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Full Length Article

Lead Induced Modulation in Growth, Chlorophyll Pigment, Nutrient Uptake, Antioxidant Enzyme Regulation, Gene Expression and Fruit Quality in two Tomato Cultivars

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Abstract

Metal stress is one of the major restrictions for agricultural production. A pot experiment was set-up to appraise modulation in growth, oxidative defense, secondary metabolism and relative gene expression of two tomato cultivars *viz*., Roma (sensitive) and Nagina (tolerant) at flowering stage in response to different lead (Pb^{2+}) regimes (160, 320, 640 and 1280 μM). The results showed that Pb^{2+} stress (1280 μM Pb) caused a considerable reduction in growth attributes, chlorophyll (*Chl*.) pigments and ascorbic acid contents, and increase in malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and total soluble protein (TSP) contents in both cultivars. A significant enhancement in ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT) activities and *Cat2* gene expression was documented in Pb^{2+} stressed tomato plants. Fruit quality of Nagina was better than Roma cultivar. In this context, higher fruit ash contents, protein contents, fructose and glucose contents were observed in Nagina while Roma was inferior in this regard when under Pb^{2+} stress. Furthermore, Pb^{2+} reduced the fresh and dry biomass, moisture, fiber content and mineral (Na^+, K^+ and Ca^{2+}) uptake in tomato fruits of both cultivars. The results indicated that significant amount of Pb^{2+} accumulates in the root compared with its concentration in shoot and leaves while only a small amount of Pb^{2+} reaches the fruit. The exposure to Pb^{2+} caused significant changes in *Cat2* gene transcripts indicating the contribution of this gene in Pb^{2+} tolerance. The sensitive cultivar exhibited higher oxidative damage, decreases in the concentration of essential nutrients, poor oxidative defense system, and thus had low quality fruit. © 2020 Friends Science Publishers

Keywords: Enzyme activity; Gene expression; Lead regimes; Oxidative stress; Tomato

Introduction

In recent times, a serious concern has developed among researchers and environmentalists regarding the metals and their harmful association with the plants. Although some metals are known to be beneficial for plants when applied in specific quantities, most of the metals are detrimental to plant growth overall (Zeng *et al.* 2008). Usually they cause Hindrance in growth, development and metabolic processes and accelerate senescence in plants (Sah *et al.* 2016). Thus, the metal pollution in soil becomes an increasing risk for human well-being and the environment (Yáñez *et al.* 2002). In this context, the whole world should worry about the metals pollution (He *et al.* 2008). The commonly contaminating metals are cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), Pb and zinc (Zn), while burning of fossil fuels, industrial residues, waste water, sewage sludge, and phosphate fertilizers are their major sources. These sources have considerably enhanced the level of these metals in the soil (Khan *et al.* 2008). The Pb^{2+} is a hazardous trace element because of its greater solubility and toxicity in the soil (Wani *et al.* 2015). Plant roots simply absorbed Pb^{2+} and then uptake it to above ground plant parts, entering the food chain *via* consumption of vegetables and causing risks to human fitness (Cao *et al.* 2010).

The Pb^{2+} negatively affects various biological processes in plants. Reacting to Pb^{2+} stress, plants over generate reactive oxygen species (ROS) (Zulfiqar *et al.* 2019) that cause oxidative stress, which leads to increase in nonspecific lipids oxidation, membrane permeability, *Chl*. degradation, damages to proteins and DNA (Hasanuzzaman *et al.* 2018). Moreover, it activates downstream signaling for transcriptional regulation (Vinocur and Altman 2005), possibly through up-regulation of mitogen-activated protein

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kinases (Hamel *et al.* 2006). In Plants, ROS signaling and calmodulin (CaM) and CaM-like proteins/signaling pathways seem to be the leading players in transcriptional regulation under abiotic stresses (Zeng *et al.* 2015). One of the ways of plant defense from ROS, the plants possess protective antioxidant enzymes and low molecular weight antioxidants (Cuypers *et al.* 2016). Ascorbate-glutathione (AsA-GSH) cycle and GHS metabolism are involved in the detoxification of H_2O_2 in various cell compartments (López-Climent *et al.* 2014). The degree of oxidative damage is assessed in the form of MDA, which is the product of lipid peroxidation in plasma membranes (Grotto *et al.* 2009). Further, activities of antioxidant enzymes like CAT, SOD, APX and GR rise in response to Pb^{2+} stress (Caverzan *et al.* 2012).

Tomato (*Lycopersicon lycopersicum* L.) plants serve as a rich source of minerals, vitamins and lycopene (Wilcox *et al.* 2003), and can grow under all climatic regions of the globe. Nevertheless, ecological stress factors limit its production (Gerszberg and Hnatuszko-Konka 2017). In Pakistan, tomato is 2nd primary vegetable crop (FAO 2009). Metals, particularly Pb^{2+} have harshly hindered the production of tomato (Mengel *et al.* 2001). Irrespective of better nutritious value, its yield per hectare is very less. However, breeding or transgenic strategies have revealed significant results in improving plant stress tolerance (El-Esawi and Alayafi 2019) Further, plants can acquire tolerance to abiotic stresses through general and specific stress components (Sung *et al.* 2003). Plants adapt to abiotic stresses by regulating the expression of several stress-responsive genes (Jiang *et al.* 2007). Accumulation of Pb^{2+} contents in various plant parts and its effect on fruit quality and production is seldom studied. However, little information is available in the literature about the impact of *Cat2* gene in Pb^{2+} tolerance in tomato plants and its role in nutritive value of tomato fruit as well. Therefore, in this study, two contrasting tomato cultivars were grown under different Pb^{2+} regimes with the hypothesis that Pb^{2+} stress might differently modulate key physio-chemical and molecular processes to affect growth and fruit quality in tomato.

Materials and Methods

Experiment site, and growth conditions

This study was conducted in experimental research site of Department of Botany, Government College University Faisalabad, Pakistan during winter season (November-2018 to March-2019). Seedlings of two tomato cultivars having differential metal tolerance capacity (Hussain *et al*. 2017), namely, Nagina (tolerant) and Roma (sensitive) were collected from vegetable section, Ayub Agriculture Research Institute, Faisalabad, Pakistan. The plants were grown under 11 h/13 h light/dark period and 24°C/12°C day/night temperatures, average relative humidity (54%), and photosynthetically active radiation (PAR 687–948 μmol m^{-2} s^{-1}) during the whole study. Selected three seedlings of Nagina and Roma were maintained in each pot (dimension 30 cm high, circumference 50 cm at bottom and 60 cm at top). At vegetative stage, plants were subjected to different Pb^{2+} regimes (160, 320, 640 and 1280 μM) for three weak. For this purpose, Pb^{2+} solutions were prepared in Hoagland nutrient solution and applied to each pot in an increment of 40, 80, 160 and 320 μM each day until desired Pb^{2+} level were achieved. Lead nitrate was used as Pb^{2+} source. An equivalent amount of NO_3 (as calcium nitrate) was added to the control plants to compensate for NO_3 content of Pb^{2+}-treated plants in nutrient solution. Experiment was laid out in completely randomized design (CRD) in triplicates. Fresh leaf samples were harvested three weeks after the application of Pb^{2+} treatments and kept in a freezer at -20^{o}C for determination of different growth and biochemical attributes. Fruit quality attributes were obtained from mature but green tomato fruits.

