INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–581/2014/16–4–789–794
http://www.fspublishers.org

Full Length Article

Chronic Cadmium Induced Oxidative Stress Not the DNA Fragmentation Modulates Growth in Spring Wheat (*Triticum aestivum*)

Sana Riaz[1], Muhammad Iqbal[1*], Iqbal Hussain[1], Rizwan Rasheed[1], M. Arslan Ashraf[1], Saqib Mahmood[1], Muhammad Younas[2] and Muhammad Zafar Iqbal[2]
[1]*Department of Botany, Government College University, Faisalabad 38000, Pakistan*
[2]*Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan*
*For correspondence: iqbaluaf@yahoo.com

Abstract

The present study was carried out to investigate the effects of Cd-stress on growth and DNA fragmentation in spring wheat. For this purpose, wheat genotype 91BT010-1and cultivars AS-2002 and Ufaq-2002 were grown in small pots filled with sand treated with different Cd concentrations (0, 50, 100, 200 and 400 µM) in Hoagland's nutrient solution and placed in a Plant Growth Chamber. The data for various attributes were collected after 30 days of germination. Higher Cd level restricted plant growth in all the genotypes as estimated in terms of decreases in plant height, leaf area and fresh and dry mass. Interestingly, Cd stress decreased chlorophyll contents while increased carotenoids contents in wheat leaves. Although genotypes differed with respect to membrane stability index and malondialdehyde contents under different Cd levels, those having more ascorbic acid concentration showed lesser lipid peroxidation and greater tolerance to chronic Cd exposure. However, no DNA fragmentation was observed in either genotype. The results suggested that the drastic effects of Cd on the growth of wheat plants were due to oxidative stress and decreased chlorophyll contents rather than DNA fragmentation. © 2014 Friends Science Publishers

Keywords: Cadmium toxicity; DNA fragmentation; Lipid peroxidation; Pigments; Wheat

Introduction

Among the factors responsible for lowering yield of wheat, heavy metal stress is of prime importance (Al-Qurani *et al.,* 2003; Jonak *et al.*, 2004). Amongst the heavy metals, cadmium (Cd) is extremely toxic for living organisms even in minute fractions. Anthropogenic activities, especially the improper industrial waste management and overuse of fertilizers have promoted Cd toxicity in the environment. Unluckily after its entrance into the rooting medium, it enters in the food chain through plants within no time (Wagner, 1993).

Cd causes decline in the growth of vegetative parts (Weigel and Jäger, 1980; Hussain *et al.*, 2012) and imbalance in nutrients uptake and carbohydrate metabolism (Monteiro *et al.*, 2009; Zulfiqar *et al.*, 2012). The reduction of biomass was due to reduced synthesis of chlorophyll in *Phaseolus vulgaris* (Padmaja *et al.,* 1990) and photosynthetic inhibition in *Brassica napus* (Baryla *et al.*, 2001). Cd affected plants showed necrosis in leaf and root and chlorosis in leaf (Hernandez and Cooke, 1997; Wahid *et al.*, 2008). Cd produces oxidative stress in plants that result in lipid peroxidation (Shah *et al.*, 2001) and disturb the oxidative defence system in plants.

Various environmental stresses such as salinity (Liu *et al.,* 2000) and metal toxicity (Meriga *et al.,* 2004) have been shown to enhance DNA degradation in plants. Short exposure of plant cells to high concentrations of Cd or chronic exposure to low concentrations of Cd can trigger cell death (Fojtova and Kovarik, 2000; Yakimova *et al.*, 2006). Reports show links between Cd-induced oxidative stress and DNA fragmentation in plants (Gichner *et al.,* 2008; Sharma *et al.,* 2012). In view of the literature, it was hypothesized that Cd retards plant growth through over-production of reactive oxygen species (ROS). Therefore, the principal objective of the present study was to assess whether chronic Cd exposure induces oxidative stress, DNA fragmentation or both to modulate growth in wheat plants.

