Original article

Ascorbate glutathione antioxidant system alleviates fly ash stress by modulating growth physiology and biochemical responses in *Solanum lycopersicum*

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ABSTRACT

Tomato plants (*Solanum lycopersicum* L.) were developed in soils with different fly ash (FA) amendments (25, 50, 75, 100% FA) to measure the effects of FA on metal accumulation, chlorophyll pigments, chlorophyll fluorescence, growth, biomass, gas exchange parameters, and the ascorbate glutathione pathway (AsA-GSH). The metal concentration was much higher in FA compared to the garden soil/(control). The observed metal translocation was higher in roots than shoots. Plants raised in soils treated with 50% or more FA showed significant decreases in growth, biomass, gas exchange parameters, protein, chlorophyll pigments, and fluorescence parameters. Additionally, a significant increase in antioxidants under higher FA-amended soils were observed. Our results showed that the ability of *Solanum lycopersicum* plants to effectively synchronize the actions of antioxidant enzymes associated in reactive oxygen species (ROS) scavenging – notably superoxidase dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) – with good maintenance of the AsA/DHA ratio, that could be connected to FA stress tolerance. The toxic metals present in FA caused oxidative stress in *Solanum lycopersicum*, as evident from the increase in electrolyte leakage (EL), lipid peroxidation (MDA), and ROS levels. Furthermore, the AsA-GSH cycle plays a key role in alleviating oxidative damage caused by FA application.

1. Introduction

The Combustion residues released during coal burning in thermal power plants (TPPs) consist of fly ash (FA), bottom ash (BA), flue gas desulfurization products, and boiler slag. FA and BA are collectively called as called pond ash. Globally, FA dumpsites are recognized as major sources of social and environmental problems due to haphazard open disposal and combining wet and dry methods (Ahmad et al., 2021). Owing to the continuous increase in dumpsites, the area covered by FA disposal sites sweeps large amounts of pollutant substances (Pandey et al., 2010) in our environment. The reported pollutants released from FA disposal sites include metals and metalloids, notably Pb, Cd, As, and Cr (Upadhyay et al., 2021). Additionally, large amounts of particulate matter and gaseous pollutants such as SO_2, CO_2, CO, HF, polycyclic aromatic hydrocarbons (PAHs), unburnt hydrocarbons (Pandey, 2015b), and other trace elements (Love et al., 2013) are also released into the atmosphere during coal combustion (Cropper et al., 2012; Qadir et al., 2016b; Qadir et al., 2019). Of all the pollutants existing in FA, metals and metalloids are of serious apprehension due to higher toxicity, contamination of soil and groundwater resources, and their bioaccumulation in plants (Pandey, 2015a, Ahmad et al., 2021).

FA generation in our country is projected to increase from prevailing 300 million tonnes to about 700 million tonnes annually by the end of 2031 (Khan and Baheera, 2013). As such, a holistic approach is needed for FA management. Revegetation, assisted phytoremediation, and bioremediation are eco-friendly techniques

employed for the removal, degradation, and detoxification of environmental contaminants with the intent to prevent the detrimental effects of FA (Gajić et al., 2016; Randelovic et al., 2016). However, factors such as poor water retention, nutrient supply capacity, high alkalinity and salinity, presence of potentially toxic metals and their leachability, are challenges for phytoremediation of FA dumps (Kumari et al., 2013; Pandey, 2015a; Gajić et al., 2016; Pandey et al., 2016, Wang et al., 2020). Therefore, these properties must be addressed before the revegetation of FA dumps to establish proper plant cover and luxuriant plant growth required for phytoremediation (Szalai et al., 2009).

Recently, several plant species with hyper-accumulative potential have been identified for remediation of FA contaminated sites. However, due to decreased growth and biomass in contaminated sites, the phytoremediation potential of many of these plant species is limited (Pandey et al., 2016; Maiti and Pandey, 2021). High metal-accumulating capacity, fast growth with large biomass, tolerance to unfavorable conditions, and rapid and easy propagation are some key features of plants suitable for restoration of FA-contaminated sites (Kumar et al., 2021). In comparison to other contaminated sites, research on the phytoremediation of FA-contaminated sites are limited. Several studies have recommended the use of wild-grown species with environmental and societal importance for *in situ* phytoremediation of contaminated sites. These plant species help in controlling atmospheric contamination and prevention of toxic elements from leaching into groundwater, there by creating a bio-aesthetically pleasing location for native dwellers by modifying the local climate (Pandey et al., 2009, Upadhyay et al., 2021).

