007), *Lupinus luteus* (Kopyra and Gwo'z'dz, 2003) and tomato (Jin *et al.*, 2009).

The plants grown under Cd toxicity produced comparatively less number of leaves. The decrease in number of leaves was directly proportional to increasing concentration of Cd in the growth medium. In 1, 2, and 4 mM Cd-treated plants the average number of leaves at 90 DAS was 67, 60 and 57, respectively, as compared to 72 in control. Different Cd concentrations also reduced the leaf size and consequently the leaf area plant^{-1} (Table 2). The decline in average leaf area plant^{-1} paralleled the decrease in leaf number plant^{-1}. Compared to controls, the average leaf area plant^{-1} at 90 DAS decreased by about 13, 34 and 51 %, respectively, in response to 1, 2 and 4 mM Cd treatments. Moreover, the leaves of plants grown under Cd toxicity showed general chlorosis symptoms which were more apparent in plants treated with 4 mM Cd concentration. The leaves showed patch work- type chlorosis pattern, with green areas neighboring pale yellow to pinkish areas. The younger plants (up to 50 DAS) appeared to be more affected by Cd than the plants at latter stages of development. Similar reduction in leaf number and leaf area for plant in response to Cd treatments has been reported by Baryla *et al.* (2001). Since dry matter production is the function of photosynthesis, the Cd induced

Table- 1: Influence of cadmium (Cd) and nitric oxide (sodium nitroprusside as donor) on various morpho-physiological characteristics of *Brassica napus* at 90 DAS

Treatments	Plant height (cm)	Root length (cm)	Root dry matter (mg)	Shoot dry matter (mg)
Control	130.6±1.87	19.3±0.27	12.5±0.20	32.5±1.78
1mM Cd	116.8±1.81	16.5±0.30	11.0±0.70	30.5±1.52
2mM Cd	110.6±1.08	12.8±0.35	9.5±0.54	26.3±1.13
4mM Cd	95.3±1.99	9.40±0.49	8.0±0.57	23.2±2.12
200μg ml^{-1} SNP	139.2±2.88	21.2±0.16	14.0±0.21	34.2±3.42
1 Cd+200μg ml^{-1} SNP	127.6±1.87	19.3±0.26	12.2±0.90	32.1±3.21
2 Cd+200μg ml^{-1} SNP	119.3±1.59	15.1±0.21	11.2±0.70	31.5±3.15
4 Cd+200μg ml^{-1} SNP	100.2±1.57	12.8±0.57	9.5±0.57	27.2±2.72
CD at p=0.05	**1.78**	**2.53**	**1.92**	**2.01**

Values are mean ± SE of ANOVA for completely randomized design (CRD); Sample size for analysis = 24; The level of significance is referred by p as depicted alongwith CD

Table- 2: Influence of cadmium (Cd) and nitric oxide (sodium nitroprusside as donor) on photosynthetic parameters of leaves in *Brassica napus* at 90 DAS

Treatments	No. of leaves plant^{-1}	Leaf area plant^{-1} (cm^2)	Total Chl (mg g^{-1} f.wt.)	Chl a / Chl b ratio	Hill reaction activity (△ OD mg^{-1}Chl hr^{-1})
Control	72.5±1.98	6408±120.9	0.81±0.09	1.39	20.0±0.32
1mM Cd	67.4±1.87	5561±100.1	0.75±0.11	1.25	17.4±0.24
2mM Cd	60.6±1.08	4200±60.5	0.68±0.08	1.19	14.2±0.25
4mM Cd	57.5±1.83	3135±60.0	0.59±0.05	1.08	11.6±0.19
200μg ml^{-1} SNP	74.4±1.90	6496±119.7	0.84±0.07	1.47	21.0±0.21
1Cd+200μg ml^{-1} SNP	71.2±1.19	5600±99.9	0.78±0.12	1.38	18.9±0.38
2Cd+200μg ml^{-1} SNP	64.1±1.06	4400±68.7	0.73±0.10	1.31	15.4±0.46
4Cd+200μg ml^{-1} SNP	60.1±1.91	3250±61.5	0.62±0.08	1.23	12.1±0.56
CD at p=0.05	**2.01**	**85.9**	**0.02**	**-**	**0.58**

