INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–311/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0242
http://www.fspublishers.org

Full Length Article

Cadmium-induced Perturbations in Growth, Oxidative Defense System, Catalase Gene Expression and Fruit Quality in Tomato

Iqbal Hussain[1*], Muhammad Arslan Ashraf[1], Rizwan Rasheed[1], Muhammad Iqbal[1], Muhammad Ibrahim[2],Tanzella Zahid[1], Sumaira Thind[1] and Farhan Saeed[3]

[1]*Department of Botany, Government College University, Faisalabad-38000, Pakistan*
[2]*Department of Applied and Biochemistry, Government College University Faisalabad-38000, Pakistan*
[3]*Department of Food Science, Government College University, Faisalabad-38000, Pakistan*
[*]For Correspondence: iqbalbotanist1@yahoo.com

Abstract

Cadmium (Cd) toxicity causes leaf chlorosis, growth inhibition, and disruption of photosynthetic machinery. A pot experiment was conducted to evaluate the changes in growth, physiochemical attributes and expression level of the catalase (*Cat2*) gene in two tomato cultivars (cv. Nagina; cv. Roma) under cadmium (Cd) stress. Plants were subjected to various concentrations of Cd in soil medium (0, 160, 320, 640 and 1280 µM Cd) in full strength Hoagland nutrient solution. Elevated Cd concentrations caused an apparent turn down in plant growth, photosynthetic pigments, and activity of CAT and increased the level of ascorbic acid, proline, H_2O_2 and malondialdehyde (MDA) and ascorbate peroxidase (APX) peroxidase (POD) activities. The fruit biomass, moisture content, total fiber, protein, glucose and fructose contents were decreased. In addition, Cd induced alterations in some nutrient elements; for instance, Ca^{2+} decreased and K^+ in fruit was increased in response to Cd toxicity. Cadmium contents in fruit decreased evidently under elevated levels of Cd. The results of the present study demonstrated that Cd toxicity severely deteriorated the fruit quality, whereas the cultivar that showed greater *Cat2* gene expression and had low H_2O_2 and MDA contents tolerated Cd stress effectively. Fruit quality of cv. Nagina was better than cv. Roma. In this context, we have recorded greater ash, fiber and protein contents in cv. Nagina, while cv. Roma was inferior in this regard under cadmium toxicity. © 2016 Friends Science Publishers

Keywords: Tomato; Nutrient; Oxidative stress; Enzyme activity; Gene expression; Cadmium

Introduction

Heavy metals have long life in soil and are non-degradable in nature, and thus contamination of economically important plants poses a threat to humans and animals. The cadmium (Cd) is greatly soluble in water and is highly mobile which allows its entry into food chains (Pedro *et al.*, 2013). For plants, Cd is a non-essential element, and its excess can lead to several toxic effects at the whole plant level (Tang *et al.*, 2013). Plant leaf chlorosis, growth reduction, water imbalance and disturb essential mineral uptake are the common symptoms observed in Cd stressed plants (Redondo-Gómez *et al.*, 2010). Among metabolic processes, photosynthesis appears to be particularly sensitive to Cd excess (Tang *et al.*, 2013). Furthermore, it alters stomatal regulation (Perfus-Barbeoch *et al.*, 2002), causes impairment to both types of photosystems and restrains the activities of some key enzymes in the Calvin cycle (Küpper *et al.*, 2007).

Cadmium triggers excessive synthesis of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH), superoxide radical (O^{2-}.), and the singlet oxygen ($^1O_2$), that in turn induce oxidative damage to plants (Choppala *et al*., 2014). The overproduction of ROS under heavy metal stress causes lipid peroxidation and instability in enzymes (Hussain *et al*., 2012; Riaz *et al*., 2014). On the other hand, balance between ROS generation and detoxification mechanisms is necessary for growth regulation. Plants have adapted to mitigate the oxidative stress through antioxidative (enzymatic and non-enzymatic) mechanisms. The catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) are antioxidant enzymes that formulate integral component of oxidative defense system (Liu *et al.*, 2015). Catalase is one of the antioxidants with a high turnover of about 0.006 billion molecules of hydrogen peroxide to molecular oxygen and water per 60 s (Lee and An, 2005). In this way, it detoxifies the major portion of H_2O_2 (Shim *et al.*, 2003).

The Cd also interferes with the uptake and translocation of mineral nutrients in different plants (Liu *et al.*, 2015). Therefore, it is important to study the effect of Cd, particularly on Ca as well as on physiologically

To cite this paper: Hussain, I., M.A. Ashraf, R. Rasheed, M. Iqbal, M. Ibrahim, T. Zahid, S. Thind and F. Saeed, 201x. Cadmium-induced perturbations in growth, oxidative defense system, catalase gene expression and fruit quality in tomato. *Int. J. Agric. Biol.*, 00: 000-000

important micronutrients, such as Zn, Cu and Mn. However, contradictory results have been found in the literature. Thus, further studies are needed to investigate the interactions between contaminants and nutrients in order to favour selection for enhanced uptake efficiency of desirable nutrients and reductions in the uptake of undesirable ones (Liu *et al.*, 2003).