Materials and Methods

The seeds of wheat genotype 91BT010-1 and cultivars AS-2002 and Ufaq-2002 were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The seeds were sown in small pots filled with sand and irrigated with Hoagland's nutrient solution having no heavy metal (control) and heavy metal (2.25, 4.5, 9 and 18 mg L^{-1} Cd). The experiment was laid-out in a completely randomized design with four replications for each treatment. The pots

To cite this paper: Riaz, S., M. Iqbal, I. Hussain, R. Rasheed, M.A. Ashraf, S. Mahmood, M. Younas and M.Z. Iqbal, 2014. Chronic Cadmium Induced Oxidative Stress Not the DNA Fragmentation Modulates Growth in Spring Wheat (*Triticum aestivum*). *Int. J. Agric. Biol.,* 16: 789–794

were placed in the Plant Growth Chamber (Sanyo, Model MLR-351H) set at 25/15°C day/night temperature, respectively with 10 h photoperiod and light intensity of 370 µmol m^{-2} s^{-1}. After 30 days of germination, the data for following parameters was recorded.

Morphological and Physiological Attributes

Plant height, shoot and root fresh and dry (after keeping in an oven at 70°C for 3 days) masses were recorded. Membrane stability index (MSI) was determined as previously described (Lutts *et al*., 1996).

Leaf relative water contents were determined as previously described (Barrs and Weatherley, 1962). Leaf chlorophyll (*a*, *b* and total) contents and carotenoids contents were determined according to the method of Arnon (1949). The malondialdehyde (MDA) concentration was determined according to the method of Dhindsa *et al.* (1981). Ascorbic acid contents were determined as previously described (Mukherjee and Choudhuri, 1983).

DNA Fragmentation

The genomic DNA was extracted as previously described (Doyle and Doyle, 1990). The PCR conditions for the amplification of DNA were optimized following the method of Dograr and Akkaya (2001). The optimized annealing temperature for SSR primer (Wmc24) was 55°C. The PCR products were electrophoresed on 2.0% agarose gels using 0.5X Tris Borate EDTA (TBE) buffer and visualized by ethidium bromide staining under UV light using gel documentation and analysis system (Syngene, Cambridge, UK).

Statistical Analysis

The data collected were statistically analysed using COSTAT computer software (COHORT Monterey, CA, USA). When the difference between means was significant ($P \leq 0.05$), the mean values were compared using the least significant difference (LSD).

Results

Cd affected plant growth significantly ($P \leq 0.05$) in wheat plants. The maximum decrease in plant height was observed at 18 mg L^{-1} Cd in all wheat plants while the minimum decrease was noted in the plants of wheat genotype 91BT010-1 (Fig. 1a). Plant fresh mass decreased as the concentration of Cd increased in the rooting medium. The maximum decrease was observed in the cultivar Ufaq-2002 (Fig. 1b). Cd stress gradually decreased plant dry mass in both wheat cultivars except in genotype 91BT010-1 in which dry mass increased at low Cd concentration and then further increase in Cd concentration decreased plant dry mass (Fig. 1c). A marked decrease in root fresh and dry mass was observed under different Cd concentrations in both cultivars except in genotype 91BT010-1 in which an increase in dry mass was observed at 9 mg L^{-1} Cd stress (Fig. 1d-e).

Plant fresh and dry mass ratio showed that the cultivar AS-2002 followed by genotype 91BT010-1 retained more water contents under Cd stress. The minimum shoot fresh and dry mass ratio was noted in the cultivar Ufaq-2002 which had the maximum dry mass (Fig. 1f). Root fresh and dry mass ratio showed that genotype 91BT010-1 retained more water contents under different Cd levels except at 9 mg L^{-1} Cd level (Fig. 1g). Increasing Cd concentrations in the rooting medium caused a decrease in leaf area in wheat plants (Fig. 1h). The genotype 91BT010-1 had the maximum and the cultivar Ufaq-2002 exhibited the minimum leaf area under control conditions.

Cd stress affected different pigments significantly ($P \leq 0.001$) in wheat plants. In general, decrease in chlorophyll *a* contents at low Cd concentration and relatively increase at higher Cd concentration was observed in wheat genotype and both cultivars (Fig. 2a). In contrast, the maximum decrease in chlorophyll *b* contents was observed at low Cd concentration in wheat plants. However, all wheat plants, particularly of genotype 91BT010-1 showed stability in chlorophyll *b* contents under higher Cd concentrations (Fig. 2b). Similarly, low Cd concentration caused major decrease in total chlorophyll contents, while further increase in Cd concentration caused less decrease in total chlorophyll contents in wheat plants. At higher Cd level (18 mg L^{-1}), cultivar AS-2002 and genotype 91BT010-1 showed an increasing trend in leaf total chlorophyll contents (Fig. 2c. Interestingly, a marked increase in leaf total carotenoids contents was observed at low Cd level (2.25 mg L^{-1}) in wheat plants. Overall, Cd stress caused increase in leaf carotenoids contents when compared with control in the genotype and both cultivars (Fig. 2d).