The presence of essential nutrients in FA is playing a favorable role in the growth progression and plant health development, which makes FA a better source with enormous potential applications in agronomic sector of India. However, plant species growing on FA dumpsites are also exposed to toxic metals and metalloids in abundance (Pb, Cd, Zn, Cr, Mn, As, and Co). These toxic metals and metalloids hamper physiological, photosynthetic, and gas exchange processes, alter the structural and functional integrity of chloroplasts (Gajic et al., 2013; Kumar et al., 2021), with the genesis of reactive oxygen radicals commonly called (ROS) (Szalai et al., 2009; Raja et al., 2017; Qadir et al., 2019; Ahmad et al. 2010, 2019), which when produced under stressful environmental conditions causes oxidative stress in different plant tissues. Chlorophyll fluorescence is the most appropriate and explanatory way to assess the effects of environmental stressors on photosynthesis (Panda et al., 2018). However, the photosynthetic and biochemical responses of *Solanum lycopersicum* to FA-amended soils remain poorly understood. In plants, a sophisticated but intricate stability exists between generation and foraging of ROS species. Metal contamination affects this study state balance by altering the antioxidative system (Yan et al., 2016). Evidence of FA-induced oxidative damage in plant species is scarce, especially for vegetables such as tomatoes. To our knowledge, the effect of FA-amended soils on antioxidant levels and photosynthetic activity in tomatoes has not been reported. The role of metals and metalloids from FA-amended soils in inducing oxidative stress has been studied under laboratory and field conditions in various plants (Love et al., 2013; Ribeiro et al., 2014; Pandey et al., 2016; Qadir et al., 2019).

The current study was undertaken to examine the connection between metals and oxidative stress in *Solanum lycopersicum* L. grown in FA-amended soils and the role of antioxidants in alleviating oxidative damage. The outcomes of this evaluation will provide details for hazard identification concerning toxic metals and metalloids (Love et al., 2013; Nayak et al., 2015; Qadir et al., 2016b). The main aim of the current investigation is to measure the impacts of FA on growth features, photosynthesis, PSII activity, stress markers, concentrations of selected metals in plant leaves, the role of different antioxidants in stress tolerance, and relative changes in variables of *Solanum lycopersicum* L. Taking into account, the evaluation of the phytotoxic effects of FA amendments is essential for phytoremediation and restoration of FA areas.

2. Materials and methods

2.1. Assortment and characterization of soil and FA

Soil and FA materials were collected from two arbitrarily selected sites. FA was taken from a dumping yard of BTTP in New Delhi and the Garden soil (GS) was obtained from the backyard of the Jamia Millia Islamia (JMI) Engineering Campus, New Delhi. The collected samples were properly air-dried, crushed to powder, and sweeped with a sieve of small pore size (2 mm). Soil and FA samples (10xg each) were kept in properly marked plastic containers with Kraft papers. Before analysis, the marked samples were comprehensively assorted. The materials were then analyzed regarding Organic matter (OM), pH, and cation exchange capacity (CEC). The sample pH was determined with the help of Decibel pH meter by suitably attenuating FA and soil material in 1:5 ratio with the help of distilled water. For evaluating OM Walkley and Black method was used (Jackson, 1958) and CEC was estimated by employing the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986).

2.2. Sample preparation for elemental analysis

Dried samples of soil, FA, and leaves (1 g each) were processed using 40 mL mixture of three different acids (HNO_3:$HClO_4$: H_2SO_4) in 5:1:1 ratio. The samples were left undisturbed overnight, later on these tubes were placed in a digestion block in properly ventilated fume hood and slowly heated from 80 °C to 160 °C by gradually increasing the temperature until the extracts appeared clear (Nawab et al., 2015; Qadir et al., 2016a). The digested materials were then used for determination of metal contents including Zn, Cu, Cr, and Cd with the use of atomic absorption spectrometer (2380 Model PerkinElmer) at the Indian Institute of Technology (IIT) Delhi. All digestions were performed in replicates of five.

2.3. Plant material and growth parameters

The seeds of *Solanum lycopersicum* L. were acquired from the Indian Agricultural Research Institute (IARI) New Delhi. These seeds were first soaked in ethanol (70%) for 1 min, washed with deionized water, sterilized for 15 min with 6% hydrogen peroxide, washed again, and soaked overnight in water containing glass jars. The surface sterilized and soaked seeds were then sowed in five earthen pots (ten per pot) containing GS (negative control) and 100% pure FA in three replicates. These pots were placed in open field conditions with 16 h of light and 8 h of darkness from March to May. The mean temperature fluctuates from a minimum of 15 °C to a maximum of 29 °C, with a relative humidity between 64 and 78%. Total rainfall of 62 mm was reported during the present study period. The pots were watered at regular intervals with 1 L of tap water. The leachate gathered in pot plates below was reverted to the corresponding container. The plant leaves were collected after three months (on the 90th day) and were assessed for FA tempted metal toxicity and oxidative stress. Instantaneously, by using atomic absorption spectrophotometer (AAS), the heavy metal analysis and uptake were also evaluated.