Tomato (*Lycopersicum esculentum* L.) is a highly nutritious diet. Therefore, nutrient and proximate analysis of vegetables and fruits is of pivotal significance in evaluating their nutritional value (Pandey *et al.,* 2006). Moisture contents, fiber contents, ash contents, and protein contents of fruits are a good indicator of its quality. In Pakistan, tomato is a second major vegetable plant (FAO, 2009). Global crop production of tomato is severely hampered by heavy metals, particularly Cd (Mengel *et al.,* 2001). In spite of better nutritional value, its yield per hectare is very low. The Cd accumulation in relation to other nutrients in the fruit and its influence on fruit quality and production is rarely studied in tomato. Furthermore, there is a lack of knowledge about the contribution of *Cat2* gene in overall reduction of Cd-induced oxidative stress and subsequent role in altering fruit nutritional value. The present study aimed to assess how different Cd level stress affects the uptake, translocation of nutrients and deteriorate the fruit quality, whereas the cultivar that showed greater *Cat2* gene expression and had low H_2O_2 and MDA contents tolerated Cd stress effectively.

Materials and Methods

Gowth Conditions, Plant Material and Treatments

The seedlings of two tomato (*Lycopersicum esculentum* L.) cultivars (Roma and Nagina) were kindly provided by Vegetables section, Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. A pot experiment was conducted in the wire house during November-March, 2014 at the Botanical Garden, Government College University Faisalabad, Pakistan (latitude 29º30N, longitude 72º11E and altitude 214 m), with 11/13 h light/dark period, average day/night temperature cycle (24/12ºC), mean relative humidity (65%), and photosynthetically active radiation 612–920 µmol m^{-2} s^{-1} during the entire course of the experiment. Six seedlings of Roma and Nagina were planted in pots (25 cm high, circumference 60 cm at the base and 72 cm at apex). A hole was made in each pot and was filled with 8 kg of washed sand. Seedlings were thinned to maintain five healthy seedlings per pot. Cadmium (0, 160, 320, 640 and 1280 µM) was applied using $CdCl_2$ to the growth medium of tomato plants in nutrient solution at flowering stage. Completely randomized design was used for this experiment with three replications per treatment. Sampling was done after three weeks of imposition of stress so as to record data for various growth and physiochemical attributes.

Growth and Yield Attributes

A measuring scale was used to determine shoot length. Shoot and fruit fresh and its dry masses were measured in grams. The maximum leaf length (L) and width (W) was measured and the leaf area was calculated by using the following formula:

$$\text{Leaf area } (cm^2) = L \times W \times 6.45$$

Where, 6.45 is a correction factor calculated by using graph squares.

Determination of Chlorophyll *a, b* and Total Carotenoid Contents

The chlorophyll *a* and *b* (Chl. *a* & *b*) contents were quantified by the method of Yoshida *et al.* (1976). 10 mL of acetone (80%) was used to homogenize the 0.1g of fresh leaf material. Then the supernatant was used to measure absorbance at different wavelengths (480, 645 and 663 nm) using UV-VIS spectrophotometer (U–2910, Hitachi Instruments Inc., Tokyo, Japan). Leaf carotenoids were quantified by using the procedure of Davies (1976).

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

$$\text{Chl. } b \text{ (mg g}^{-1} \text{ fwt)} = \{12.9\ (OD_{645} - 4.68\ (OD_{663}) \times V/1000 \times W\}$$

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

$$\text{Carotenoids (mg g}^{-1}\text{fwt)} = OD_{480} + (0.114 \times OD_{663}) - (0.638 \times OD_{645})$$

Determination of H_2O_2 Contents

Leaf hydrogen peroxide levels were quantified using the procedure of Velikova *et al.* (2000). Fresh leaf tissue (0.15 g) was homogenized ground in 1 mL of TCA (0.1%) (w/v). Centrifuged the homogeneous material at 10,000*g* for 10 min for collection of supernatant. The mixture of supernatant and potassium phosphate buffers (0.5 mL each) reacted with 1 mL of KI solution (1M). Vortexed the mixture thoroughly. Then absorbance was noted at 390 nm by using UV-VIS spectrophotometer (U–2910, Hitachi Instruments Inc., Tokyo, Japan) and TCA (0.1%) solution was used as blank. The hydrogen peroxide was determined from the standard curve of known concentration of hydrogen peroxide.

Estimation of MDA Contents

MDA contents were estimated using the procedure of Hodges *et al.* (1999). One mL of 5% TCA solution (w/v) was used to homogenize the 0.1 g of fresh leaf material. Centrifuged the homogeneous material at 12,000 *g* for

collection of supernatant and 1 mL of it was reacted with 1mL of 20% TCA prepared in 0.5% (w/v) TBA. This mixture was heated at 95°C for 30 min in a water bath. After heating, the mixture was centrifuged at 7500 *g* for 5 min. The absorbance of material was noted at 532 and 600 nm using UV–VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan) using TCA (5%) as a blank.

Ascorbic Acid Content

Ascorbic acid content in the leaf was measured according to protocol using by Mukherjee and Choudhuri (1983). 0.25 g fresh leaf material was homogenized in 10 mL of TCA (6%). The 2 mL of dinitrophenyl hydrazine (2%) solution (in acidic medium) was mixed extract (4 mL) for a reaction and then added 1-2 drop of thiourea (prepared in 70% ethanol to attain 10% concentration) to the mixture. The mixture was incubated in a water bath at 95°C for 15 min. The mixture was cooled down to 25°C placed on the ice box and then added sulfuric acid (80%) to the mixture slowly. The absorbance was measured at 530 nm with UV–VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan). Ascorbic acid content was determined from a standard curve of known concentration of ascorbic acid.