Cd stress altered leaf water contents significantly ($P \leq 0.01$) in wheat plants. In general, leaf relative water contents increased by increasing Cd concentrations in all wheat plants except those of cultivar Ufaq-2002 in which an increase at low Cd and a decrease at high Cd concentration was observed (Fig. 3a). A differential change in membrane stability index was found in all Cd stressed wheat plants. The cultivar AS-2002 showed membrane stability at low Cd levels while genotype 91BT010-1 and cultivar Ufaq-2002 showed membrane stability at high Cd levels (Fig. 3b). Malondialdehyde (a lipid peroxidation product) concentration increased even at low Cd stress in wheat plants. However, a decrease in MDA concentration was observed at higher Cd levels in genotype 91BT010-1. In the cultivars AS-2002 and Ufaq-2002, fluctuations in MDA concentration suggested their ability to tolerate Cd stress by decreasing harmful effects of Cd through various mechanisms (Fig. 3c). Imposition of low Cd stress decreased ascorbic acid contents in wheat plants.

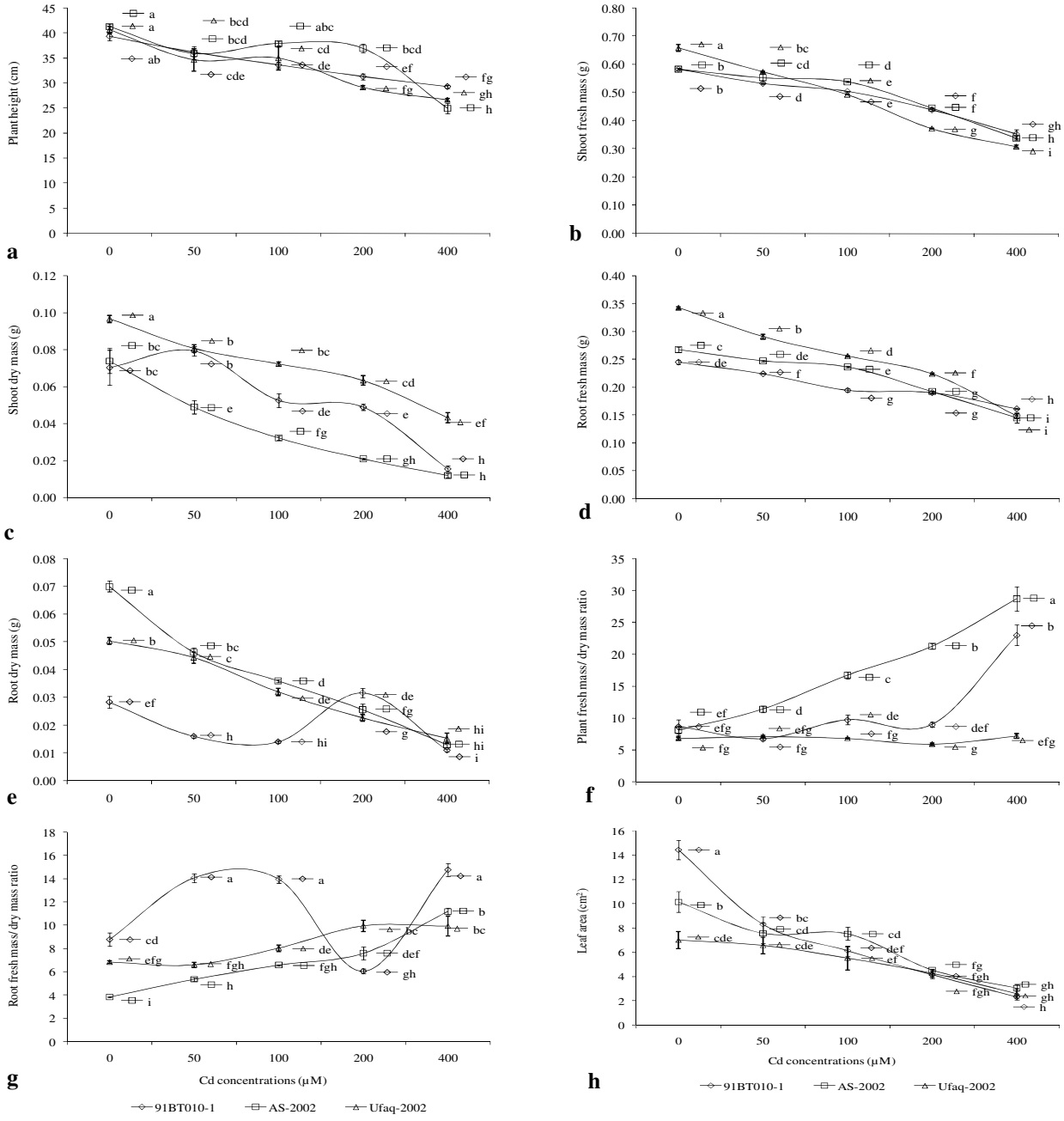

Fig. 1: Growth responses of 30 days old wheat plants subjected to different concentrations of cadmium stress (mean ± standard deviation). Means sharing the same letter do not differ significantly (P>0.05)

However, a further increase in Cd stress affected genotype and both cultivars differently. The maximum leaf ascorbic acid contents were observed in the cultivar AS-2002 both under Cd stress and control conditions (Fig. 3d).

In all the fifteen samples of one wheat genotype and two cultivars, DNA bands were found quite visible but no DNA fragmentation or laddering of DNA was observed after 30 days of Cd stress (Fig. 4).

Discussion

Exposure to Cd stress caused a gradual decrease in fresh and dry mass of shoots and roots in both wheat cultivars. In contrast, in wheat genotype 91BT010-1, low Cd concentration increased while further increase in Cd concentration decreased these attributes. The stimulatory effects of Cd on growth at low concentrations and inhibitory at high concentrations had already been reported in *Brassica juncea* (Singh and Tewari, 2003), *Oryza sativa*