Values are mean ± SE of ANOVA for completely randomized design (CRD); Sample size for analysis = 24; The level of significance is referred by p as depicted alongwith CD

Table- 3: Influence of cadmium (Cd) and nitric oxide (sodium nitroprusside as donor) on yield components at final harvest i.e. 120 DAS in *Brassica napus*

Treatments	No. of branches	No. of siliquae plant^{-1}	Seed no. siliqua^{1}	1000 seed weight (g)	Seed yield plant^{1} (g)
Control	35±1.01	337±6.90	13.0±0.60	3.15±0.11	13.80±0.60
1mM Cd	30±2.00	298±6.01	12.3±0.81	2.92±0.22	10.81±0.54
2mM Cd	27±2.82	267±5.99	11.5±1.09	2.80±0.26	8.96±0.51
4mM Cd	23±0.70	246±5.86	10.3±0.04	2.30±0.29	7.09±0.45
200μg ml^{-1} SNP	44±1.19	365±6.51	14.0±0.19	3.20±0.14	16.35±0.89
1Cd+200μg ml^{-1} SNP	38±1.91	322±6.41	13.1±0.58	3.10±0.12	13.07±0.17
2Cd+200μg ml^{-1} SNP	34±1.41	288±6.14	12.0±0.95	2.90±0.27	10.02±0.51
4Cd+200μg ml^{-1} SNP	30±2.82	265±5.99	11.3±0.70	2.85±0.29	8.53±0.51
CD at p=0.05	**2.58**	**3.62**	**1.93**	**1.87**	**2.03**

Values are mean ± SE of ANOVA for completely randomized design (CRD); Sample size for analysis = 24; The level of significance is referred by p as depicted alongwith CD

Table- 4: Influence of cadmium (Cd) on some structural features of root at 120 DAS in *Brassica napus*

Treatments	Diameter (cm)	No. of vessel elements per unit area	Transectional area of a vessel element (μm^2)
Control	0.71±0.08	12.20±0.61	318.30±6.82
1mM Cd	0.52±0.06	9.10±0.54	290.90±6.17
2mM Cd	0.47±0.05	7.50±0.57	255.10±5.24
4mM Cd	0.38±0.04	5.70±0.49	197.40±3.49
CD at p=0.05	**0.02**	**0.51**	**17.73**

Values are mean ± SE of ANOVA for completely randomized design (CRD); Sample size for analysis = 24; The level of significance is referred by p as depicted alongwith CD decreases in leaf number and leaf area appear to result in overall reduced photosynthetic efficiency of oilseed rape plants.

Nitric oxide enhanced the number of leaves as well as leaf area plant^{-1} in control and Cd-treated plants. However, this chemical could only partially ameliorate the Cd induced inhibition in number of leaves and leaf area plant^{-1}. Low micro molar concentrations of NO have been found to increase the rate of leaf expansion in pea (Leshem and Haramaty, 1996) and effect leaf cell ultrastucture of common flax (Batasheva, 2010).