Antioxidants Enzymes Assay

POD and CAT activities were estimated according to the protocol of Cakmark *et al.* (1993). The reaction mixture (3 mL) contained guaiacol (20 mM), phosphate buffer (50 mM) contained pH (5.0), H_2O_2 (40 mM) and enzyme extract (0.1 mL) for POD. Change in absorbance at 470 nm was recorded using a spectrophotometer (IRMECO U2020, Geesthact, Germany) after every 20 s for 2 min. The activity of enzyme was estimated in units/mg protein (U = 1 mM of H_2O_2 reduction/min/mg protein). CAT reaction mixture (3 mL) included phosphate buffer (50 mM) (pH 7.0), H_2O_2 (5.9 mM) and enzyme extract (0.1mL). Enzyme extract was added to mixture to start the reaction. Change in absorbance after every 20 s was recorded for 2 min at 240 nm using a spectrophotometer (IRMECO U2020, Geesthact, Germany). Leaf APX activity was estimated by using the procedure of Asada and Takahashi (1987). The reaction mixture (1 mL) comprised H_2O_2 (0.1 mM), potassium phosphate buffer (50 mM) (pH 7.0), ascorbic acid (0.5 mM) and enzyme extract (200 µL). The absorbance of mixture was noted as reduced at 290 nm and the correction was done by H_2O_2. The activity of these enzymes was expressed in U/mg protein.

Determination of Catalase Gene Expression with RTq-PCR

For determination of *Cat2* gene expression, total RNA was isolated from the shoot of tomato (0.1 g) by using Trizol (Invitrogen, USA). Real time RTq-PCR was performed with SYBER ExScript RT PCR Kit (TaKaRa, Inc., Japan) to determine the expression level of Catalase *(Cat2)* gene. The expression level of target gene (*Cat2*) was normalized to the level of *Actin (Act)*. The sequences of primers of the target gene *Cat2* and *Act* are listed in Table 1.

Determination of Moisture and Ash Content from Fruit

The fruit moisture contents were measured with air forced draft oven (Memmet Germany) and ash content by the procedure described in AACC (2000) method no. 44-15A and method no. 08-01, respectively.

Determination of Total Protein and Fibre Contents from Fruit

The fruit nitrogen contents were measured with Kjeldhal's method and crude fibre as described in AACC (2000) with method no. 46-10A and 32-10, respectively. Protein (%) contents were also estimated from nitrogen (%) with a factor 5.7.

Determination of Mineral Nutrients

Fruit samples (0.5 g) of each variety were digested with a di-acid mixture such as $HCLO_3$ and HNO_3 (1:3) for 2-3 h at hot plate. Dilute digested mixture was used to determine the mineral nutrients, according to the method of Yoshida *et al.* (1976). Sodium, potassium and cadmium contents of fruit were determined with help of polarized zeeman atomic absorption spectrophotometer (Hitachi, Z-2000, Tokyo, Japan).

Statistical Methods

The results were considered statistically significant at $P \leq 0.05$. The experiment was carried out in a completely randomized design (CRD) with three replications. Difference among means was calculated using least significant difference (LSD) at $P \leq 0.05$. Computer software COSTAT (CoHort software, 2003, Monterey, California, USA) was used for all statistical analysis, and MS-Excel was used to graphically present the data.

Results

Growth Attributes

Exposure of tomato plants to increasing concentrations of Cd (0, 160, 320, 640 and 1280 µM Cd) had a marked inhibitory effect on different growth attributes. In this context, we recorded a dose-dependent decline in shoot length, shoot, root dry weights and leaf area, where an erratic pattern of increase or decrease was observed. Cd-induced decline in various growth attributes was more evident in cv. Roma than those in cv. Nagina (Fig. 1).

Table 1: Nucleotide sequences of primers for real-time RT-PCR gene expression analyses

Plant	Gene	Accession No.	Amplicon size (bp)	Sequence
Tomato	*Act*	NM_00111149	160	fw 5′- tct gtt tcc cgg ttt tgc tat tat; rv 5′- tgc atc agg cac ctc tca ag
	Cat2	NM_001247257.1	185	fw 5′- ctt tcc tct tcg acg ata ttg gta; rv 5′- gtg att tgc tcc tcc gac tc