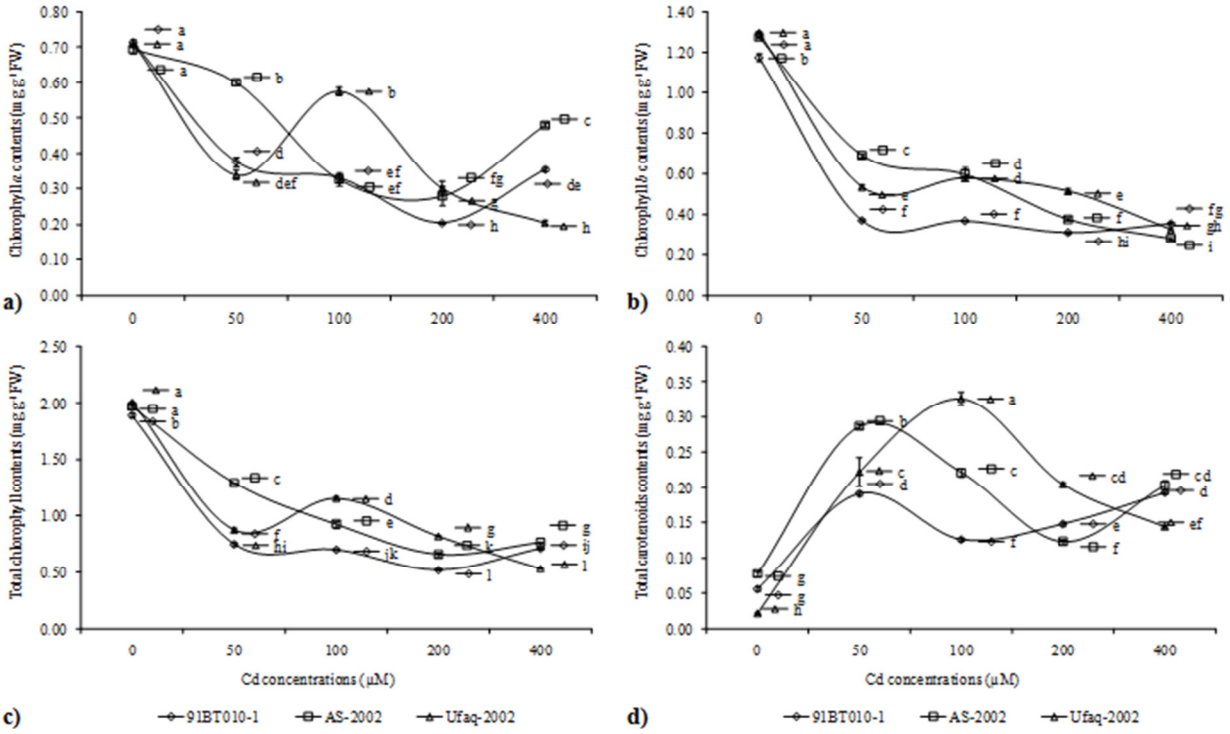

Fig. 2: Leaf pigments of 30 days old wheat plants subjected to different concentrations of cadmium stress (mean ± standard deviation, $n = 3$). Means sharing the same letter do not differ significantly (P>0.05)

(Aina *et al.*, 2007) and *Zea mays* (Hussain *et al.*, 2012). Previous studies have shown differential effects of Cd on shoots and roots of plants. For instance, Cd toxicity inhibited shoot and root growth in maize seedlings (Hussain *et al.*, 2012) and root growth in garlic (Liu *et al.*, 2000) and rice (Rascio *et al.*, 2008). Increase of Cd concentrations in the rooting medium caused a decrease in leaf area in wheat plants. Cd stress altered water relations (Tran and Popova, 2013), cell turgor potential, transpiration rate (Gu *et al.*, 2007) and biomass accumulation (Wahid and Ghani, 2008) in different plants. All these processes ultimately affect leaf area and fresh and dry mass ratio both in the shoots and roots. Such decrease in plant growth has been observed in tomato (Dong *et al.,* 2005).

Generally Cd decreased chlorophyll *a*, *b* and total contents in wheat plants. Interestingly, a marked increase in leaf total carotenoids contents was observed at low Cd level (2.25 mg L^{-1}) in wheat plants. Overall, Cd stress caused increase in leaf carotenoids contents when compared with control. Carotenoids can act as light-harvesting pigments (accessory pigments) as well as antioxidants (Young and Lowe, 2001), and thus can protect chlorophylls against ROS (Ramel *et al*., 2013). The effects of Cd toxicity on different pigments could be attributed to the inhibitory effects of Cd on the synthesis of photosynthetic pigments (Baszynski *et al.