Data collection

Growth attributes: The growth parameters including shoot length, leaf area, shoot and fruit biomasses of two plants per replicate were studied. Leaf area was determined by multiplying the maximum length (L) and width (W) of leaf with the correction factor (6.45) obtained by using graph squares.

Leaf area (cm^2) = L × W × 6.45 (Hussain *et al.* 2017)

Analysis of photosynthetic pigments

Tomato fresh leaves (0.1 g) were used for pigments extraction in 80% acetone. The mixture was centrifuged at 4000 rpm for 10 min. The optical density (OD) was assessed at 480, 645 and 663nm spectrophotometrically (Lichtenthaler 1987) and *Chl*. pigments were calculated by using the following formulas.

Chl. a (mg g^{-1} FW) = 12.7 (OD663 –2.69 (OD645) × (V/1000 × W)

Chl. b (mg g^{-1} FW) = 12.9 (OD645–4.68 (OD663) × (V/1000 × W)

Total *Chl*. (mg g^{-1} FW) = 20.2 (OD645–8.02 (OD663) × (V/1000 × W)

Car. (mg g^{-1} FW) = OD480 + (0.114 × OD663) - (0.638 × OD645)

Estimation of H_2O_2 contents

Determination of H_2O_2 content in fresh leaves was done according to the method of Velikova *et al.* (2000). For this purpose, 0.2 g fresh leaves were grinded in 0.1% TCA (5 mL) and then centrifuged for 15 min at 12000 g, the supernatant mixed with potassium phosphate buffer (10 m*M*; pH 7.0) and potassium iodide (1 mL; 1 *M*). Optical density was determined at 390 nm.

Analysis of malondialdehyde (MDA) contents

Tomato leaves (0.2 g) were grinded in 5.0 mL of 5% (w/v) TCA, and centrifuged. The MDA was estimated at 532 and 600 nm spectrophotometrically using thiobarbituric acid assays (Heath and Packer 1968).

Analysis of ascorbic acid content

Ascorbic acid was measured by using the method of Mukherjee and Choudhuri (1983). Tomato fresh sample (0.2 g) was grinded in ice bath with 5.0 mL of TCA (6%) and centrifuged. Two mL of supernatant was dissolved with 2,4-DNP hydrazine (1 mL). Then one drop of thiourea (10%) was added to the mixture and heated (95°C) for 20 min, then cool down the mixture at 25°C. Thereafter, cool 80% H_2SO_4 (2.5 mL) was added to the mixture placed on ice bath and the absorbance was read at 530 nm.

Protein and antioxidant enzymes assays

The total soluble proteins in fresh leaves of tomato were estimated by Bradford method using Coomassive blue dye (Bradford 1976). The reaction mixture contained 100 μL of protein sample (leaf extract) and 5 mL of Bradford reagent while the blank had 100 μL of distilled water. These test tubes were incubated for 20 min in dark and then absorbance measured at 595 nm. For the antioxidant enzyme, fresh leaf material homogenized with 10 mL of chilled potassium phosphate buffer (50 m*M*; pH 7.5) was poured down. The mixture was centrifuged at 12,000 *g* at 4°C. The supernatant was collected and used for the determination of following antioxidant enzyme activities.

Estimation of POD activity

The reaction volume (3 mL) consisted of phosphate buffer (50 m*M*; pH 7.5), guaiacol (20 m*M*) and H_2O_2 (5.9 m*M*) and enzyme extract (100 μL). The POD contents were assessed at 470 nm after every 20 seconds for 2 min spectrophotometrically (Chance and Maehly 1955)

Estimation of CAT activity

The CAT activity was analysed with method of Chance and Maehly (1955). The reaction solution contained 1 mL H_2O_2 (5.9 m*M*), phosphate buffer (50 m*M*; pH 7.5) and enzyme extract (100 μL). The change in absorbance of reaction mixture was noted at 240 nm after every 20 seconds for 2 min.

Estimation of APX activity

The reaction volume for APX (1 mL) contained phosphate buffer (50 m*M*; pH 7.5), AsA (0.5 m*M*), H_2O_2 (0.1 m*M*) and enzyme extract (200 μL). The readings of the mixture were taken at 290 nm after every 20 seconds for 2 min (Nakano and Asada 1987).

Cat gene expression with RTq-PCR analysis

Fresh leaves (0.1 mg) were weighed from each sample, and obtained using the total RNA extraction kit (Takara Bio Inc. Japan). The RNA purity and integrity was confirmed by using micro-spectrophotometer (Nano Drop ND-1000) and agarose gel electrophoresis, respectively after extraction. The cDNA was synthesized from total RNA (1 μg) using a Prime Script RT Reagent Kit (Takara Bio Inc.) and subjected to RT-PCR. The KAPA SYBR FAST qPCR Kit (Kapa Bio, Boston, MA, USA) was used for amplification to observe the *Cat2* gene expression level by using RTq-PCR (Bio-Rad, Hercules, CA, USA). The amplification conditions were: pre-denaturation at 95°C for 30 sec; denaturation at 95°C for 5 sec; annealing at 60°C for 20 sec; extension at 72°C for 10 sec, 40 cycles. After the completion of reaction, verified the amplification curve and melting curve, and Ct value was calculated from the amplification curve. Actin (NM_00111149) was used as a control or data normalization. Transcript level of the specific gene was calculated using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen 2001). Sequences of *Cat 2* and *Actin* primers are given in Table 1.

Fruit moisture and ash contents determination

The moisture contents in fruit were calculated after drying using air forced draft oven and ash content by the method (44-15A and 8-1) explained in AACC (2000), respectively.

Fruit total protein and fibre contents determination

The nitrogen contents in fruit were calculated with Kjeldhal's procedure and crude fibre according to method (46-10A and 32-10) described in AACC (2000), respectively. The protein content in fruits measured by their total N content and multiplied by factor 5.7 (N×5.7) to obtain protein content in crude form (Jones 1931)

Estimation of mineral nutrients from tomato fruits

Fruit samples (500 mg) of each tomato variety were digested with HNO_3 and $HCLO_3$ (3:1) at hot plate for 2–3 hours. Digested material was used to measure the nutrients with the procedure of Yoshida *et al*. (1976). Calcium (Ca^{2+}), potassium (K^+) and sodium (Na^+) contents in the fruit of tomato were measured using atomic absorption spectrophotometer (Hitachi, Z-2000, Tokyo, Japan). Similarly, Pb^{2+} concentration was determined in root, shoot, leaves and fruit samples of tomato.