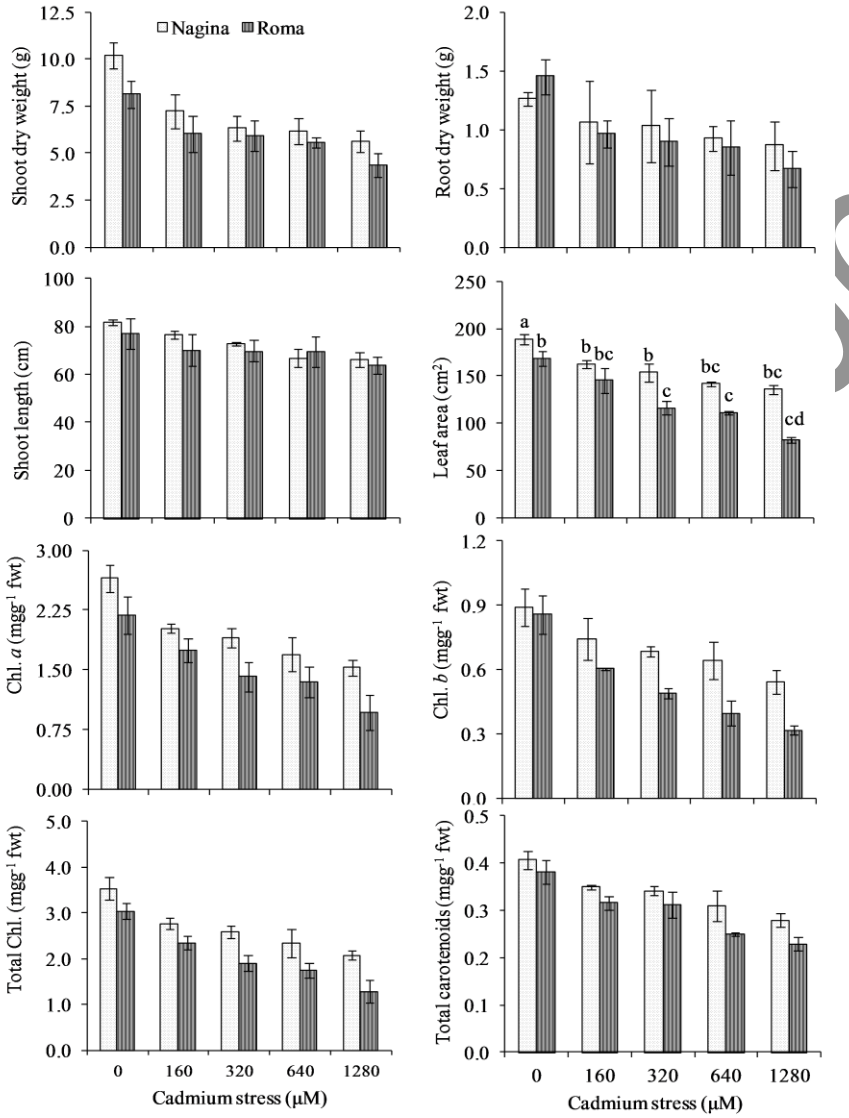

Fig. 1: Changes in various growth attributes and photosynthetic pigments in two tomato cultivars (Nagina and Roma) exposed to varying concentrations of $CdCl_2$. (n = 3; Mean ± SD)

Photosynthetic Pigments

When two tomato cultivars were subjected to Cd toxicity in the growth medium, a significant degradation of photosynthetic pigments was recorded. In this context, Chl. *a*, *b*, total Chl. and carotenoids decreased significantly with increasing Cd concentration in the growth medium. Of two cultivars, cv. Nagina had minimal degradation of above-mentioned pigments (Fig. 1).

Malondialdehyde, Hydrogen Peroxide and Ascorbic Acid Contents

Cd toxicity also triggered a significant rise in ascorbic acid contents of both tomato cultivars, but this rise was more in cv. Roma. The presence of toxic metal (Cd) in the root zone caused a conspicuous increase in the cellular levels of MDA and H_2O_2 which in turn cause oxidative damage to tomato plants. This oxidative damage was more in cv.