*, 1980; Padmaja *et al.*, 1990). Photosynthetic pigments have the tendency to absorb light energy and serve as a power source for the light reactions of photosynthesis. Thus, any change in their quantities is likely to affect photochemical efficiency in plants (Taiz and Zeiger, 2010).

Malondialdehyde (a lipid peroxidation product) concentration increased even at low Cd stress in wheat plants. However, a further increase in Cd caused gradual decrease in MDA concentration in the genotype 91BT010-1 whereas its concentration fluctuated in both wheat cultivars. The results suggested variable ability and detoxifying mechanisms of wheat genotype and cultivars to tolerate Cd stress. The peroxidation of lipids in membranes constructs them permeable to the leakage of solutes (Sharma *et al.*, 2012; Hossain *et al.*, 2012). The MDA is a membrane lipid bilayer peroxidation product and its greater accumulation is taken as a measure of sensitivity to Cd stress (Lin *et al.,* 2007; Xiao-Juan *et al.,* 2011). Imposition of low Cd stress decreased ascorbic acid contents while a further increase in Cd stress increased ascorbic acid contents in wheat plants being the maximum in wheat cultivar AS-2002. Ascorbic acid acts both as antioxidant and chelating agent and is the most abundant water-soluble antioxidant in the plant cells (Gallie, 2013).An increased concentration of ascorbic acid in the present studies indicated its role in the reduction of Cd-induced ROS production. Tissue ascorbic acid concentration has been shown to regulate Cd toxicity in rice seedlings (Chao *et al.*, 2010). Thus, greater accumulation of ascorbic acid in the present study increased tolerance to Cd in wheat plants.