Cd induced reduction in leaf number and leaf area was accompanied by a simultaneous decrease in total Chl content in leaves. The total Chl content in leaves of Cd-treated plants decreased consistently with increasing concentrations of the heavy metal in the growth medium. Leaf chlorophyll content decreased by about 7, 16 and 27 % in response to 1, 2 and 4 mM Cd treatments, respectively, as compared to control (Table 2).The Chl *a* and *b* contents decreased differentially in leaves with increase in Cd concentrations. However, Chl *a* was more sensitive to Cd toxicity as evidenced by greater reduction in its content than Chl *b*. Consequently, Chl a/ Chl b ratio also showed decrease due to Cd treatments. Moreover, Hill reaction activity of chloroplasts in leaves also decreased following Cd treatments. Earlier it has been reported that high Cd concentrations in leaf tissues affect indirectly the chlorophyll level via metabolic perturbations and accelerated senescence and oxidative damage (Sandalio *et al.,* 2001), enhancement of chlorophyllase catabolic activity (Abdel-Basset *et al.*, 1995) or deficiency of some essential elements such as Fe or Mg (Siedlecka and Krupa, 1999). A considerable decrease in the level of chlorophyll, Chl *a*/Chl *b* ratio and Hill reaction activity of chloroplasts occurred in leaves of Cd-treated plants. The photosynthetic machinery is extremely sensitive to Cd and the use of chlorophyll content has been shown to be highly reliable and fairly sensitive measure of Cd stress in various crop species. Therefore, the reductions in chlorophyll content and its above mentioned characteristics seem to affect plant photosynthetic efficiency, and thus could partially be responsible for the decrease in dry matter production and plant growth. The decrease in chl a/chl b ratio due to Cd treatments is possibly due to faster hydrolysis of Chl *a* compared to Chl *b* when plants are under stress(Drazkiewicz, 1994). The Hill reaction activity of isolated chloroplasts (indicating PS II activity) is very sensitive to Cd treatments (Simonova *et al.*, 2007)

SNP treatments enhanced the levels of total Chl, Chl *a*, *b*, Chl a/Chl b ratio and Hill reaction activity of chloroplasts in the leaves of control and Cd-treated plants (Table 2). Similar results were also reported by He *et al.* (2004) and Pahwa *et al.* (2009a) in which NO exerted promotive effect on chlorophyll content. NO donors are reported to stimulate de-etiolation and increase chlorophyll content in lettuce, potato, *Arabidopsis* and dark grown wheat seedlings (Beligni and Lamatina, 2000) and in pea leaves particularly guard cells (Leshem *et al.*, 1996), and retard chlorophyll loss in *Phytophthora infestans* infected potato leaves (Laxalt *et al.*, 1997).

Table 3 shows the effect of Cd and SNP treatments on yield and yield components at final harvest. The number of branches per plant decreased by 14, 22 and 34 %, respectively, in 1, 2 and 4 mM Cd treated plants as compared to controls. Concomitant with the decrease in branching, the number of siliquae plant^{-1} also decreased significantly in response to Cd treatments and the decline in number of siliquae was directly proportional to increasing concentrations of Cd. The average number of siliquae plant^{-1} in controls was 337, and it decreased to 298, 267 and 246 plant^{-1}, respectively, in 1, 2 and 4 mM Cd treated plants. Seed number siliqua^{-1} and 1000-seed weight also declined significantly with Cd treatments. Consequently, the seed yield plant^{-1} also decreased by about 21, 35 and 48 % in response to 1, 2 and 4 mM Cd treatments, respectively, as compared to control. In *Brassica* sp., the seed yield depends primarily on the number of branches and the number of siliquae plant^{-1}, seed number siliqua^{-1} and average seed weight (Pahwa *et al.*, 2009b). These yield parameters were adversely affected by Cd treatments. The suppression of development of reproductive structures in response to Cd has also been reported in pea and wheat (Setia *et al.*, 1993). The low seed yield due to Cd treatments in *Brassica napus* seems to be the result of insufficient availability of photoassimilates during seed development due to decreased photosynthetic efficiency of plants.

SNP treatments resulted in enhancement of siliqua number and seed yield plant^{-1} in control as well as Cd- treated plants. Due to SNP treatments the increase in siliqua number plant^{-1} was by about 27 and 8 %, and the seed yield plant^{-1} was by about 18 and 20 % in control and 4 mM Cd treated plants, respectively, over their respective controls. The significant increase in yield with SNP treatments could be the result of enhanced supply of assimilates towards developing siliqua. Several studies have also reported an increase in yield and yield components of *B. napus* with plant growth regulators as a result of altered source: sink ratio (Yadav *et al.*, 2009). Further, it has been well documented that NO is also a phytohormone that interacts with different plant hormones at different steps of signaling cascades to evoke various responses (Neill *et al.*, 2003).