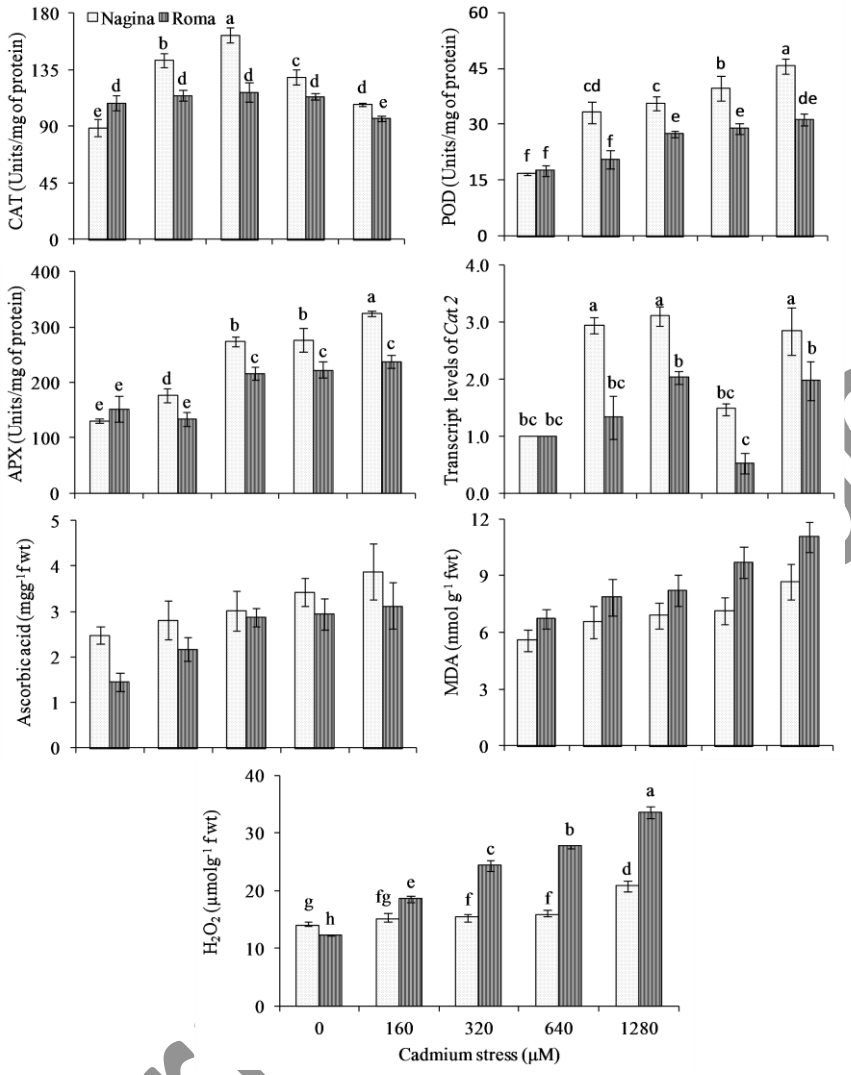

Fig. 2: Changes in various biochemical attributes and transcript level of *Cat* gene in two tomato cultivars (Nagina and Roma) exposed to varying concentrations of $CdCl_2$. (n = 3; ± SD)

Roma than that in cv. Nagina in terms of elevated cellular levels for MDA and H_2O_2. The concentration of ascorbic acid was maximal in plants of cv. Roma (Fig. 2).

Antioxidants Enzymes

Antioxidant enzymes; POD and APX exhibited a consistent rise in their activities with increasing concentration of Cd, while CAT increased significantly at 320 µM of Cd^{+2} in the tomato plants. Plants of cv. Nagina had showed greater activity of CAT, while cv. Roma was inferior in this context. In addition, Quantitative gene expression of *Cat* also depicted a significant rise in the transcription level of this enzyme but this rise was greater in cv. Nagina than that in cv. Roma. Plants of cv. Nagina had greater activities of different antioxidant enzymes (Fig. 2).

Catalase Gene Expression

In this report, at mRNA level, *Cat2* gene expression was probed under Cd toxicity. The plethora of *Cat2* gene transcripts was compared in plants subjected to 160, 320, 640, and 1280 µM concentrations of Cd^{+2}. The *Cat2* gene expression levels were examined by real-time PCR (Fig. 2). To stay away from error, real-time PCR is stabilized with a housekeeping (reference) gene usually and also with control samples. To appraise the stability of our results, *Cat2* transcript levels of 160, 320, 640 and 1280 µM of Cd^{+2} samples were measured three times, which pointed out steady-state mRNA levels for both *Cat2* and housekeeping gene (*Act*) in the cells. The 160, 320 and 1280 µM of Cd^{+2} treatments led to a increased in catalase gene expression level compared to the control, *Cat2 gene* expression levels were increased significantly at 320 µM of Cd^{+2},

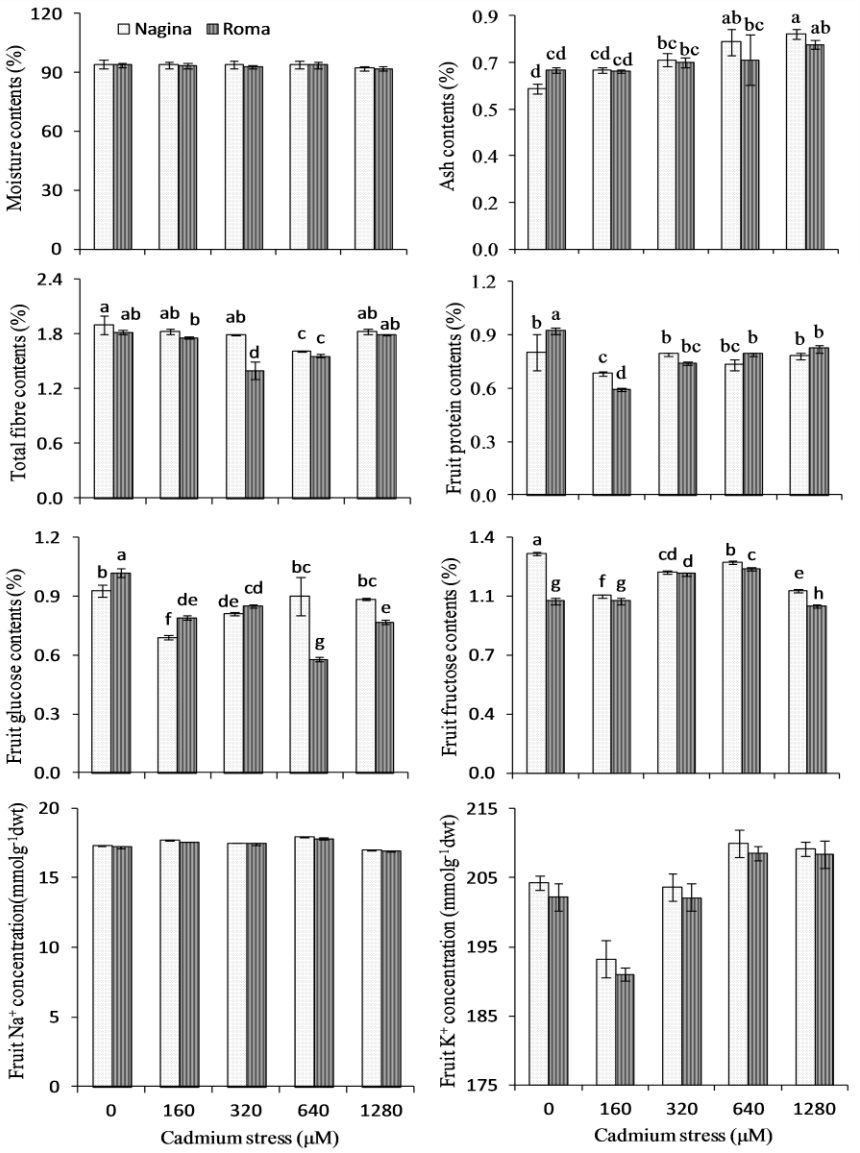

Fig. 3: Fruit quality of two tomato cultivars (Nagina and Roma) exposed to varying concentrations of $CdCl_2$. (n = 3; ± SD)

