International Scholars Journals

African Journal of Agriculture ISSN 2375-1134 Vol. 7 (5), pp. 001-010, May, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Cytogenetic and biochemical investigations to study the response of *Vigna radiata* to cadmium stress

Sowbiya Muneer[1], Tabasum Nazir Qadri[2], Mahmooduzaffar[2] and T. O. Siddiqi[2]*

[1]Integrative Division of Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang, South Korea.

[2]Department of Botany, Faculty of Sciences, Jamia Hamdard, New-Delhi, India.

Accepted 18 January, 2020

A study was carried out on root tips and leaves of *Vigna radiata*, 15 days old plantlets grown in nutrient Hoagland media were exposed to various levels of cadmium chloride (0.05, 0.10 and 0.50 mM) for 48 and 72 h. The plant tissues were analyzed for mitotic index, chromosomal variations, root-shoot ratio, leaf area, chlorophyll estimation, and the activity of nitrate content, nitrate reductase (NR), soluble protein and proline content. A number of chromosomal variations such as laggard chromosomes, anaphasic bridges, and unidistribution of chromatin material were observed in root tips. A reduction in mitotic index, root-shoot ratio, area of leaf and chlorophyll estimation was observed at all concentrations of cadmium. Moreover, activity of nitrate reductase (NR), nitrate content and soluble protein content was also found to be decreased. However, rate of proline content was found to be increased on increasing the concentration of cadmium. An increase in the levels of proline suggests its possible incorporation in synthesis of the phytochelatins and metallothioneins to sequester and combat Cd-stress.

Key words: *Vigna radiata*, phytochelatins, cytogenetics, cadmium.

INTRODUCTION

Cadmium (Cd) is the fifth most toxic metal to vertebrates and the fourth most toxic metal to vascular plants (Oberlunder et al., 1978). Even at low concentration, Cd may adversely affect the plant reproduction by inhibiting pollen germination and tube growth (Xiong et al., 2001). Various authors reported and reviewed the toxic effect of cadmium on biological systems (Baryla et al., 2001; Qureshi et al., 2010). In plants, the symptoms of cadmium-toxicity are easily identifiable ranging from slight injury to lethality or crop failure. The most general symptoms are stunting growth, chlorosis and alteration of anatomical, morphological, physiological and biochemical properties

Metal toxicity and tolerance in plants is a subject that has been broadly reviewed on several occasions over the past 30 years (Brown and Jones, 1975; Foyer et al., 1997; Ernst et al., 1992; Das et al., 1997; Hall, 2002; Clemens et al., 2001).

One of the major consequences of Cd toxicity is oxidative stress (Okamoto et al., 2001; Agaehi et al., 2009) mediated by increased levels of reactive oxygen species (ROS), but in contrast with other toxic metals, such as Cu, it does not seem to act directly on the production of ROS through Fenton-type reactions or Haber–Weiss reactions. Evidence that Cd causes the production of ROS (Foyer et al., 1997) in plants came from observations where new isozymes of peroxidases were detected in both root and leaves of *Phaseolus vulgaris* (Van and Assche, 1990) treated by Cd. Exposure of plants and plant cell to cadmium result in a large number of physiological, biochemical, and molecular changes, in addition to the synthesis of metal binding polypeptides. Some of these responses are similar to those elicited by other abiotic stresses (Edelman et al., 1988). Some responses may result from the requirement for the

*Corresponding author. E-mail: tariq117@rediffmail.com. Tel: 00-91-9810770943.

Abbreviations: mM, (millimolar); **NR,** (nitrate reductase); **HAT,** (hours after treatment); **MI,** (mitotic index); **ROS,** (reactive oxygen species); **DDW,** (double distilled water); **Cd,** (cadmium), **cm;** (centimetres).

synthesis of the polypeptide precursors but many other have no clear relationship to the production of these molecules. The mechanism of cadmium tolerance and the response of plants to this toxic metal are quite complex and cannot be explained by the production of only one class of molecules. Genetic toxicity bioassays have to be very useful in environmental monitoring and assessment of cadmium toxicity. The advantages of using plant assays for genetic toxicological testing are that a number of plants having long and low number of chromosomes offer excellent cytogenetic system with a wide range of genetic end points, from gene mutation to mitotic and meiotic chromosome aberrations and DNA-damage as well as division aberrations, micronuclei (MNC), sister chromatic exchange (SCE) and the comet assay that evaluates DNA damage.

In general, accumulation of a given metal is a function of uptake, capacity and intercellular binding sites. At every level, concentration and affinities of chelating molecules as well as the presence and selectivity of transport activities affect metal accumulation rates (Clemens et al., 2002). The strategies for avoiding toxic metal toxicity are diverse. A first barrier against Cd stress, operating mainly at the root level can be immobilization of Cd ions seems to be mostly bound by pectic sites and hystidyl groups of the cell wall (Leita et al., 1996). However, the importance of these mechanisms may vary in accordance with the concentration of Cd supplied, the species involved, the exposure time etc. preventing Cd ions from entering the cytosol through the action theoretically represent the best defence mechanism.