Data analysis

Experiment was conducted in CRD with triplicate. Obtained data were subjected to two-way analysis of variance (ANOVA) using COSTAT 6.2, (Cohort software, 2003,

Table 1: Primer for real-time PCR

Plant	Accession No.	Gene	Amplicon size (bp)	Sequences (5´→3´)
Tomato	NM_001247257.1	Cat *2*	185	F 5′- ctt tcc tct tcg acg ata ttg gta R 5′- gtg att tgc tcc tcc gac tc
	NM_00111149	Actin	160	F 5′- tct gtt tcc cgg ttt tgc tat tat R 5′- tgc atc agg cac ctc tca ag

Table 2: Mean square values from ANOVA of data for growth, chlorophyll pigments, oxidative defense system, catalase gene expression and fruit quality in tomato subjected to different Pb^{2+} regimes

SOV	df	SDW	RDW	SL	LA	*Chl. a*	*Chl. b*	Tot. *Chl.*	Car.	MDA	H_2O_2	AsA
Lead regimes (T)	4	9.75 ***	0.33 ***	74.64 ***	6940.48 ***	0.57 ***	0.087 ***	1.104 ***	0.015 ***	279.96 ***	3.6624 ***	5.677 ***
Cultivars (C)	1	2.58 **	0.28 ***	1219.2 ***	633.88 ***	0.36 ***	0.038 ***	0.637 ***	0.009 ***	25.357 **	13.668 ***	5.502 ***
T×C	4	0.05 ns	0.00 ns	5.38 ns	3.81 ns	0.007 ns	0.000 ns	0.007 ns	0.000 ns	6.839 ns	1.325 ***	0.396 ***
Error	20	0.47	0.03	8.81	35.78	0.012	0.002	0.017	0.000	3.848	0.254	0.142
SOV	df	TSP	CAT	APX	POD	*Cat2*	Moisture in fruit	Ash in fruit	Fiber in fruit	Protein in fruit	Glucose in fruit	Fructose in fruit
Lead regimes (T)	4	11.28 ***	5989.0 ***	614.809 ***	33697.3 ***	7.679 ***	10.47 ***	0.047 ***	0.074 **	0.036 ***	0.027 ***	0.032 ***
Cultivars (C)	1	1.981 ***	11901.7 ***	686.634 ***	27686.3 ***	1.843 ***	5.70 **	0.007 **	0.129 **	0.001 **	0.000 ns	0.004 ***
T×C	4	0.153 ***	44.6 ns	21.853 ***	2235.8 ***	0.187 ns	0.260 ns	0.017 ***	0.026 ns	0.005 ***	0.004 ns	0.001 ***
Error	20	0.0196	129.7	5.179	144.1	0.144	1.700	0.001	0.019	0.000	0.004	0.032
SOV	df	FFW	FDW	Na^+ in fruits	K^+ in fruits	Ca^{2+} in fruits	Pb^{2+} in roots	Pb^{2+} in shoots	Pb^{2+} in leaves	Pb^{2+} in fruits		
Lead regimes (T)	4	706.049 ***	4.269 ***	1.018 ***	1342.47 ***	2.066 ***	74809.4 ***	25421.3 ***	40607.1 ***	0.230 ***		
Cultivars (C)	1	192.027 ***	0.358 ***	0.100 ***	11.19 *	1.756 **	1182.1 ***	218.4 ***	860.6 ***	0.343 ***		
T×C	4	13.724 ns	0.077 ns	0.477 ***	97.65 ***	0.194 ns	155.7 ***	52.9 ***	153.6 ***	0.009 ns		
Error	20	8.330	0.108	0.001	3.05	0.302	27.1	6.3	10.7	0.007		

Montery, CA, USA). Difference among means was ascertained using least significant difference (LSD) at $P \leq 0.05$.

Results

Growth attributes

Interaction between tomato cultivars and Pb^{2+} regimes had significant effect on morphological attributes including shoot and root biomasses, shoot length and leaf area of tomato (Table 2). The exposure of tomato plants to Pb^{2+} stress (160–1280 μM) produced a considerable decline in various morphological attributes including biomass of tomato shoot and root, shoot length and leaf area (Fig. 1a–d). The response of tomato cultivars was diverse regarding to shoot and root dry weights. The shoot dry weight was reduced in both tomato cultivars, though cv. Roma (10–43%) showed a more decline than cv. Nagina (15–41%) under different Pb^{2+} regimes (160–1280 μM) over control plants (Fig. 1a). Lead stress (160–1280 μM) decreased the root dry weight in both cv. Nagina (20–39%) and Roma (18–50%) over control plants, respectively (Fig. 1b). Lead inhibited (>8%) elongation of shoot growth was more evident as compared to controls, though cv. Nagina showed a less shoot length reduction (1–8%) than cv. Roma (1–10%) at 160–1280 μM level of Pb^{2+}, respectively (Fig. 1c). Lead stress (160–1280 μM) reduced the leaf area in both cv. Nagina (23–52%) and Roma (23– 54%) over control plants, respectively (Fig. 1d). Lead stress induced a visible decline in plant dry weights, shoot length and leaf area in the present study, and this Pb^{2+} induced decreased was more in cv. Roma (Fig. 1a–d).

Chlorophyll (*Chl.*) and carotenoid (Car.) contents

In case of *Chl.* pigments and Car. contents, no significant ($P > 0.05$) interaction of Pb^{2+} regimes and cultivar was noted (Table 2). The Pb^{2+} regimes differentially modulated the contents of *Chl.* and total Car. in both tomato cultivars. Although the two cultivars showed similar trends, the degree of increase or decrease in photosynthetic pigment content was not the same, and the change in degree of decrease or increase in Roma was significantly higher than that in Nagina. Lead stress significantly reduced the *Chl. a* content (11–37% and 5–41%), *Chl. b* content (9–37% and 8–42%) and total *Chl.* (10–37% and 6–41%) in both cv. Nagina and cv. Roma, respectively (Fig. 1e–g). Nevertheless, the reduction in *Chl.* content was higher in cv. Roma than that in cv. Nagina. In contrast, cultivar's response was similar regarding Car. contents. Between two cultivars, lesser contents of total Car. (13–34%) were recorded in cv. Nagina under Pb^{2+} stress (160–1280 μM) leaves (Fig. 1h).