which indicates that *Cat2* was strongly up regulated by this concentration (Fig. 2). Plants of cv. Nagina had showed greater *Cat2* gene expression levels as compared to cv. Roma.

Fruit Moisture, Ash, Fiber, Protein, Glucose and Fructose Contents

The Cd application influenced fruit quality of tomato plants. In this context, increasing levels of Cd caused a consistent decline in moisture contents, while ash contents more increased in response to Cd toxicity in cv. Nagina. The difference between two cultivars with respect to moisture and ash content was non-significant. A dose dependent decrease in total fiber content was observed under Cd toxicity in cv. Roma. Likewise, fruit protein content decreased due to Cd toxicity, particularly in cv. Roma. In contrast, cv. Nagina had greater protein contents at 320 and 1280 µM $CdCl_2$. The stress generated by Cd toxicity caused a decline in fruit glucose and this decline was more evident in cv. Roma at 640 µM $CdCl_2$. Similarly, toxicity generated by Cd decreased fruit fructose content, but this decrease was more in cv.Nagina. Minimal values for fruit fructose were evident under elevated Cd level (1282 µM) in cv. Roma (Fig. 3). In the present study, Cd stress exhibited non-significant effect on the moisture content of the fruit.

Fruit Fresh and Dry Masses, Mineral Quality and Cd Contents

Tomato plants subjected to Cd toxicity showed a marked perturbation in fruit fresh and dry masses as well as fruit mineral quality. The fruit Na^+ content remained unaffected by $CdCl_2$ application except for a slight non-significant decline at an elevated Cd concentration (1280 µM). In contrast, toxicity generated by Cd induced rise in fruit K^+ content and cv. Roma had greater fruit K^+ concentration than that of cv. Nagina under elevated Cd levels (640 and 1282 µM). Fruit Ca^{2+} content decreased significantly in response to Cd toxicity and this decrease was being more maximal in cv. Roma at 640 µM $CdCl_2$. Fruit Cd^{2+} content increased significantly under elevated levels of Cd. Plants of cv. Nagina had significantly accumulated Cd^{2+} in fruit at 1280 µM $CdCl_2$, while cv. Roma was inferior in this context. Cd toxicity also induced a significant decline in fruit biomass (Fig. 4). Plants of cv. Roma had greater fruit dry masses under varying levels of Cd.

Discussion

In the present study, Cd toxicity induced a conspicuous decrease in shoot fresh and dry masses and this Cd-induced decline was greater in cv. Roma. Ample information is available in the literature that highlights the negative influence of Cd on plant growth (Xie *et al.,* 2014). The decrease in tomato growth under Cd stress could be due to Cd-induced altered water relations and photosynthetic pigments (Riaz *et al*., 2014), oxidataive damage (Saidi *et al.,* 2013), altered nutrients uptake and thus reduced cell expansion. The stress generated by Cd toxicity significantly degraded chlorophyll *a* and *b*, and carotenoid content. This decline was being greater in the cv. Roma compared with cv. Nagina. Earlier studies reported that Cd suppressed the activity of enzymes such as δ-aminolevulinic acid dehydratase and proto-chlorophyllide reductase required for the biosynthesis of pigments (Qian *et al.,* 2009). Under diverse environmental conditions, plants maintained a balance between ROS generation and elimination (Tang *et al.,* 2013). Plants under environmental constraints accumulate excessive levels of ROS that in turn trigger oxidative damage. The Cd toxicity inhibits physiochemical processes in plants such as chlorophyll biosynthesis, photosynthesis and nutrient uptake that in turn retard growth and productivity (Ali *et al.,* 2013). A significant rise in MDA and H_2O_2 contents in plants under Cd stress was observed in the present study. The higher cellular levels of H_2O_2 and MDA were associated with growth inhibition (Collén *et al.,* 2003). In our study, Cd increased the activities of CAT, POD and APX possibly to cope with oxidative stress. However, sensitive cultivar had comparatively low activities of antioxidant enzymes that in turn reduced its growth. Antioxidant enzymes are crucial cellular components that maintain cellular redox potential.