Vigna radiata (green gram) is among the most important in semiarid regions. A decoction of seeds is used as an effective in Beri-Beri. The mung extract is said to have protective and curative properties of certain diseases such as polyneuritis glanarium. It is also used in the production of several antibiotics such as pencilline and thus we selected it for our study. Our study aimed to examine the impact of different concentration of cadmium on mitotic index, chromosomal variations, morphological parameters such as root-shoot ratio, leaf area and certain biochemical analysis including chlorophyll estimation, soluble protein content, activity of NR and proline content.

MATERIALS AND METHODS

Plant material and treatment conditions

Seeds of *V. radiata* variety Pusa vishal were collected from IARI (Indian Agricultural Research Institute) New-Delhi, India. Experiments were conducted in 250 ml beakers containing Hoagland nutrient solution of half strength in a culture room with 16 h photoperiod, a day/night temperature and relative humidity regimes of 25 ± 2°C and 55 to 75%, respectively. Healthy seeds of uniform size were surface sterilized with 4% sodium hypochlorite in double distilled water (DDW) for 5 min. The sterilized seeds were sown on wet paper towels in petriplates. Three-day-old seedlings were transferred to beakers containing nutrient media (half strength). Hoagland solution was changed after every third day (Figure 10). Fifteen day-old seedlings were subjected to various concentrations of $CdCl_2$ (0.05, 0.10 and 0.50 mM) for 48 and 72 h after treatment (HAT). The plant tissues were harvested and were stored at -80°C until use.

Mitotic index

Mitotic index was studied according to the method described by Sharma et al. (1994). The root tips were cut from the plantlets and were preserved in chlochine for 24 h and then kept in acetocarmine for few hours to accomplish a stain. The root tips were slashed and a drop of glycerine were dropped on a slide, covered with cover slip then were tabbed with a blunt end of a needle and heated for sometimes on a sprit lamp till it became bearable to touch and were observed under multi probe scanning microscope. Mitotic index was calculated as the percentage of number of dividing cells in a particular field to the total number of cells to the same field.

$$MI = \frac{\text{Number of dividing cells in a microscopic field}}{\text{Total number of cells in the same field}} \times 100$$

Root-shoot ratio

The root-shoot ratio indicates that the length of plant arise from the root tip to upper most growing tip of the main axis. For root-shoot ratio plants were measured from the base to apex in cm. Plants were uprooted carefully washed with DDW and were kept on moist filter paper to avoid desiccation. The plants were measured with the help of measuring scale in cm and were recorded.

Leaf area

Fully opened leaves of individual plants were counted. The areas of leaves were measured by using a leaf area meter (Model 3000A, L1COR, USA) in cm.

Chlorophyll estimation

Chlorophyll content was estimated by the method of Hiscox and Israelstam (1979). Fresh leaves were collected and kept in vials to which 10 ml DMSO were added and were kept in an oven at 65°C for 1 h. Absorbance was taken at 480, 645, 520 and 663 nm on the Beckman DU 640 B spectrophotometer. The chlorophyll concentrations in mg fresh samples were calculated by the formula given by Arnon (1949).

Nitrate content

Nitrate estimation was done by the method given by Grover et al., (1978). Fresh leaves were taken in a test tube to which 50 mg of charcoal and 10 ml DDW were added and were boiled for 10 min. After filtration, the volume was made up to 50 ml by adding DDW. 1 ml of aliquot were taken and 0.5 ml $CuSO_4$-$ZnSO_4$ solution, 0.25 ml hydrazine sulphate, 0.25 ml NaO_4 and 1.5 ml DDW were added to it. The test tubes were kept in water bath incubator for 10 min at

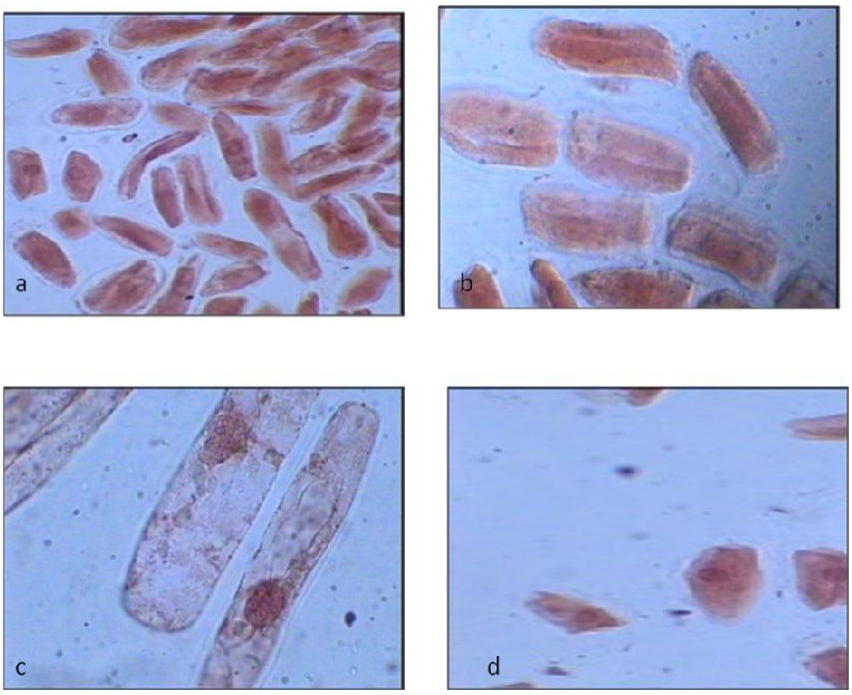

Figure 1. Chromosomal aberrations under Cadmium stress (a) control (b) anaphasic bridges (c) Laggard chromosomes (d) Unidistribution of chromosomes.