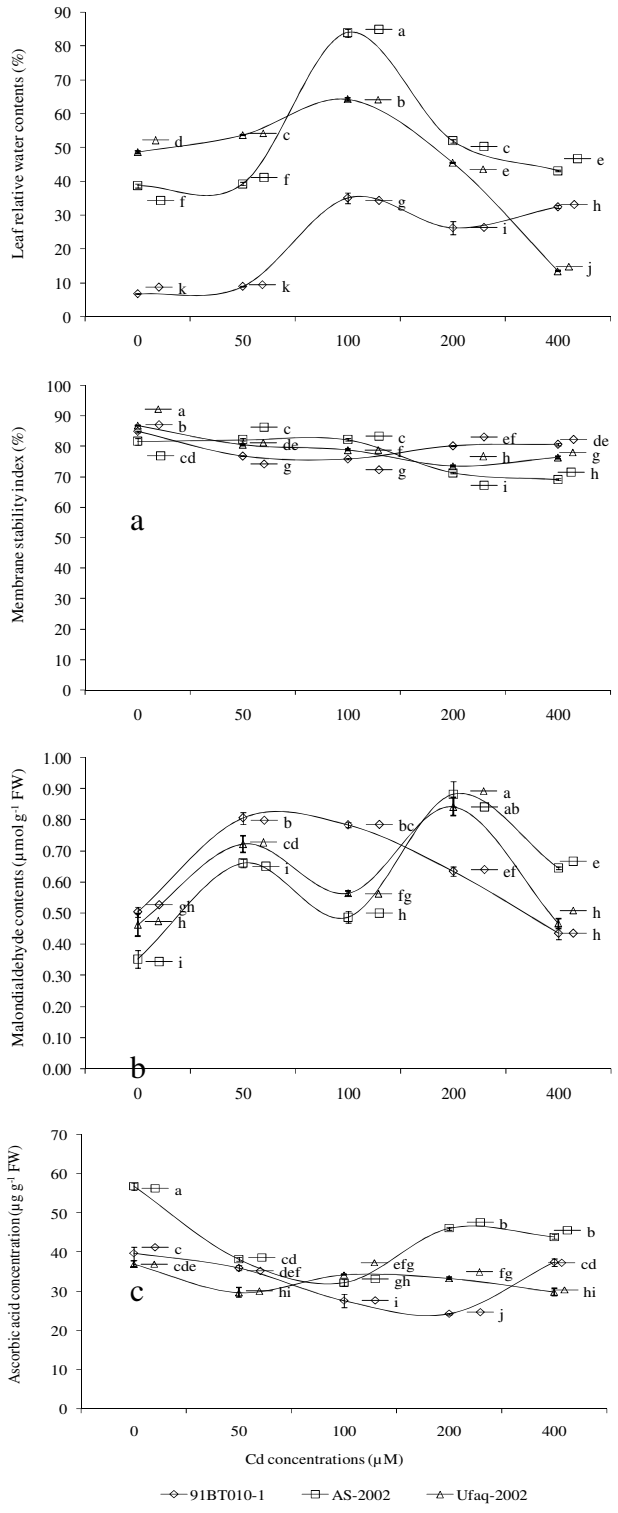

Fig. 3: Leaf relative water contents a), malondialdehyde contents b), membrane stability index c) and ascorbic acid contents d) of 30 days old wheat plants subjected to different concentrations of cadmium stress (mean ± standard deviation, n = 3). Means sharing the same letter do not differ significantly (P>0.05)

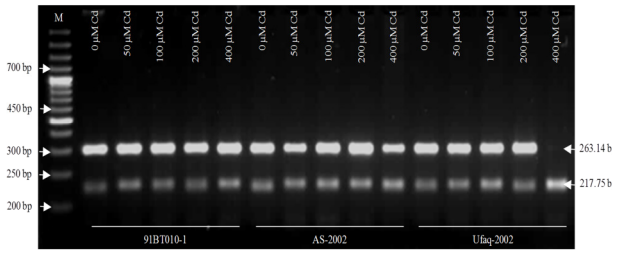

Fig. 4: Amplification profile using SSR primer (WMC24) resolved on a agarose gel of one genotype (91BT010-1) and two cultivars (AS-2002 and Ufaq-2002) of wheat exposed to different concentrations of cadmium (0, 50, 100, 200 and 400 µM)