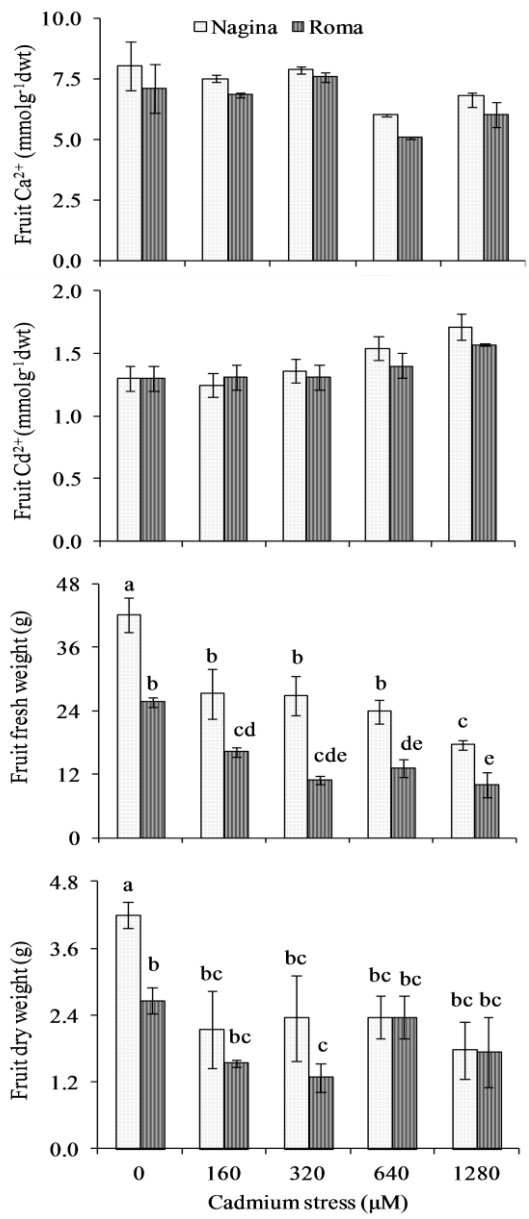

Fig. 4: Fruit Ca and Cd contents and biomass of two tomato cultivars (Nagina and Roma) exposed to varying concentrations of $CdCl_2$. (n = 3; ± SD)

Irfan *et al.* (2014) found a dose dependent increase in the activities of antioxidant enzymes in *Brassica juncea* subjected to Cd stress. Likewise, Mohamed *et al.* (2012) reported that *Brassica juncea* plants with greater activities for antioxidant enzymes presented a better ROS detoxification potential. The Cd stress resulted in a significant increase in the cellular contents of AsA in tomato plants.

Cat2 gene expression was probed under Cd toxicity at mRNA level. In this context, Iannone *et al.* (2015) evaluated the response of wild type tobacco (*SR1*) plants

and CAT-deficient plants (*CAT1AS*) to Cd toxicity. These authors further reported that *CAT1AS* plants accumulated more Cd than wild type plants and this was also associated with inhibited plant growth. In our study, we recorded greater transcript levels of *Cat2* in the tolerant cv. Nagina possibly due to its better performance under Cd toxicity.

The Cd stress caused a conspicuous rise in the ash content (%) of tomato fruits. Ash content of fruit provides information about the inorganic content that could be used to calculate the mineral content (Abitogun *et al.,* 2010). Proteins are considered as an important part of the diet that ensures the adequate supply of amino acids. In our study, Cd stress caused a decline in protein and fibre content that significantly reduced the fruit quality. It is emphasized that the amount of fibre content should be low in the diet of infants and school going children. Higher concentration of fibres in weaving diet causes irritation in the gut mucosa, reduced mineral and vitamin bioavailability as well as digestibility (Eromosele and Eromosele, 1993). In our study, cv. Roma had lower fibre contents as compared with cv. Nagina. Nonetheless, a diet with high fibre content is good for anti-constipation purpose (Eromosele and Eromosele, 1993). In our study, Cd stress reduced essential micronutrients, glucose and fructose contents in tomato fruits. In almond seedlings, Cd toxicity caused a significant decline in essential macronutrients including Ca, K, Mg (Nada *et al.,* 2007). Liu *et al.* (2003) reported that Cd had synergistic interaction with the uptake and translocation of different nutrients, including Fe, Cu and Zn in rice. Similarly, Yang *et al.* (2011) recorded Cd-induced decline in macronutrients (K and P) in *Potamogeton criprus*. In contrast, we found increase in K content of fruit, particularly in the cv. Roma under higher Cd regimes. However, fruit Ca content decreased due to Cd toxicity and this decrease was being much more in the cv. Roma at 640 µM $CdCl_2$.

Conclusion

The results of the present study demonstrated that Cd toxicity severely deteriorated the fruit quality, whereas the tolerant cultivar (Nagina) that showed greater *Cat2* gene expression and had low H_2O_2 and MDA contents tolerated Cd stress effectively. The sensitivity of cv. Roma to Cd was associated with the greater cellular levels of H_2O_2 and MDA under Cd toxicity that in turn triggered more oxidative damage, suggesting a lack of systemic oxidative defence mechanism in the cultivar.

Acknowledgement

This work was partially supported by Higher Education Commission (HEC), Islamabad, Pakistan through Project grant No. PM-IPFP/HRD/HEC/2011/0579).