33°C and the reaction was terminated in ice to which 0.5 ml chilled acetone and 1 ml suophanilamide and NEDD were added and absorbance was taken at 540 nm on Beckman DU 640 Spectrophotometer.

Nitrate reductase activity

NR activity was determined by the method given by Klepper et al., (1971). Fresh leaves with 3.0 ml phosphate buffer (pH = 7.2) and KNO_3 were kept in vials in vacuum desicator for 30 s interval till the leaves settle down. The vials were then kept in hot water for 5 min to stop the reaction. 0.2 ml aliquot was taken to which 1.0 ml of suophanilamide and NEDD solution were added, kept in dark for 20 min. Absorbance was taken at 540 nm on Beckman DU 640 spectrophotometer.

Soluble protein content

Soluble protein content was evaluated by the method of Bradford (1976), using bovine serum albumin as a standard. 0.5 g of fresh plant material were homogenized in 1 ml phosphate buffer and were centrifuged at 5,000 rpm from which 0.5 ml supernatant were taken to which 0.5 ml TCA was added. The samples were again centrifuged at 3,300 rpm and the supernatant were discarded and the remaining was washed with NaOH to which 5 ml Bradford was added and the absorbance was taken at 640 nm.

Proline content

The proline content was determined by the method given by Bates et al. (1973). Fresh leaf material was homogenized in 10 ml of aqueous sulphosalylic acid and the homogenate were centrifuged at 3,300 rpm, and supernatant were collected to which 2 ml of acid ninhydrin glacial acetic acid were added. The vials were then incubated for 1 h at 100ºC and were terminated in ice bath, then 4 ml toluene was added and vortexed. Absorbance was taken at 520 nm on DU spectrophotometer. The concentration of proline in the sample was computed from a standard curve of L-Proline and expressed in mg^{-1}g fresh weight.

Statistical analysis

The data obtained were statistically analyzed using one way ANOVA to check the authenticity of results.

RESULTS AND DISCUSSION

Cytogenetical analysis

Cd exhibited inhibitory effect on cytogenetical studies namely, mitotic index and chromosome number considerably in a dose and time dependent manner. Reduction in mitotic index varied from 8 to 11%, 19 to 20%, 22 to 23% at 48 and 72 HAT respectively when compared to their respective control (Figure 2) was observed. Chromosomal studies showed various chromosomal abnormalities such as laggard chromosomes, anaphasic bridges, and unidistribution of chromosomes with respect to various treatments of $CdCl_2$ studied under multiprobe scanning microscope as shown in Figure 1. *V. radiata* has been used widely in scanning for the clastogenetic effects of different chemicals, due to

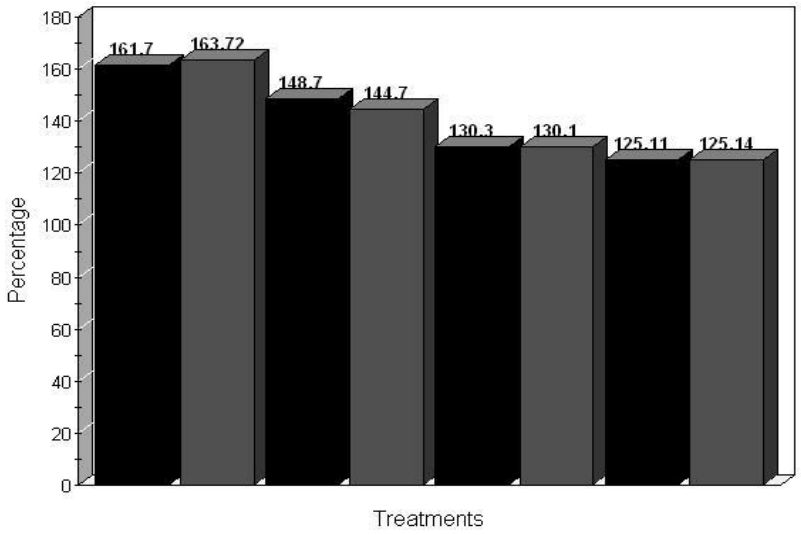

Figure 2. Effect of on Mitotic index in *Vigna radiata* to various concentrations of Cd.

their relative simplicity. Cadmium did not only brought down the frequency of dividing cells, but also produced a good number of anomalies in mitotic cells. There was a marked decrease in the mitotic index and gradual increase in the percentage of chromosomal abnormalities as the concentration of the experimental solution increased. Our results are in agreement with the finding of other workers who worked on higher plants with different toxic metals which suggests that such reduction in the mitotic activity could be due to inhibition of DNA synthesis. Schnaderman et al. (1971) and Beu et al. (1976) also showed that exposing the root tips of *V. radiata* to higher concentration of the cadmium led to inhibition of DNA synthesis which supports our findings.