The growth of plant could be affected either by oxidative stress (Hussain *et al.*, 2012), DNA fragmentation or by both of the processes. In the present study, no DNA fragmentation or laddering of DNA was observed after 30 days of Cd stress in the wheat genotype and both cultivars. Plants have an efficient DNA repair system that might have repaired Cd-induced any changes in DNA. Moreover, the plants were grown in Cd-treated medium for long time (30 days) and it's quite possible that plants had detoxified the Cd mainly through chelation and sub-cellular compartmentalisation (Yadav, 2010). In contrast, Gichner *et al.* (2008) found increase in leaf DNA damage under continuous (for 2 weeks) Cd treatments in potato. The accumulation of ROS could damage the cellular components such as DNA (Lopez *et al.*, 2006) because both the sugar and base moieties of DNA are susceptible to oxidation by ROS (Sharma *et al.*, 2012). Therefore, contrasting results of Gichner *et al.* (2008) could be due to relatively lack of antioxidative potential in potato plants that resulted DNA fragmentation. Moreover, shoot and root may also differ in this respect. For instance, Gichner *et al.* (2004) found DNA fragmentation in the tobacco roots, but not in the leaves.

In conclusion, although the varietal difference was evident, both low and high levels of Cd restricted growth in wheat genotypes. High level of Cd (18 mg/L) decreased chlorophyll while increased carotenoids contents. Wheat genotypes having more ascorbic acid contents showed less lipid peroxidation and greater tolerance to Cd. Results of the present study supported the hypothesis that the growth of wheat genotypes was affected by the chronic Cd-mediated oxidative stress rather than fragmentation in DNA. Overall, the results suggested that ascorbic acid accumulation might have detoxified Cd possibly through chelation under higher Cd stress.

Acknowledgements

The research presented in this paper was partially supported by Higher Education Commission (HEC), Islamabad, Pakistan through Project grant No. 20-1522/R&D/09.

References

Aina, R., M. Labra, P. Fumagalli, C. Vamnini, M. Marsoni, U. Cucchi, M. Bracale, S. Sgorbati and S. Citterio, 2007. Thiol peptide level and proteomic changes in response to cadmium toxicity in *Oryza sativa* L. roots. *Environ. Exp. Bot*., 59: 381–392

Al-Qurani, F., A.A. Alameri and S. Khan, 2003. RAPD profile for the assessment of genotoxicity on a medicinal plant; *Eruca sativa*. *J. Med. Plants Res.,* 4: 579–586

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1–15

Barrs, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian J. Biol. Sci.,* 15: 413–428

Baryla, A., P. Carrier, F. Franck, C. Coulomb, C. Sahut and M. Havaux, 2001. Leaf chlorosis in oilseed rape (*Brassica napus*) grown on cadmium polluted soil: causes and consequences for photosynthesis and growth. *Planta,* 212: 606–709

Baszynski, T., L. Wajda, M. Krol, D. Wolinska, Z. Krupa and A. Tuken-Dorf, 1980. Photosynthetic activities of cadmium-treated tomato plants. *Physiol. Plant*, 48: 365–370

Chao, Y-Y., Hong, C-Y., C.H. Kao, 2010. The decline in ascorbic acid content is associated with cadmium toxicity of rice seedlings. *Plant Physiol. Biochem.,* 48: 374–381

Dhindsa, R.S., D.P. Plumb, and T.A. Thorpe, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 32: 93–101

Dograr, N. and M.S. Akkaya, 2001. Optimization of PCR amplification of wheat simple sequence repeat DNA markers. *Turk. J. Biol*., 25: 153–158

Dong, J., B.O.W. Fei and G.P. Zhang, 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang Univ. Sci.,* 6: 974–980

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus,* 12: 13–15

Fojtova, M. and A. Kovarik, 2000. Genotoxic effects of cadmium is associated with apoptotic changes in tobacco cells. *Plant Cell Environ*., 23: 531–537

Gallie, D.R., 2013. L-ascorbic acid: a multifunctional molecule supporting plant growth and development. *Scientifica*, 2013, Article ID 795964

Gichner, T., Z. Patkova, J. Szakova and K. Demnerova, 2004.Cadmium induces DNA damage in tobacco roots, but no DNAdamage, somatic mutations or homologous recombination intobacco leaves. *Mutat. Res*., 559: 49–57

Gichner, T., Z. Patkova, J. Szakova, I. Znidar and A. Mukherjee, 2008. DNA damage in potato plants induced by cadmium, ethyl methanesulphonate and γ-rays. *Environ. Exp. Bot.,* 62: 113–119

Gu, J., L. Qi, W. Jiang, D. Liu, 2007. Cadmium accumulation and its effects on growth and gas exchange in for *Populus* cultivars. *Acta Biol. Cracov. Ser. Bot.,* 2: 7–14