References

AACC, 2000. *Approved Methods of American Associations of Cereal Chemists*, 10th edition. American Association of cereal chemists, incorporation, street Paul, Minnesota, USA

Ali, B., B. Wang, S. Ali, M.A. Ghani, M.T. Hayat, C. Yang and W.J. Zhou, 2013. δ- Aminolevulinic acid ameliorates the growth, photosynthetic gas exchange capacity, and ultrastructural changes under cadmium stress in *Brassica napus* L. *J. Plant Growth Regul.*, 32: 604–614

Asada, K. and M. Takahashi, 1987. Production and scavenging of active oxygen in chloroplasts. *In*: *Photoinhibition*, pp: 227–287. Kyle, D.J., C.B. Osmond and C.J. Arntzen (eds.) Elsevier Science Publication Co

Cakmark, I., D. Strboe and H. Marschner, 1993. Activities of hydrogen peroxide scavenging enzymes in germinating wheat seeds. *J. Exp. Bot.,* 44: 127–132

Choppala, G., Saifullah, N. Bolan, S. Bibi, M. Iqbal, Z. Rengel, A. Kunhikrishnan, N. Ashwath and Y.S. Ok, 2014. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. *Crit. Rev. Plant Sci*., 33: 374-391

Collén, J., E. Pinto, M. Pedersen and P. Colepicolo, 2003. Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutant metals. *Arch. Environ. Contam. Toxicol.,* 45: 337–342

Davies, B.H., 1976. Carotenoids. *In*: *Chemistry and Biochemistry of Plant Pigments*, pp: 199–217. Goodwin, T.W. (ed.). Academic press, London, UK

Eromosele, I.C. and T.J. Hamagadu, 1993. Graft copolymerization of methylmethacrylate onto caesarweed fibers by the potassium permanganate–toluene redox system. *J. Appl. Poly Sci.,* 50: 645–649

FAO, 2009. Faostat database collection, http://apps.fao.org/pagc/collection

Hodges, D.M., J.M. DeLong, C.F. Forney and R.K. Prange, 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207: 604–611

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

Iannone, M.F., M.D. Groppa and M.P. Benavides, 2015. Cadmium induces different biochemical responses in wild type and catalase-deficient *tobacco* plants. *Environ. Exp. Bot.,* 109: 201–211

Irfan, M., A. Ahmad and S. Hayat, 2014. Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. *Saudi. J. Biolog. Sci.,* 21: 125–131

Küpper, H., A. Parameswaran, B. Leitenmaier, M. Trtílek and I. Šetlík, 2007. Cadmium- induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. *New Phytol.*, 175: 655–674

Lee, S.H. and C.S. An, 2005. Differential expression of three catalase genes in hot pepper (*Capsicum annuum* L.). *Mol. Cells*, 20: 247–255

Liu, J., K. Li, J. Xu, J. Liang, X. Lu, J. Yang and Q. Zhu, 2003. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. *Field Crops Res.*, 83: 271–281

Liu, W.X., S.H. Shang, X. Feng, G.P. Zhang and F.B. Wu, 2015. Modulation of exogenous selenium in cadmium-induced changes in antioxidative metabolism, cadmium uptake, and photosynthetic performance in the 2 tobacco genotypes differing in cadmium tolerance. *Environ. Toxicol. Chem.*, 34: 92–99

Mengel, K., E.A. Kirkby, H. Kosegarten and T. Appel, 2001. Elements with more toxic effects. *In*: *Principles of Plant Nutrition*, pp: 657-673. Springer Netherlands

Mohamed, A.A., A. Castagna, A. Ranieri and L.S. di Toppi, 2012. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. *Plant Physiol. Biochem.,* 57: 15–22

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

Nada, E., B.A. Ferjani, R. Ali, B.R. Bechir, M. Imed and B. Makki, 2007. Cadmium-induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. *Acta Physiol. Plant.*, 29: 57–62

Pandey, M., A.B. Abidi, S. Singh and R.P. Singh, 2006. Nutritional evaluation of leafy vegetable paratha. *J. Hum. Ecol.*, 19: 155-156

Pedro, C.A., M.S. Santo, S.M. Ferreira and S.C. Gonçalves, 2013. The influence of cadmium contamination and salinity on the survival, growth and phytoremediation capacity of the salt marsh plant *Salicornia ramosissima*. *Mar. Environ. Res.*, 92: 197–205

Perfus- Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier, 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32: 539–548

Qian, H., J. Li, L. Sun, W. Chen, G.D. Sheng, W. Liu and Z. Fu, 2009. Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. *Aquat. Toxicol.*, 94: 56–61

Redondo-Gómez, S., E. Mateos-Naranjo and L. Andrades-Moreno, 2010. Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum*. *J. Hazard. Materials*, 184: 299–307

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

Shim, I.S., Y. Momose, A. Yamamoto, D.W. Kim and K. Usui, 2003. Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. *Plant Growth Regul.*, 39: 285–292

Tang, L., R.R. Ying, D. Jiang, X.W. Zeng, J.L. Morel, Y.T. Tang and R.L. Qiu, 2013. Impaired leaf CO_2 diffusion mediates Cd-induced inhibition of photosynthesis in the Zn/Cd hyperaccumulator *Picris divaricata*. *Plant Physiol. Biochem.*, 73: 70–76

Velikova, V., I. Yordanov and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. *Plant Sci.*, 151: 59–66

Xie, W., M. Liu, X. Lv, W. Lu, J. Gu and H. Yu, 2014. Construction of a controllable β- carotene biosynthetic pathway by decentralized assembly strategy in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.*, 111: 125–133

Yang, H.Y., G.X. Shi, Q.S. Xu and H.X. Wang, 2011. Cadmium effects on mineral nutrition and stress-related induces in *Potamogeton criprus*. *Russ. J. Plant Physiol.*, 58: 253–260

Yoshida, S., D.A. Forno, J.H. Cock and V. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*. IRRI, Los Banos

(Received 22 June 2016; Accepted 20 October 2016)