Anaphasic bridges were induced by the toxic metals these may be formed due to unequal exchange or dicentric chromosomes. The occurrence of breaks and their lateral fusion leads to the formation of dicentric chromosomes. The dicentric chromosome in pulled equally to both the poles at anaphase and a bridge is formed. The occurrence of lagging chromosomes may be explained on the basis of abnormal spindle formation and failure of chromosomal breakage by binding of DNA regions rich in GC pairs causing these to become unstable (Lowley and Brooke, 1963). A good number of metals, those having high affinity for S-H group preferentially associated with tubulin protein, impair spindle fibre causing chromosome condensation and aneuploidy.

Leaf area

The leaf area was eventually decreased at all concentrations of Cd treatments from 11 to 17%, 76 to 90%, 78 to 93% at 48 and 72 HAT, respectively as shown in Figure 3. The reduction in leaf area may be due to increase in concentration of ROS which reduces the level of proteins and certain enzymes which are responsible for the growth of leaves. Several studies have also reported that due to presence of metal toxicity the chlorosis and necrosis in leaves reduces the leaf area (Qureshi et al., 2005).

Root-shoot length

Compared to control the length of root and shoot were 70 and 74 to 73% declined both at 48 and 72 HAT. Maximum decline was found at 0.50 mM Cd exposure (76 to 77%) (Figure 4). Several studies have been carried out on various plants under different metal toxicity on root-shoot ratio which supposed to decrease the height of plant. This might be due to inactivation and interruption of proteins accountable for growth and development of plants. It has been observed that in *Brassica juncea* Cadmium exhibited inhibitory effect on plant growth, plant height and biomass accumulation in a dose- and time-dependent manner (Qadir et al., 2004).

Chlorophyll estimation

Chlorophyll (chl a + chl b and carotenoid) content in the leaf samples decreased both at 48 and 72 HAT at all concentrations of (reduce space) cadmium in a dose-dependent manner, showing a greater decline in plants under 0.50 mM cadmium treatment at 72 HAT (Figure 5). Chlorophyll is a vital pigment for photosynthesis in plants. Recent studies have suggested that the formation of

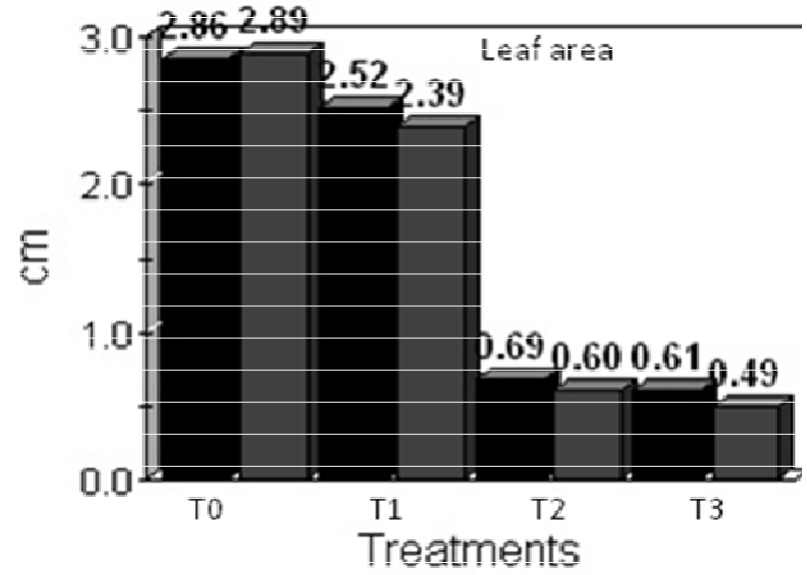

Figure 3. Effect of on leaf area in *Vigna radiata* to various concentrations of Cd.

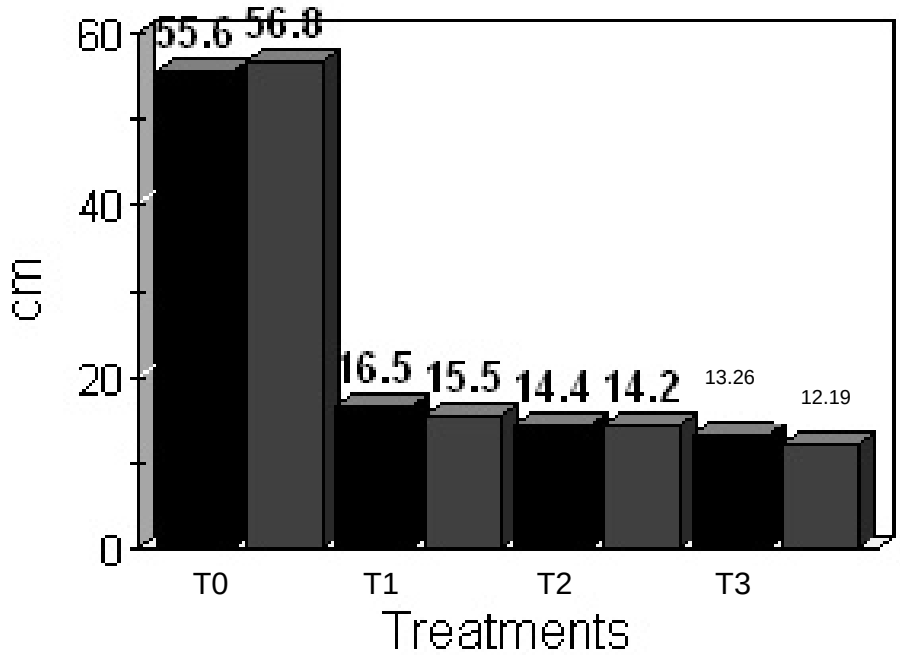

Figure 4. Effect of on root-shoot in *Vigna radiata* to various concentrations of Cd.