Cd treatments greatly suppressed the differentiation of various tissues with concomitant decrease in root diameter (Table 4).Compared to controls, roots of Cd treated plants showed decrease in number and transactional area of vessel elements. The inhibitory effect of Cd was directly proportional to its increasing concentrations in the growth medium. Figure 1 shows the transverse section of root depicting the pattern of differentiation of ground and vascular tissues in control and 4mM Cd treated plants. The differentiation of xylem fibers was suppressed and the ray cells became indistinguishable. Further, in roots of plants treated with 4mM Cd, the most of the cells remained undifferentiated except for a few partially differentiated vessel elements. Cd induced abnormalities in the root structure have also been observed in 8-day-old Cd-treated *Vigna mungo* seedlings (Molina *et al.*, 2008).The availability of water and nutrients to the crop depends primarily on the root system.

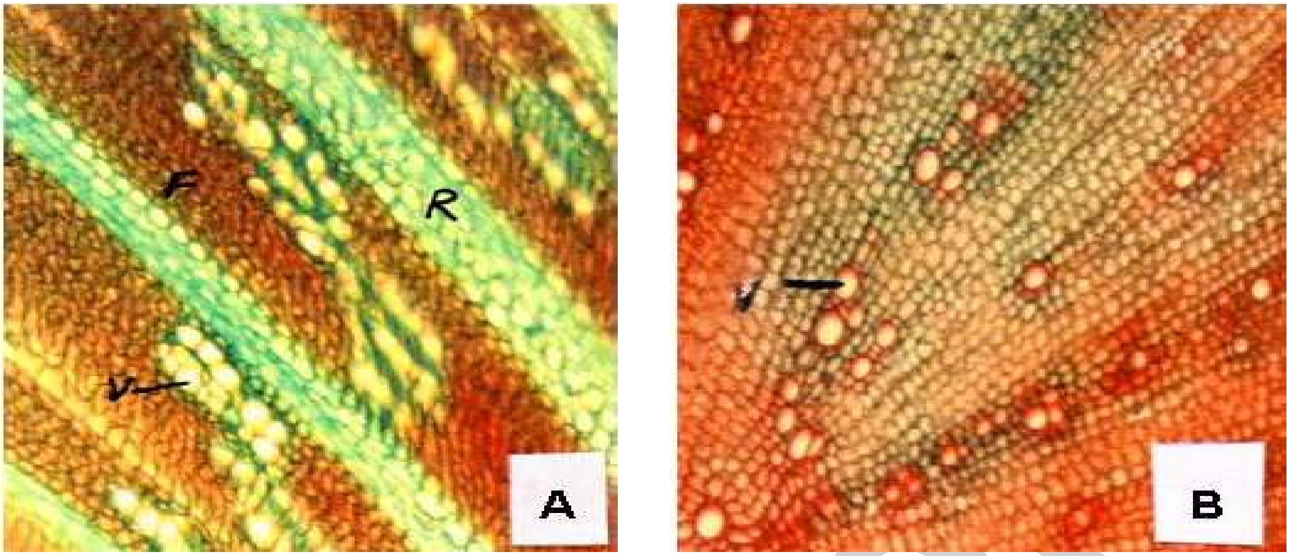

Fig.1: Transverse sections of the root of *Brassica napus* **(A)** Control: Note vessel elements, fibres and ray parenchyma, **(B)** 4 mM Cd-treated root. The differentiation of vessel elements is suppressed as evident from their apparent number. (V-vessel elements, F-fibres, R- ray parenchyma) (A-B x 400)

The inhibition of shoot growth of *B. napus* plants in response to Cd treatments might be the result of restriction of water and nutrient supply and/or from other factors associated with histological disorders induced by Cd in roots and decreased rooting.

Thus, the results indicated that the increasing concentrations of Cd in the soil inhibited growth of *Brassica napus* plants by affecting morphological, physiological and anatomical aspects. The Cd induced inhibition of various plant growth and yield parameters were at least partially ameliorated by SNP treatments as evident by improvements in the these parameters.. The protective effect of NO might be due to maintenance of appropriate levels of reactive oxygen species as a result of up regulation of activities of antioxidant enzymes.