Hernandez, L.E. and D.T. Cooke, 1997. Modifications of the root plasma membrane lipid composition of cadmium-treated *Pisum sativum. J. Exp. Bot.,* 48: 1375–1381

Hossain, M.A., P. Piyatida, A. Jaime, T.D. Silva and M. Fujita, 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J. Bot*., 2012, Article ID 872875

Hussain, I., M. Iqbal, S. Qurat-Ul-Ain, R. Rasheed, S. Mahmood, A. Perveen and A. Wahid, 2012. Cadmium dose and exposure-time dependent alterations in growth and physiology of maize (*Zea mays*). *Int. J. Agric. Biol*., 14: 959–964

Jonak, C., H. Nakagami, and H. Hirt, 2004. Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. *Plant Physiol.,* 136: 3276–3283

Lin, R., X. Wang, Y. Luo, W. Du, H. Guo and D. Yin, 2007. Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum L.*). *Chemosphere,* 69: 89–98

Liu, T., J. Van Staden and W.A. Cress, 2000. Salinity induced nuclear and DNA degradation in meristematic cells of soybean (*Glycine max* (L.)) roots. *Plant Growth Regul.*, 30: 49–54

Lutts, S., J.M. Kinet and J. Bouharmont, 1996. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regul.,* 19: 207–218

Meriga, B., B.K. Reddy, K.R. Rao, L.A. Reddy and P.B.K. Kishor, 2004. Aluminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). *J. Plant Physiol.*, 161: 63–68

Monteiro, M.S., C. Santos, A.M.V.M. Soares, R.M. Mann, 2009. Assessment of biomarkers of cadmium stress in lettuce. *Ecotox. Environ. Safe.*, 72: 811–818

Mukherjee, S.P. and M.A. Choudhuri, 1983. Implication of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant*, 58: 166–170

Padmaja, K., D.D.K. Prasad and A.R.K. Prasad, 1990. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* seedlings by cadmium acetate. *Photosynthetica,* 24: 399–405

Ramel, F., A.S. Mialoudama, M. Havaux, 2013. Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. *J. Exp. Bot*., 64: 799–805

Rascio, N., F.D. Vecchia, N. La-Rocca, N. Barbato, R. Pagliano, C. Raviolo, C. Gonnelli and R. Gabbrielli, 2008. Metal accumulation and damage in rice (cv. *Vialone nano*) seedlings exposed to cadmium. *Environ. Exp. Bot.*, 62: 267–278

Shah, K., R.G. Kumar, S. Verma and R.S. Dubey, 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedling. *Plant Sci.,* 161: 1135–1144

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli, 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot*., 2012, Article ID 217037

Singh, P.K. and R.K. Tewari, 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *J. Environ. Biol*., 24: 107–112

Taiz, L. and E. Zeiger, 2010. *Plant Physiology*. 5th edition, Sinauer Associates Inc., Publishers, Sunderland, Massachusetts, USA.

Tran, T.A. and L.P. Popova, 2013. Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turk. J. Bot.,* 37: 1–13

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

Wahid, A. and A. Ghani, 2008. Varietal differences in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation. *Ann. Appl. Biol.,* 152: 59–69

Weigel, H.J. and H.J. Jäger, 1980. Subcellular distribution and chemical forms of cadmium in bean plants. *Plant Physiol*., 65: 480–482

Xiao-Juan, B., L. Li-Juan, Z. Chun-Hua, G. Ying, and C. Wang-da, 2011. Effect of H_2O_2 pretreatment on Cd tolerance of different rice cultivars. *Rice Sci.*, 18: 29-35

Yadav, S.K., 2010. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S. Afr. J. Bot.,* 76: 167–179

Yakimova, E.T., V.M. Kapchina-Toteva, L.J. Laarhoven, F.M. Harren, E.J. Woltering, 2006. Involvement of ethylene and lipid signalling in cadmium-induced programmed cell death in tomato suspension cells. *Plant Physiol. Biochem.,* 44: 581–589

Young, A.J. and G.M. Lowe, 2001. Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys*., 385: 20–27

Zulfiqar, Z., A. Wahid, M. Farooq, N. Maqbool and M. Arfan, 2012. Phytoremediation of soil cadmium by using *Chenopodium* species. *Pak. J. Agric. Sci*., 49: 435–445

(Received 30 April 2013 Accepted 23 January 2014)