light harvesting complex is disturbed in Cd treated leaves due to the inhibition of LHC protein synthesis at transcriptional level. It has been observed that chlorophyll undergoes photoxidative break down. It is likely observed that the catabolism of chlorophyll starts with the chlorophyll still bound to the membrane protein within the chloroplast, with the removal of phytol tail by an enzyme, chlorophylls. The Mg atom is then removed by Mg dechelatase, the ring is opened by a dioxygenase and the binding protein is released for degradation. The remaining chlorophyll catabolise is then transported to the vacuole where further metabolism takes place. Similar results were analyzed in *Artemesia* and *Brassica* under cadmium stress (Qureshi et al., 2005, 2010).

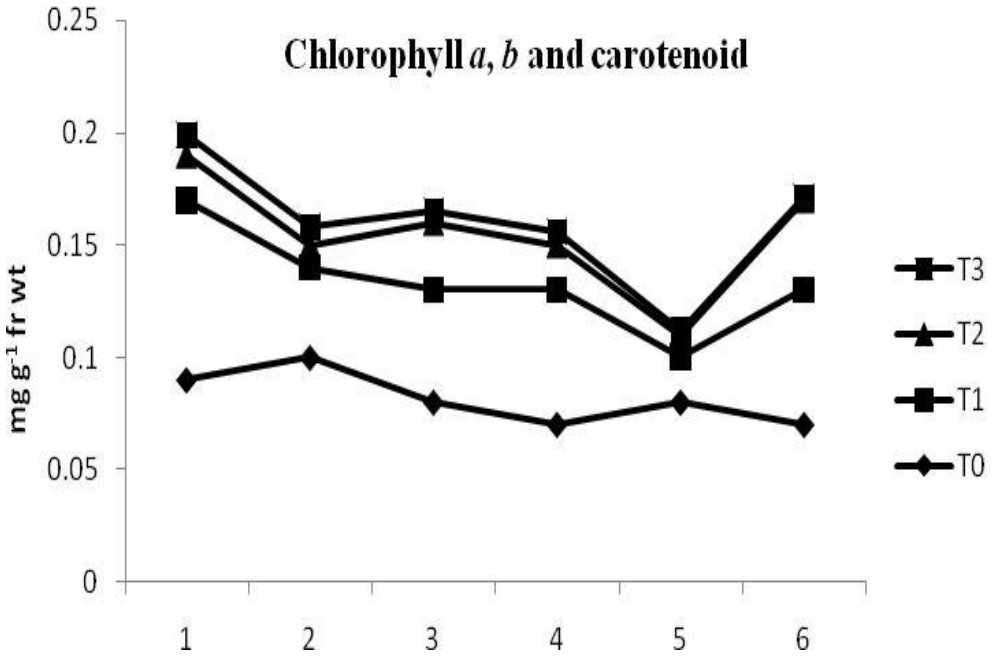

Figure 5. Effect of on chl *a*, chl *b* and carotenoid in *Vigna radiata* to various concentrations of Cd.

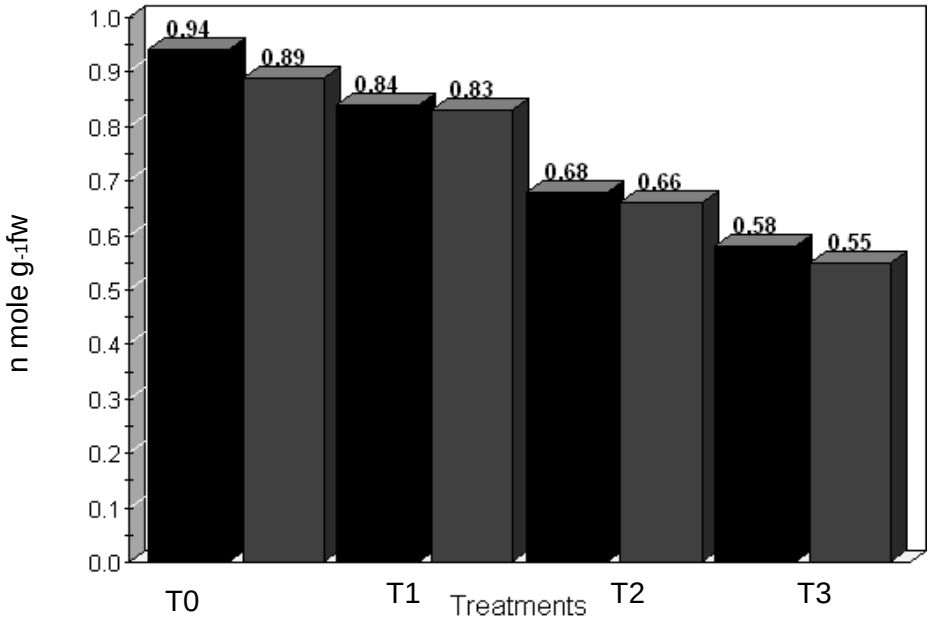

Figure 6. Effect of on nitrate content in *Vigna radiata* to various concentrations of Cd.