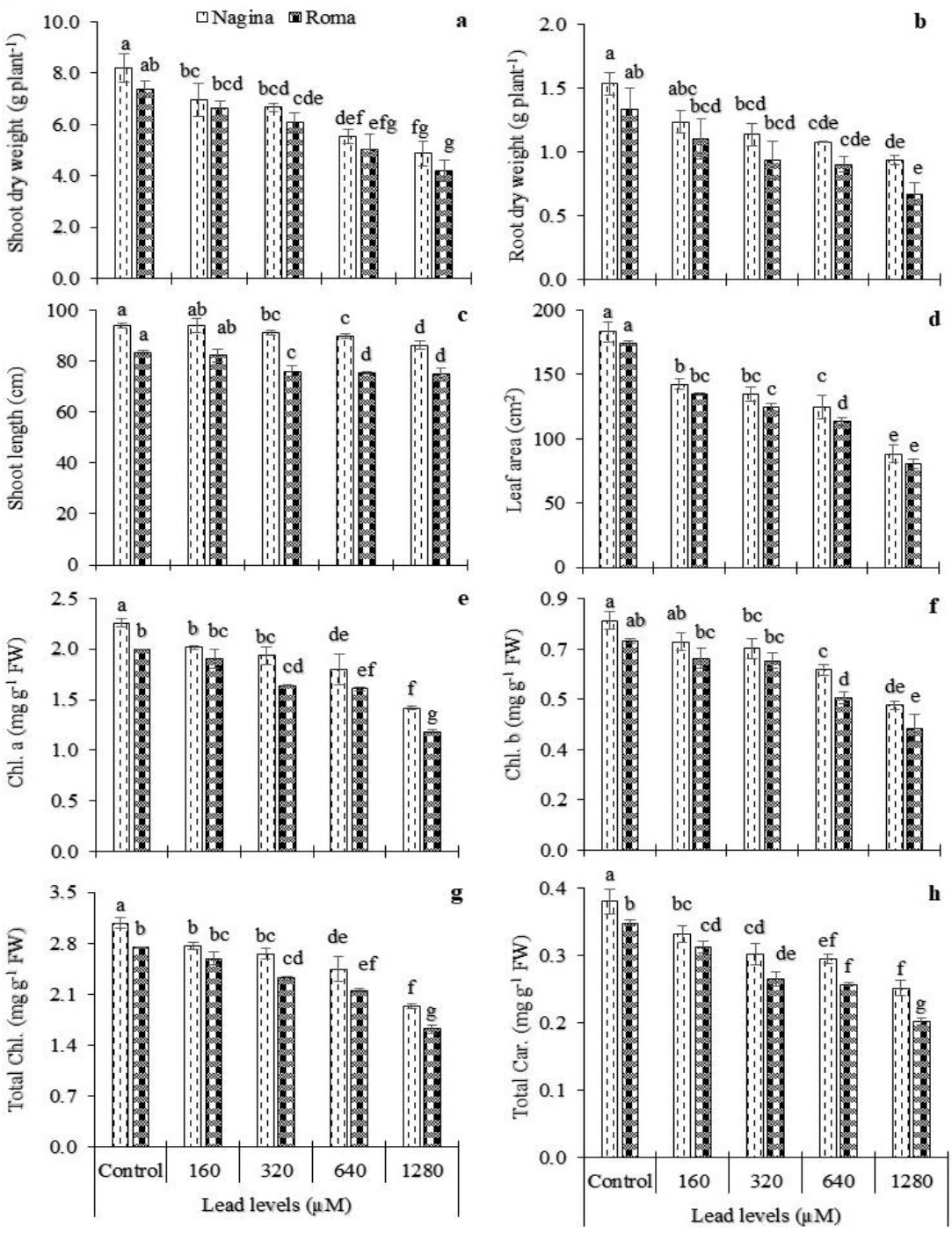

Fig. 1: Changes in growth characteristics and photosynthetic pigments in two tomato cultivars subjected to different Pb^{2+} regimes (n=3± SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

Oxidative stress and ascorbic acid (AsA) contents

Lead stress led to enhance in membrane disruption reflected by higher MDA content. Interaction between tomato cultivars and Pb^{2+} regimes had non-significant ($P > 0.05$) effect in MDA contents (Table 2). When tomato plants were subjected to Pb^{2+} stress (160–1280 μM), the MDA content of cv. Roma and Nagina increased by (43–122%) and (9–86%), respectively as compared to control plants (Fig. 2a). For H_2O_2, the interaction of Pb^{2+} regimes and cultivars were

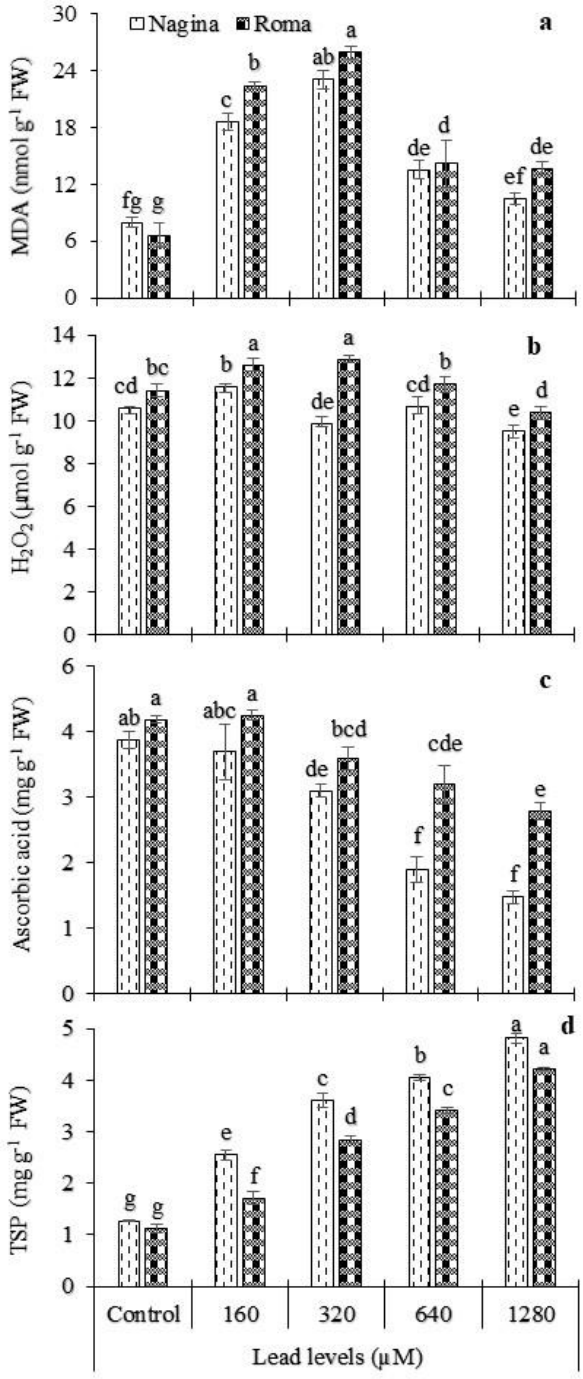

Fig. 2: Changes in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and ascorbic acid contents in two tomato cultivars subjected to different Pb^{2+} regimes (n =3± SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

more significant ($P < 0.001$). The H_2O_2 content also markedly increased under Pb^{2+} stress as well as during plant senescence. The Pb^{2+} stress (160–1280 μM) in growth medium caused a significant increase in H_2O_2 contents in cv. Nagina (9.45–84.13%) and Roma (10.48–96.06%) as shown in Fig. 2b.

Interaction between tomato cultivars and Pb^{2+} regimes had significant ($P < 0.05$) effect on ascorbic acid contents (Table 2). A substantial decrease in ascorbic acid levels was present in tomato plants subjected to Pb^{2+} stress in growth medium. Toxic effect of Pb^{2+} stress (160–1280 μM) also triggered a significant decline in ascorbic acid contents of cv. Nagina (4–28%) and Roma (14–65%), but this reduction was more apparent in cv. Nagina (Fig. 2c).