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References

Abdel-Basset, R., A.A. Issa and M.S. Adam: Chlorophyllase activity: Effects of heavy metals and calcium. *Photosynthetica*, **31**, 421-425 (1995).

Anderson, J.H. and N.K. Boardman: Studies on greening of dark brown bean plants. VI. Development of photochemical activity. *Aust. J. Biol.*, **17**, 93-101 (1964).

Artexe, U., J.L. Gareias-Plazoals, A. Hernandez and J.M. Becerril: Low light grown duckweed plants are more protected against the toxicity induced by Zn and Cd. *Plant Physiol. Biochem.*, **40**, 859-63 (2002).

Baryla, A., P. Carrier, F. Fabrice, C. Coulomb, C. Sahut and H. Michel: Leaf chlorosis in oilseed rape (*Brassica napus*) plants grown on cadmium polluted soil: Causes and consequences for photosynthesis and growth. *Planta*, **212**, 696-709 (2001).

Batasheva, S.N., F.A. Abdrakhimov, G.G. Bakirova, E.V. Isaeva and V.I Chikov : Effects of sodium nitroprusside, the nitric oxide donor, on photosynthesis and ultrastructure of common flax leaf blades. *Russian Plant Physiol.*, **57**, 376-381 (2010).

Beligni, M.V. and L. Lamattina: Nitric oxide stimulates seed germination and de-etiolation and inhibits hypocotyl elongation, three light inducible responses in plants. *Planta*, **210**, 215-21 (2000).

Cherry, J.H.: Molecular Biology of Plants, A Text Manual. Columbia University Press, New York and London, p. 46-49 (1973).

Dong, J. and G. Zhang: Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang. Univ. Sci.*, **6**, 974-980 (2005).

Drazkiewicz, M.: Chlorophyllase: Occurrence, functions, mechanism of action, effects of external and internal factors. *Photosynthetica*, **30**, 321-331 (1994).

Gabbrielle, R., T. Pandolfini, O. Vergnamo and M.R. Palandri: Comparision of two serpentine species with different nickel tolerance strategies. *Plant Soil*, **122**, 271-277 (1990).

He Y., R.H. Tang, Y. Hao, R.D.Stevans, C.W. Cook, S.M. Ahn, L. Jing, Z. Yang, L. Chen, F.Guo, F.Fionari, R.B.Jackson, N.M.Crawford and Z.M.Pei: Nitric oxide represses the *Arabidopsis* floral transition. *Science*, **305,** 1968-1971 (2004).

Hsu, Y.T. and C.H. Kao: Cadmium toxicity is reduced by nitric oxide in rice leaves. *Plant Growth Regul.*, **42**, 227-238 (2004).

Ismail, M.A.: Involvement of calcium in allevation of cadmium toxicity in common bean (*Phaseolus vulgaris* L.) plants. *Asian J. Biol. Sci.*, **1**, 26-32 (2008).

Jensen, W.A.: Botanical Histochemistry. Freeman Publishers, San Francisco. (1962).

Jin, C.W., S.T. Du, Y.S.Zhang, X.Y. Lin and C.X. Tang: Differential regulatory role of nitric oxide in mediating nitrate reductase activity in roots of tomato (*Solanum lycocarpum*). *Ann. Bot.*, **104**, 9-17 (2009).

Kopyra M. and E.A. Gwo´z´dz´: Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiol. Biochem.*, **41**, 1011–1017 (2003).

Laspina, N.V., M.D. Groppa, M.L. Tomara and M.P. Benavides: Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Sci.*, **169**, 323-30 (2005).

Laxalt, A., M.V. Beligni and L. Lamattina: Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*. *Eur. J. Plant Pathol*., **103**, 643-651(1997).

Leshem, Y.Y. and E.Haramaty: The characterization and contrasting effects of nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn foliage. *J. Plant Physiol.,* **148**, 258-263 (1996).

Leshem, Y.Y., E. Haramaty, D. Iluz, Z. Malik, Y. Sofer and L. Roitman: Effect of stress on nitric acid (NO) interaction between chlorophyll fluorescence, galactolipid fluidity and lipoxygenase activity. *Plant Physiol. Biochem.,* **35**, 573-79 (1996).