Nitrate content and NR activity

Nitrate content and NR activity under various concentrations of cadmium decreases up to 29 to 30 and 32 to 34% at 0.10 mM, 10 to 12 and 16 to 20% at 0.01 mM and 38 to 40 and 24 to 25% at 0.50 mM at 48 and 72 HAT respectively (Figures 6 and 7). Nitrogen promotes growth and development by increasing nitrogenous metabolites (Mishra et al., 1994) and also a regulator of hormonal level (Singh et al., 2000). Nitrogen metabolism is an important process which has a special relevance to legumes. NR is usually considered to perform the rate

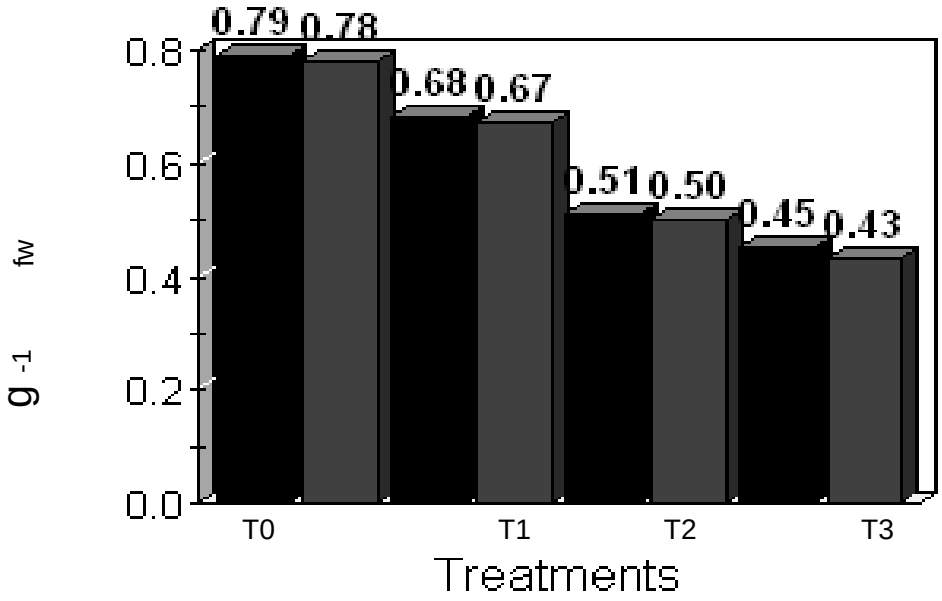

Figure 7. Effect of on NR activity in *Vigna radiata* to various concentrations of Cd.

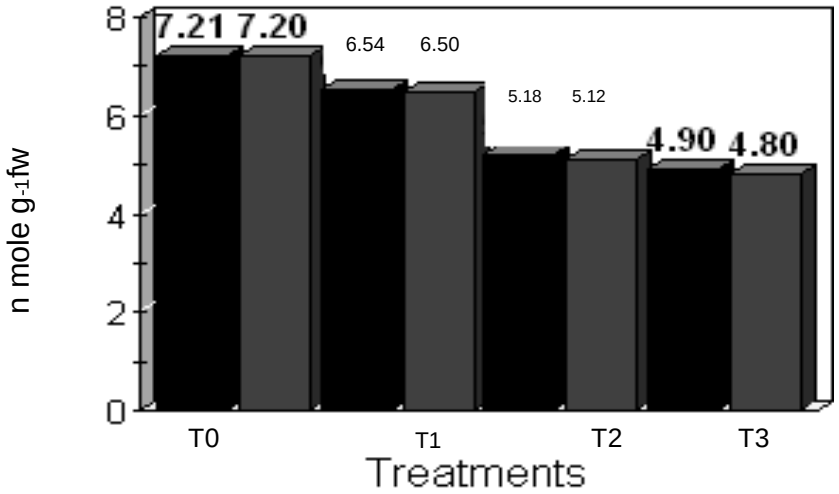

Figure 8. Effect of on soluble protein content in *Vigna radiata* to various concentrations of Cd.

limiting step of the nitrate assimilation pathway owing to the low turnover of NR compared with other enzymes in the pathway and to play a control rule regulation (Marwaha, 1998). This activity of nitrate reductase has often been correlated to the status of the plant. In addition, NR is very convenient genetic marker that has the potential to be selected or counter selected in many organisms be they plants or microorganisms (Rouze and Caboche, 1992). The leaf nitrate content is probably the most decisive factor controlling NR expression and activity in shoot and root (Abd-El Baki et al., 2000). Decline in nitrate content and nitrate reductase activity due to stress might be a result of a reduced uptake of nitrogen or adverse effect of NR activity enzyme structure itself.