Total soluble protein (TSP) contents

Interaction between Pb^{2+} regimes and tomato cultivars had significant ($P < 0.001$) effect on TSP contents of tomato fruits (Table 2). Total soluble protein content was significantly affected due to Pb^{2+} regimes. Lead stress markedly increased the TSP in fruit of Nagina (100–278%) and Roma (52–274%) over control plants, but this enhancement was more in cv. Nagina (Fig. 2d).

Antioxidant enzyme activity and Cat gene expression

Interaction between tomato cultivars and Pb^{2+} regimes had significant ($P < 0.001$) effect APX and POD activities of tomato cultivars except for CAT enzyme and *Cat2* gene (Table 2). After Pb^{2+} treatments on tomato, the activities of antioxidant enzymes of the both cultivars increased first and then decreased, and these changes were different between cultivars. Plants of cv. Nagina showed a rise (7–74%) in CAT activity, while Roma (13–94%) was superior in this context under various Pb^{2+} regimes (160–1280 μM) (Fig. 3a). Lead stress caused a markedly increase in APX contents of cv. Nagina (60–166%) and Roma (45–135%), respectively over control plants (Fig. 3b; Table 2). In this study, we have recorded Pb^{2+} (160–1280 μM) caused a considerable decline in POD activity of both cv. Nagina (2–50%) and cv. Roma (14–120%) can be seen in Fig. 3c. Compared with control, after Pb^{2+} treatment (320 μM), the relative expression of *Cat2* gene of cv. Nagina and Roma increased by 316 and 241%, respectively, but the degree of change of cv. Nagina is greater than Roma (Fig. 3d).

Moisture contents and Ash contents of tomato fruit

Plants subjected to Pb^{2+} stress had non-significant ($P > 0.05$) effect in fruit moisture contents of both tomato cultivars (Table 2). The Pb^{2+} stress affected the quality of tomato fruit. In this regard, Pb^{2+} (160–1280 μM) induced a steady decrease in moisture contents in cv. Nagina (2–3%) and Roma (3–4%), while interaction of Pb^{2+} regimes and cultivars ($P < 0.001$) was significant in ash contents (Table 2). The Pb^{2+} stress was increased the ash contents (13–40%) more significantly in plants of Nagina when subjected to

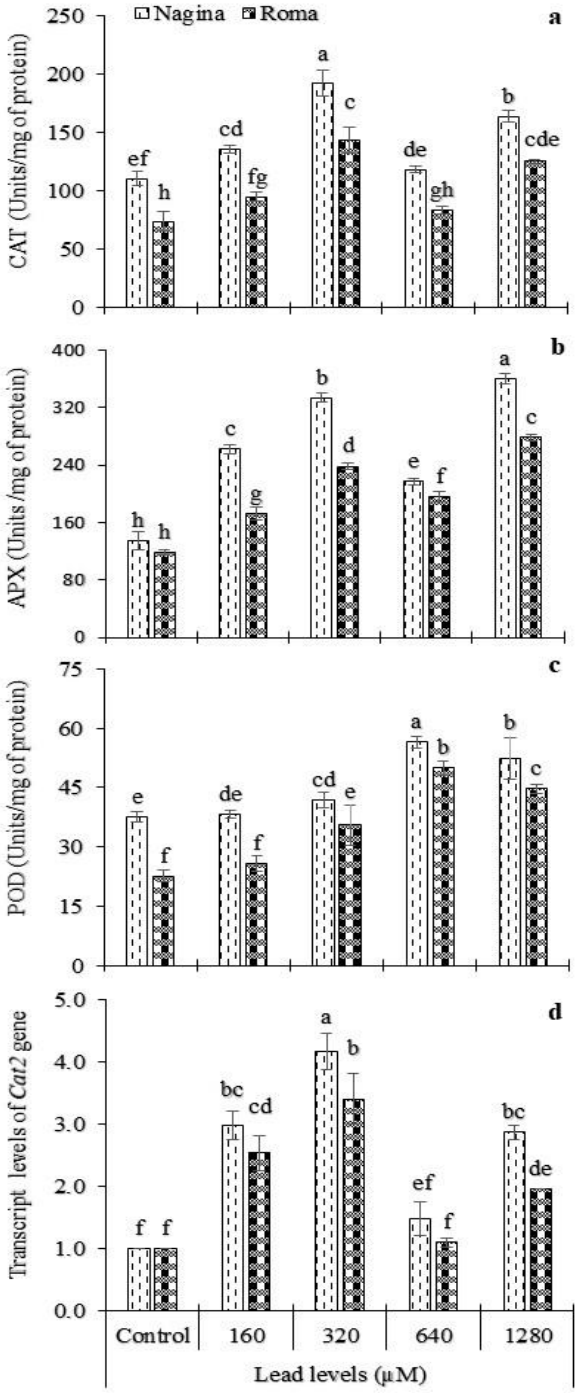

Fig. 3: Changes in antioxidant enzymes activities (CAT, APX and POD) and transcript level of *Cat2* gene in two tomato cultivars subjected to different Pb^{2+} regimes (n =3± SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

Pb^{2+} regimes (Fig. 4a–b). Moisture contents are important to storage life of the fruits. The results of present study also exhibited that lower moisture contents in fruit of tomato were recorded in Nagina plants (Fig. 4a).

Total fiber and total protein contents of tomato fruit

Lead caused non-significant ($P > 0.05$) effect on fruit total fiber contents of both tomato cultivars (Table 2). A dose dependent Pb^{2+} toxicity decline the total fiber (0.5–5% and 2.87–19%) under different Pb^{2+} regimes (160–1280 μM) in both Roma and Nagina fruits, respectively. The interaction of Pb^{2+} regimes and cultivars had significant ($P < 0.001$) effect on fruit protein contents in both tomato cultivar (Table 2). Lead stress markedly increased the total protein content in fruit of Nagina (7–35%) and Roma (5–23%).

Glucose contents and fructose contents of tomato fruit

Interaction between tomato cultivars and Pb^{2+} regimes had non-significant ($P > 0.05$) effect on glucose contents of both tomato cultivar (Table 2). Glucose contents were increased in Nagina (4.65 and 4.65%) and Roma (9.41 and 14.12%) fruits under 320 and 1280 μM Pb^{2+} levels, respectively (Fig. 4e). The interaction between Pb^{2+} regimes and cultivars was significant ($P < 0.001$) for fructose contents. Fructose content was also affected significantly due to Pb^{2+} regimes. High concentration of fructose contents (14%) were found in cv. Roma at 1280 μM (Fig. 4f).