Meng, H., S. Hua, I.H. Shamsi, G. Jilani, Y. Li and L. Jiang: Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L. and its allevation through exogenous plant growth regulators. *Plant Growth Regul*., **58**, 47-59 (1993).

Molina, A.S., C. Nievas, M.V.P. Chaca, F. Garibotto, U. González, S.M. Marsá, C. Luna, M.S. Giménez and F. Zirulnik: Cadmium-induced oxidative damage and antioxidative defense mechanisms in *Vigna mungo* L. *Plant Growth Regul*., **56**, 285-295 (2008).

Neill, S.J., R. Desikan and J. T. Hancock : Nitric oxide signaling in plants. *New Phytol*., **159**, 11-35 (2003).

Pahwa, S., R.C. Setia and N. Setia: Effect of exogenous nitric oxide on chlorophyll content and Hill reaction activity in leaves of *Brassica napus* L. *Environ. Ecol*., **27**, 278-280 (2009a).

Pahwa, S., R.C. Setia and N. Setia: Influence of sodium nitroprusside (Nitric oxide releaser) on yield components and seed quality of *Brassica napus* L. *Environ. Ecol*., **27**, 278-280 (2009b).

Pandolfini, T.R., R. Gabbrelli and C. Camparini: Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. *Plant Cell Environ*., **15**, 719-725 (1992).

Sandalio, L.M., H.C. Dalurzo, M. Gomez, M.C. Romero-Puertas and L.A. Del Rio: Cadmium induced changes in the growth and oxidative metabolism of pea plants. *J. Expt. Bot*., **52**, 2115-2126 (2001).

Setia, N. and R.C. Setia: Nitric oxide (NO) as a regulator and signaling molecule in plants. In: Plant Molecular Phisiology Current Scenario and Future Prospects (Ed.: P.C. Trivedi). Aavishkar Publishers, Jaipur, p. 32-57 (2006).

Setia, R.C., R. Bala, N. Setia and Anuradha.: Photosynthetic characteristics of heavy metal treated wheat (*Triticum aestivum* L.) plants. *Plant Sci. Res*., **9**, 47-49 (1993).

Siedlecka, A. and Z. Krupa: Cd/Fe interaction in higher plants-its consequence for the photosynthetic apparatus. *Photosynthetica*, **36**, 321-331 (1999).

Simnonova, E., M. Henselova, H.P.M. Singh, D.R. Batish, G. Kaur, K. Arora and R.K. Kohli: Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environ. Exp. Bot*., **63**,158-67 (2008).

Singh, S., M.L. Bansal, T.P. Singh and R. Kumar: Statistical Methods for Research Workers. Kalyani Publishers, New Delhi, India (1991).

Tian, Q.Y., D.H. Sun, M.G. Zhao and W.H. Zhang: Inhibition of nitric oxide synthase (NOS) underlies aluminium-induced inhibition of root elongation in *Hibiscus moscheutos*. *New Phytol*., **174**, 322-31 (2007).

Wagner, G.J.: Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron*., **51**, 173-212 (1993).

Wang, Y.S. and Z.M. Zang: Reduced aluminium toxicity by preventing oxidative stress in the roots of *Cassia toria* L. *Plant Cell Physiol*., **46**, 1915-23 (2005).

Xiong, Z.T., F. Zhao and M. Li: Lead toxicity in *Brassica pekinensis* Rupr.: Effect on nitrate assimilation and growth. *Environ. Toxicol*., **21**, 147-153 (2006).

Yadav, K.G., R.V. Singh and R. Pundir: Yield and economics of *Brassica* species as influenced by growth regulators and fertility levels under semi-arid area of Indo-Gangetic plain. *Prog. Agri*., **9**, 54-58 (2009).

Zhang, F., W. Shi, Z. Jin and Z. Shen: Response of antioxidative enzymes in cucumber chloroplasts to toxicity. *J. Plant Nutri*., **26**, 1779-1788 (2003).