Soluble protein content

Soluble protein decreases at all concentrations of cadmium exposure with respect to their control. At 0.01 mM Cd exposure soluble protein decreases up to 9 to 10%, 28 to 30% at 0.10 mM and 32 to 35% at 0.50 mM collectively at 48 and 72 HAT (Figure 8). Plants

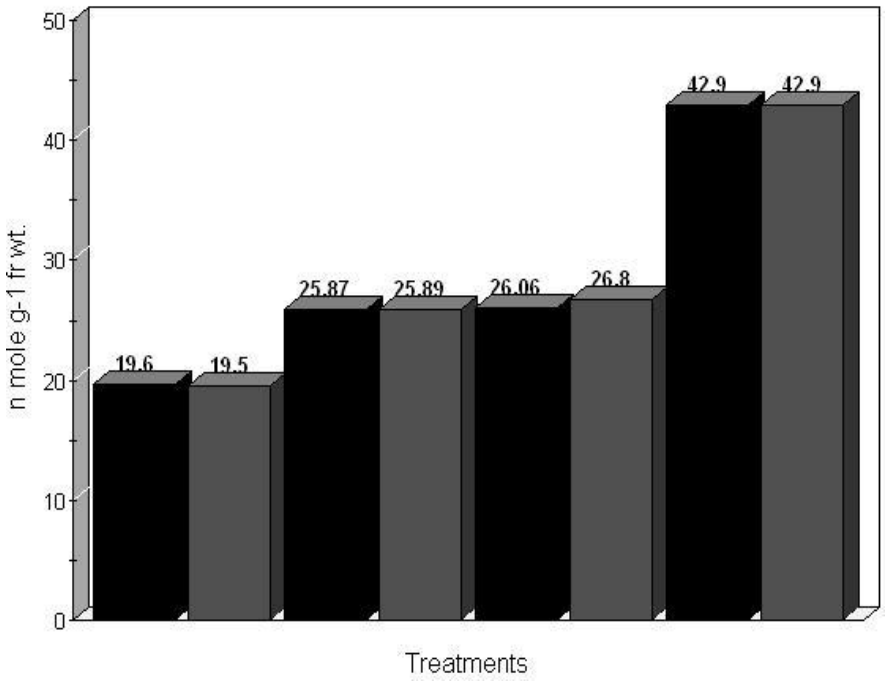

Figure 9. Effect of on proline content in *Vigna radiata* to various concentrations of Cd.

Figure 10.*Vigna radiata* grown under nutrient Hoagland media.

synthesize metal binding replicas when exposed to metal stress (Steffens, 1990). The total soluble protein content in the present study declined slightly with the increasing concentration of Cadmium stress. The reduction in protein content of Cd treated leaves might have resulted from decreased photosynthesis low nitrate reductase activity may also decrease the protein synthesis.

Proline content

Compared to that of control, proline content increase at all concentrations of cadmium exposure eventually at 48 and 72 HAT, at 0.01 mM proline was 32 to 33% increased while at 0.10 mM it was 40 to 46% increased. However, the maximum increase of proline content was

at 0.50 mM (119 to 120%) (Figure 9). There is a great controversy over proline accumulation which appears to be more of a symptom of susceptibility to stress than of adoptive response (Cordovillan et al., 1996). The increase in proline content due to different stresses particularly drought and salinity is quite common. It may be argued that proline accumulation helps to conserve nitrogenous compounds and protect plant against metal stress. Proline could perhaps provide extra protection to these plants. Since a rapid stress to which there in no time for adoptive response could lead to oxygen radicals damages (Smirnoff and Cumbes, 1989). Alia and Pradha (1991) have shown that plants exposed to metal stress accumulate proline in larger quantities and suggested that the synthesis of proline under heavy metal stress could be an adoptive mechanism to regulate NAD^+ to NADH + H^+ ratio as the level of NADH (H^+) increases due to suppression in mitochondrial electron transport. In the present investigation, proline content also increased highly with the growing concentration of cadmium. Proline has been reported to play an important role in osmo-regulation protecting enzyme denaturation, acting as a reservoir of carbon and nitrogen source regulating the cytosolic acidity (Venekamp et al., 1989) and scavenging hydroxyl radicals (Smirnoff and Cumbes, 1989).

Conclusion

Summarizing our results, we can conclude that on different concentrations of cadmium, inhibits growth and development of *V. radiata*. The data presented in this paper have demonstrated a coordinated decrease in mitotic index, photosynthetic pigments, leaf area, activity of nitrate reductase, contents of nitrate and soluble protein, while the proline content increases. The response of *vigna* may be deviated in soil conditions but hydroponic culture provide better opportunity to study the plant response under specific stresses with uniform distribution of stressor in nutrient media.

REFERENCES

Abd- El BGK, Slefritz F, Man HM, Winer H, Kadenhoff, R, Kaiser WM (2000). NR in *Zea mays* (L.) under salinity. Plant Cell Environ., 23: 515-512.

Aghaei K, Ehsanpour AA, Shah AH, Komatsu S (2009). Proteome analysis of soybean hypocotyl and root under salt stress. Amino Acids, 36: 91-98.

Alia A, Pardha SP (1991). Proline accumulation under heavy metal stress. J. Plant Physiol., 138: 554-558.

Arnon JD, Israelstam GF (1949). Copper enzymes in isolated chloroplast oxidase in *Beta vulgaris*. Plant Physiol*.,* 42: 287-292.

Baryla A, Carrier P, Franck F, Columb C, Sakut C, Havaux M (2001). Leaf chlorosis in rape plants (*Brassica napus*) grown on cadmium polluted soil: Causes and consequences for photosynthesis and growth. Planta, 212: 697-709.

Bates LS, Waldren RP, Teare JD (1973). Rapid determination of free proline for water stress studies*.* Plant Soil, 39: 205-207.