Fresh and dry biomass of tomato fruit

Interaction between tomato cultivars and Pb^{2+} regimes had non-significant ($P > 0.05$) effect on fruit biomass (Table 2). Lead toxicity also resulted in a substantial decrease in biomasses of tomato fruit. Nagina cultivar showed maximum reduction in fruit fresh and dry weight (43–68% and 38–53%) than Roma (49–66% and 29–65%) under different Pb^{2+} regimes (160–1280 μM), respectively (Fig. 4g–h).

Mineral (Na^+, K^+ and Ca^{2+}) contents in tomato fruits

Interaction between tomato cultivars and Pb^{2+} regimes had significant ($P < 0.001$) effect on Na^+ and K^+, while non-significant ($P > 0.05$) on Ca^{2+} contents of both tomato cultivar (Table 2). The response of both cultivars was inconstant regarding Na^+ contents. Lead stress (160–1280 μM) substantially increased the Na^+ content in the fruit of cv. Nagina (3–7%), respectively and this increase was 6% in Roma at 320 μM of Pb^{2+} (Fig. 5a). In contrast, Pb^{2+} toxicity increased the K^+ content significantly in cv. Roma (7–20%) than of Nagina (1–15%) under higher Pb^{2+} levels (640–1280 μM), respectively (Fig. 5b). The Ca^{2+} contents were reduced in response to Pb^{2+} toxicity and this reduction was being more maximum in Roma (13–26%) at 160–1280 μM of Pb^{2+} (Fig. 5c).

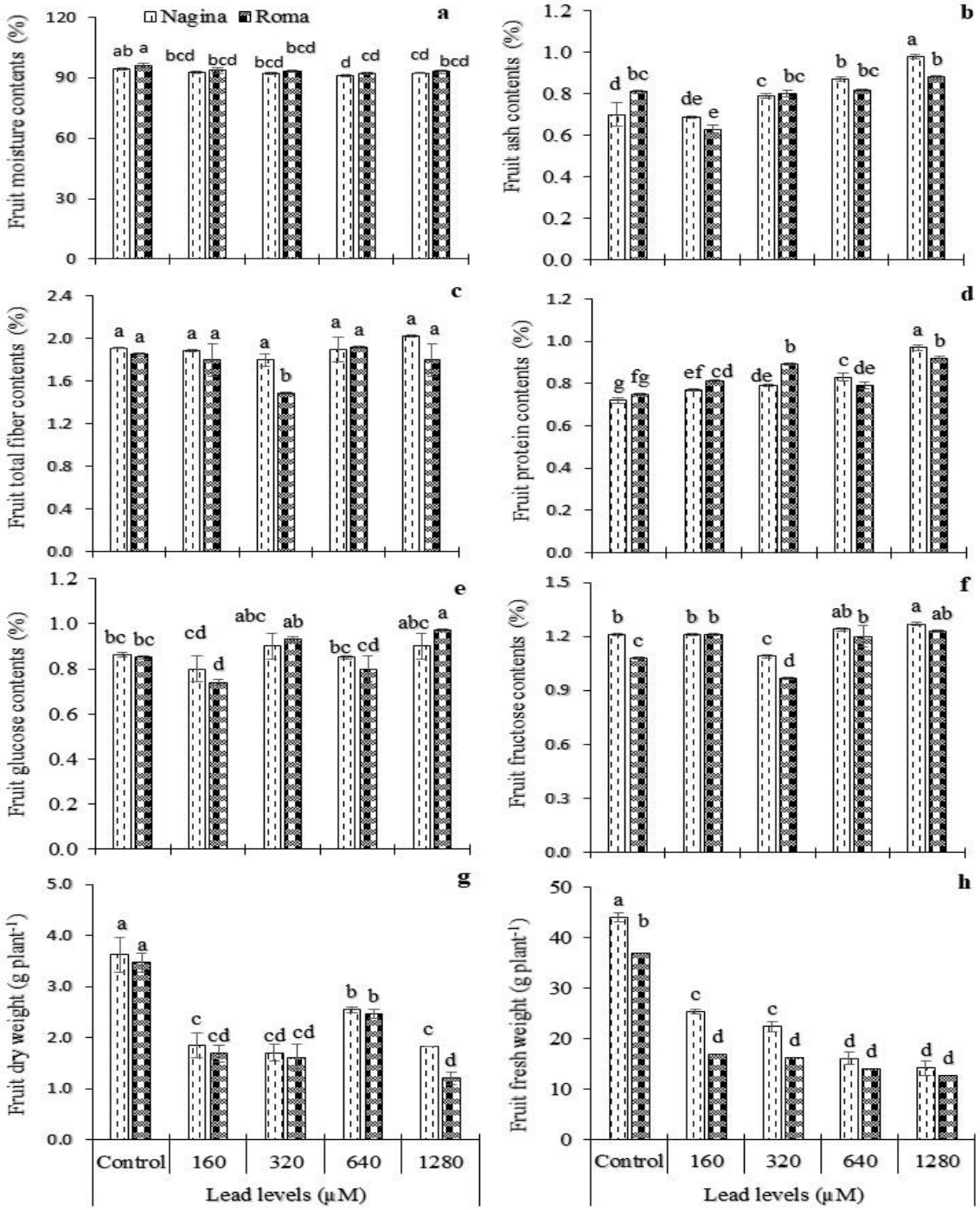

Fig. 4: Changes in the fruit quality and biomass of two tomato cultivars subjected to different Pb^{2+} regimes (n =3± SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

Lead (Pb^{2+}) uptake and accumulation in root, shoot, leaf and fruit of tomato

A significant increase in root, shoot, and leaf ($P < 0.001$) Pb^{2+} content was apparent in tomato cultivars under Pb^{2+} regimes. The differences in fruit Pb^{2+} content were not significant ($P > 0.05$) between cultivars and Pb^{2+} regimes (Table 2). After Pb^{2+} treatments (160–1280 *µM*), compared with control, the accretion of Pb^{2+} contents in root, shoot leaf and fruit was more significant in tomato plants under Pb^{2+} stress. Lead contents were increased in root (2750–7114% and 2596–6758%), shoot (569–4265% and 536–