Beu SL, Schwarz OJ, Hughes KW (1976). Studies of the herbicide "Paraquat" effects on cell cycle and DNA Synthesis in *Vigna radiata*. Can. J. Gent. Cytol., 18: 93-99.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Brown JC, Jones WE (1975). Heavy metal toxicity in plants 1. A crisis in embryo communication. Soil. Sci. Plant Anal., 6: 421-348.

Clemens S (2001). Molecular mechanisms of plant metal tolerance and homeostasis. Brazilian J. Plant Physiol., 212: 475-486.

Das P, Samantaray S, Rout GR (1997). Studies on cadmium toxicity in plants: A review. Environ. Pollut., 98: 29-36.

Edelman L, Czarnecka E, Key JL (1988). Mechanism of metal tolerance in plants. Plant Physiol., 86: 1048-1056.

Ernst S, Verkley J, Schat H (1992). Metal tolerance in plants. Acta Bot. Neerl, 41: 229-248.

Foyer CD, Changey RL, White MC (1997). The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol., 29: 11-566.

Grover HL, Nair TVR, Abrol YP (1978). Nitrogen metabolism of the three leaf blades of wheat at different soil nitrogen levels. I. Nitrate reductase activity and content of various nitrogenous constituents. Physiol. Plant, 42: 287-292.

Hall JL (2002). Cellular mechanism for heavy metal detoxification and tolerance. J. Exp. Bot., 53: 1-11.

Hiscox JD, Israclstam GF (1979). A method for interaction of chlorophyll from leaf tissue without maceration. Can. J. Bot., 57: 1332-1334.

Klepper LA, Flesher D, Hageman RH (1971). Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol., 48: 580-590.

Leita L, De Nobu M, Cesco S, Mondmi C (1996). Analysis of intercellular cadmium forms in roots and leaves of Bush bean. J. Plant Nutr., 19: 527-533.

Liu J, Reid RJ, Smith FA (2000). The mechanism of cobalt toxicity in mung beans. Physiol. Plant, 110: 104-110.

Lowley PO, Brookes P (1963). Further studies on the alkylation of nucleic acids and their constituent nucleotide. Biochem. J., 89: 137-138.

Marwaha RS (1998). Nitrate assimilation in potato cultivation during plant growth. Indian J. Plant Physiol., 3: 147-151.

Mishra SN, Bhutani S, Singh DB (1994). Influence of nitrate supply on cadmium toxicity in *Brassica juncea* during early seeding growth. Ind. J. Plant Physiol., 37: 12-16.

Oberlunder HE, Roth K (1978). Toxic metals in soil–plant system. In: Sheila M Ross (Ed.), Wiley, New York, pp. 12293-12301.

Okamoto OK, Pinto E, Latorie LR, Becharie EJH, Colepicola P (2001). Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts Arch. Environ. Contam. Toxicol., 40: 18-24.

Qadir S, Qureshi MI, Javed S, Abidin MZ (2004). Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. Plant Sci., 167: 1171-1181.

Qureshi MI, Amici GMD, Fagioni M, Rinalducci S, Zolla L (2010). Iron stabilizes thylakoid protein-pigment complexes in Indian mustard during Cd-phytoremediation as revealed by BN-SDS-PAGE and ESI-MS/MS. J. Plant Physiol., 167: 761-770.

Qureshi MI, Israr M, Abdin MZ, Iqbal M (2005). Responses of *Artemisia annua* L. to lead and salt induced oxidative stress. Environ. Exp. Bot., 53: 185-193.

Qureshi MI, Israr M, Abidin MZ, Iqbal M (2004). Responses of *Artemisia annua* L. to lead and salt-induced oxidative stress. Environ. Exp. Bot., 53: 185-193.

Rouze P, Caboche M (1992). NR in higher plant molecular approaches to function and regulation in inducible plant proteins. Wray JL Ed., Cambridge University Press, pp. 45-77.

Schnaderman MH, Devey WC, High field DP (1971). Inhibition of DNA synthesis in synchronized Chinese hamster cell treated in GI with cyclohexamide. Exp. Cell Resp., 67: 147-155.

Sharma AK, Sharma A (1994). Chromosome Technique - A Manual. Chur: Harwood Academic, pp. 305-306.

Singh DB, Makkar K, Verma S, Mishra SN (2000). Effects of pulrescine, ammonium nitrate and IAA in ameliorating metal and salinity induced stress in mustard seedlings. Indian J. Plant Physiol., 5: 257-263.

Smirnoff N, Cumlber J (1989). Hydroxyl radical scavenging activity of compatible solutes. Photochem., 28(4): 1057-1060.

.

Steffens JC (1990). The heavy metal binding peptide of plants. Plant Mol. Biol., 41: 553-575.

Van F, Assche HC (1990). Effects of metals on enzyme activity in plants. Plant Cell Environ., 13: 195-206.

Venekamp JH, Lampe JEM, Koot JTM (1989). Organic acids as sources for drought induced proline synthesis in field bean *(Vicia faba* Linn.) plants. J. Plant Physiol., 133: 654-659.

Xiong ZT, Peng YH (2001). Response of pollen germination and tube like growth to cadmium with special reference to low concentration exposure. Ecotoxicol. Environ. Saf., 48: 51-55.