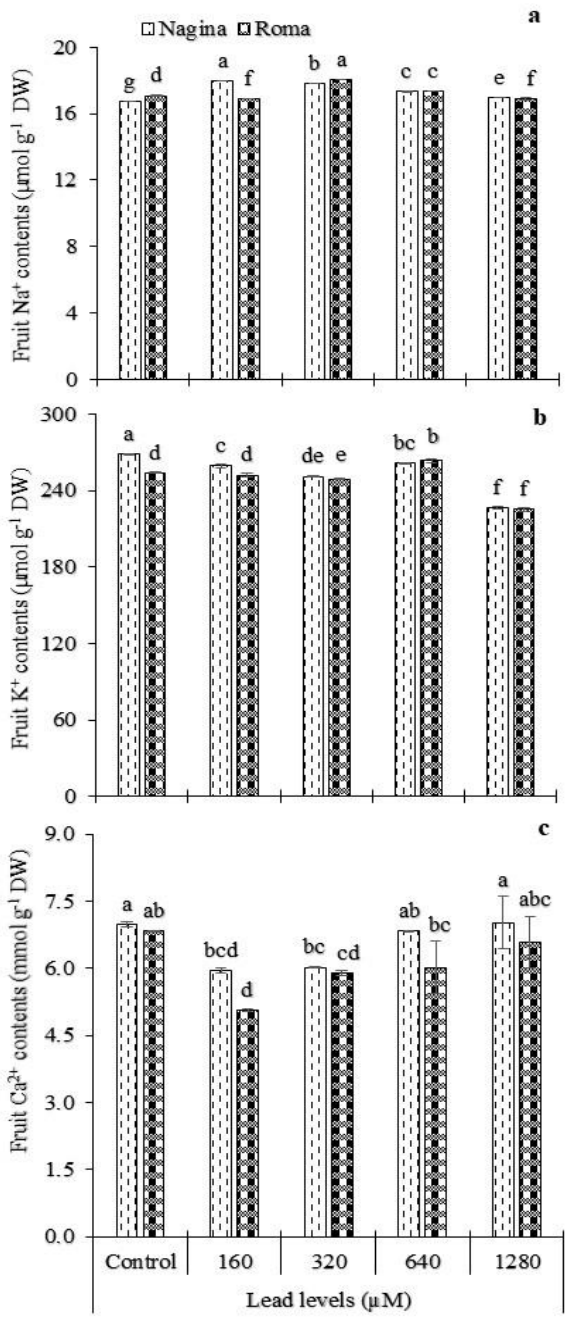

Fig. 5: Changes in mineral ions content (Na^+, K^+ and Ca^{2+}) in fruit of two tomato cultivars subjected to different Pb^{2+} regimes (n=3± SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

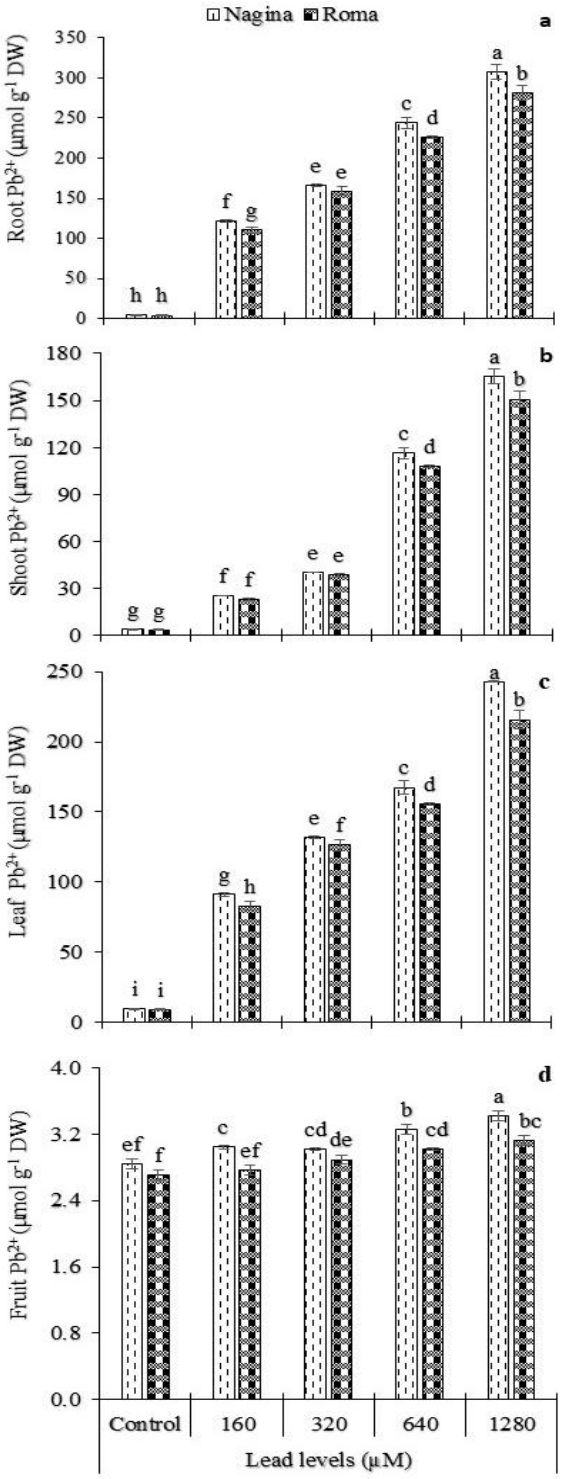

Fig. 6: Changes in Pb^{2+} contents in roots, shoot, leaves and fruit of two tomato cultivars subjected to different Pb^{2+} regimes (n=3±SD). Bars expressed with different letters are significantly different according to using least significant difference (LSD) at $P \leq 0.05$

4073%) and leaves tissues (863–2467% and 817–2288%) of both Nagina and Roma cultivars under Pb^{2+} regimes (160–1280 μM Pb^{2+}), respectively. After Pb^{2+} treatments, 7–20% Pb^{2+} contents accumulation was increased in cv. Nagina and 1–15% in cv. Roma fruit (Fig. 6a–d).

Discussion

In the present study, Pb^{2+} toxicity reduced shoot fresh and dry masses, and this Pb^{2+}-induced decrease was more prominent in the sensitive cultivar. A number of reports are available in the literature where Pb^{2+} stress induced decrease in plant growth (Zhou *et al.* 2018). The Pb^{2+} has the ability to affect the nutrients uptake, photosynthesis, and disturb the structure and properties of membrane (Hadi and Aziz 2015). It is well known that Pb^{2+} stress causes increased generation of ROS that in turn may provoke oxidation damage (Lopes *et al.* 2016). In this study, reduction in dry weights, shoot length and leaf area of plant might also be the consequence of oxidative stress. Likewise, Pb^{2+} toxicity inhibits the growth of plant by limiting the water uptake that altered the extensibility of cell wall, which in turn decreased the growth in plants (John *et al.* 2008).

Lead toxicity considerably declined the *Chl. a*, *b* and total Car. contents being lesser in the tolerant cultivar. This decline in *Chl*. and total Car. contents might be due to increase in chlorophyllase activities under metal toxicity and/or photo-oxidation (Malar *et al.* 2016). There are several reasons that Pb^{2+} induced decline in the *Chl*. contents. In this perspective, Pb^{2+} stress motivates chloroplast disorganization; thus caused decline in *Chl*. contents. The Pb^{2+} toxicity also decreased *Chl*. contents by reducing uptake and buildup of certain nutrient contents such as Mg^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+} that take part in the synthesis of *Chl*. contents (Malar *et al.* 2016). Under Pb^{2+} stress, Mg^{2+} in Chl. content is substituted by Pb^{2+} is the main reason for degradation of *Chl*. contents in plants grown-up in metal polluted area (Iqbal *et al.* 2017). This replacement of Mg^{2+} by Pb^{2+} hinders the capacity of *Chl*. pigment to catch light that declines the photosynthetic process. Moreover, excessive production of H_2O_2 may interrupt the process of photosynthesis (Gopal and Khurana 2011).

Under diverse environmental constraints, plants produce reactive oxygen species, which cause oxidative stress that damages proteins, DNA, membranes, pigments and enhance lipid peroxidation causing cell dysfunction and death (Xie *et al.* 2019). Similarly, greater MDA content was ascribed in chickpea and *Macrotyloma uniflorum* subjected to Pb^{2+} stress (Reddy *et al.* 2005). This study also indicated that increasing concentration of Pb^{2+} mediated more MDA and H_2O_2 in both tomato cultivars, respectively. Kumar and Prasad (2015) reported that Pb^{2+} stress interrupts the photosystem (PSII), which results in the production of more ROS and peroxidation of lipid. Ascorbic acid is an essential component of non-enzymatic antioxidant system and carries out detoxification of ROS, especially of H_2O_2 in plants under Pb^{2+} stress. Our results also exhibited a remarkable rise in the endogenous levels of ascorbic acid in plants under abiotic stresses. Similar results are also described in water deficit wheat plants (Roy *et al.* 2017). Ghorbanli *et al.* (2013) described the increase in ascorbic acid in tomato plants under drought. Similarly, Pb^{2+} stress exhibited a significant increase in ascorbic acid in wheat plants (Alamri *et al.* 2018). Plants display substantial accumulation of osmolytes to improve cell turgor and circumvent injuries to cell membranes and proteins from Pb^{2+} induced oxidative stress (Sahoo *et al.* 2015). This study also indicated TSP in tomato plants increased under Pb^{2+} stress regimes. Further, our results manifested a significant negative correlation between growth attributes and oxidative stress markers (MDA and H_2O_2) under drought (Fig. 1a–d and 2a–b).

In general, to overcome oxidative damage caused by increased cellular levels of H_2O_2 and MDA, plants display variations in antioxidant enzyme activities (Gupta *et al.* 2009). In this study, higher CAT, APX and POD activity was seen in the tolerant cultivar while the sensitive cultivar displayed lower POD activity due to Pb^{2+} stress. Our results are consistent with earlier reports in radish (He *et al.* 2008), Jatropha seedlings (Shu *et al.* 2012), cotton (Bharwana *et al.* 2013) and okra (Tiwari and Lata 2018). Numerous genes are induced by metal stress. Modifications in the level of catalase gene have been detected in several plant species when subjected to metal stress (Aydin *et al.* 2016). In this situation, Azpilicueta *et al.* (2008) assessed the gene expression of catalase (*CATA1* to *CATA4*) in sunflower under metal stress. These authors also further described that *CATA1* and *CATA2* accumulated more cadmium in cotyledon and roots and result preventing plant growth. In our study, we documented that qRT-PCR analysis of *Cat2 gene* shows upregulation of the *Cat2* in shoot of cv. Nagina under Pb^{2+} stress.

In this perspective, fruit having higher water content may not be saved for longer period being perishable (Miranda *et al.* 2019). In this study, higher ash contents was seen in cv. Nagina, while cv. Roma showed lower ash contents due to Pb^{2+} stress. Our results are mirrored in the findings by Garuba *et al.* (2018), who described that ash content of tomato fruit gives evidence about good mineral contents. It is well documented that greater fiber contents, less vitamin and mineral contents in the diet of infants and children can cause trouble in stomachs (Eromosele and Hamagadu 1993). Lead toxicity reduced the fiber content in the fruit of tomato and this lessens the quality of fruit more in cv. Roma. In this case, more fiber contents were observed in the tolerant cultivar. Furthermore, higher fiber content in the food of adult are also considered better for anti-constipation (Bae 2014). Protein contents are important part of food, which are necessary for humans and animals health. Proteins provides ample amount of amino acids during metabolism (Zhang *et al.* 2017). Our results also revealed marked increase in the accumulation of proteins contents in the tolerant cultivar.

Results of this study highlighted a significant enhancement in fructose and glucose contents in fruits of tomato plants, and this increase was more significant in the sensitive cultivar. Lead stress brought a significant decline in grain yield in rice (Ashraf *et al.* 2017). Results of this study are correlated with Hung *et al.* (2014), who reported

that Pb^{2+} stress caused substantial decline in the yield of okra plants. However, in the present study, we found a considerable decline in fruit biomasses of both cultivars and this decline was superior in the sensitive than tolerant cultivar.

Lead structurally resembles with some nutrients, and therefore, it strongly competes with the uptake of P, K^+, Mg^{2+}, and Ca^{2+} contents (Pourrut *et al.* 2011). Decrease in K^+ content induced by Na^+ is a well-known competitive process exhibited by plants under stress condition (Ashraf *et al.* 2018). In this study, a significant decrease in uptake of K^+ and Ca^{2+} contents and increase of Na^+ content in cv. Roma fruits exposed to Pb^{2+} stress in nutrient solution. These results are correlated with Lamhamdi *et al.* (2013), who reported Pb^{2+}-induced reduction in the uptake of macronutrients (K^+, Ca^{2+}, Mg^{2+} and P) and micronutrients (Fe, Zn, and Cu) in wheat plants. Excess Pb^{2+} activated its accumulation in the tissues of tomato plants. The toxicity of Pb^{2+} stress depends on Pb^{2+} accumulated plant species, which influences the uptake, accumulation, and translocation of Pb^{2+} contents (Akinci *et al.* 2010). Although the Pb^{2+} concentration elevated, the order of Pb^{2+} distribution within the plant are as followed root>shoot>leaf>fruits. Akinci *et al.* (2010) and Dahmani *et al.* (2000) found similar results in tomato and sea thrift, respectively. They stated that differences in Pb^{2+} accumulation in roots, shoots, leaves and fruits showed significant restriction of the interior transport of heavy metals from roots to fruit tissues. Furthermore, Gothberg *et al.* (2004) indicated that maximum Pb^{2+} contents accumulation occurred in the roots, followed by shoots. In agreement with these findings, we found that Pb^{2+} contents accumulated more in the roots, shoots, leaves and fruit tissues, respectively in tomato plants.

Conclusion

Taken together, results indicated a significant reduction in growth attributes, photosynthetic pigments, yield attributes, and calcium ions uptake in tomato cultivars under lead stress, although the damaging effects were more evident in the sensitive cultivar. Moreover, lead stress enhanced oxidative damage in terms of higher malondialdehyde and hydrogen peroxide contents in tomato plants. Nonetheless, lead stress decreased fruit quality, whereas the tolerant tomato cultivar displayed upregulation of *Cat2* gene expression, and thus had less malondialdehyde and hydrogen peroxide contents, and thus showed higher lead stress tolerance than the sensitive one. Further, the tolerant cultivar showed better antioxidant system, lesser degradation of chlorophylls and better growth under lead stress.

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Author Contributions

ZA, IH and MRA planned the research work and IH supervised the whole research work. ZA performed the experiment, done sampling and analyses. MAA, RR, SA, MTJ and MI contributed in data analysis. IH and ZA write up the paper. All authors read and approved the final paper.

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