From naturally grown *Solanum lycopersicum* L, plants with identical age, size, and height were marked from three replicates. The branches completely exposed to solar light were used for the

collection of leaf samples. The leaves of equal size, maturity, and free from diseases and injuries were chosen carefully for evaluation of different parameters. These leaf samples were collected randomly in two intervals from different plants. The various morphological, biochemical and physiological characteristics were assessed from the first batch of leaves. The leaf samples after collection were instantly frozen in liquid nitrogen for biochemical and enzyme assays. The second batch of leaves was kept at 4 °C after collection in marked plastic bags for heavy metal detection, lipid peroxidation (MDA), electrolyte leakage (EL), and concentration of photosynthetic pigment.

2.4. Metal tolerance index (MTI) and plant growth parameters

Growth-related parameters, such as fresh and dry weight of shoots (SFW & SDW) and roots (RFW & RDW) were investigated from all replicates after three months (90 days) of growth. Total plant biomass (TPBM) was measured after dehydrating the plant material at 80 °C till a constant weight. Metal tolerance index (MTI) was premeditated by using the formula of (Panda et al., 2018),

$$MTI = (Plant\ biomass\ under\ different\ FA\ treatments/Plants\ biomass\ under\ (GS)/Control) \times 100$$

2.5. Determination of morphological parameters

Leaf plant morphological parameters, such as length of plants, shoots, roots (cm), and leaf area (cm^2) were dignified by fixing the marked leaves in portable leaf area meter (SYSTRONICS) and the respective values of the parameters were recorded.

2.6. Determination of physiological parameters

Physiological parameters, such as photosynthetic rate (P_n), transpiration rate (E-), and stomatal conductance (gs) were measured using an open system photosynthetic gas analyzer (CI-304, CID, USA) under normal ecological conditions at 8–12 ha. Three healthy plants with fully expanded leaves under natural environmental circumstances were picked and the second leaf from the upper plant portion was placed inside the chamber till a constant value was attained. All dimensions were measured under temperature conditions 34 ± 4 °C, 78% relative humidity, 1028 ± 34 μmol active radiation, 370 μmol CO_2 m^{-2}s^{-1}, and 23% O_2.

2.7. Chlorophyll Pigments, chlorophyll Fluorescence, and soil plant analysis development index

The freshly prepared leaf samples were examined with the help of standard colorimetric methods using UV-spectrophotometer (PerkinElmer) and Spectrum (SP-UV 500 DB). The photosynthetic chlorophyll pigments Chlorophyll a, (Chl a), Chlorophyll b, (Chl b), Total Chlorophyll (TChl), and Carotenoids (Car) were determined by the procedure described by Hiscox and Israelstam (1979).

Similar leaves were used for quantifying of Chlorophyll fluorescence, gas exchange parameters with the help of portable Chlorophyll Fluorometer (PAM 2500; Germany). The initial (F_o), maximal (F_m), variable ($F_v = F_m$-F_o) fluorescence parameters, and photochemical efficiency of PS II (F_v/F_m) were dignified in leaves adapted for 20 min under conditions of darkness (Maxwell and Johnson, 2000). Steady-state (Fs) and maximal fluorescence (F''_m) was dignified after a 3 s white light pulse in a state of light-adaptation (for 20 min) at 1200 μmol m^{-2} s^{-1}. The minimal fluorescence (F''_o) was assessed after the exclusion of the actinic light source. Further, the Yield of PS II Photochemistry (Y II) = $(F'_m - F_s)/F''_m$, quenching value due to non-photochemical degeneracy of captivated light energy (NPQ) = $(F_m - F'_m)/F_m$, and the quenching coefficient (qP) = $(F'_m - F_s)/(F'_m - F'_o)$ were evaluated using the method of Shrestha et al. (2012). chlorophyll index was determined from healthy and fully stretched leaves of *Solanum lycopersicum* L from each replicate using a soil plant analysis development (SPAD) 502 chlorophyll meter (KONICA, JAPAN).

2.8. Determination of ROS and MDA

H_2O_2 concentration in freshly prepared plant leaves were evaluated following the procedure of Zhang et al. (2016). Firstly 0.5 g leaf material was centrifuged at 4 °C for 20 min at 1,000 × g after its homogenization in 3 mL of 50 mM potassium phosphate buffer solution of pH 6.5. Next, 3 mL supernatant material was pooled with 1 mL of 20% H_2SO_4 to which 0.1% of $TiCl_4$ was mixed. The absorbance value of a supernatant material was recorded at 410 nm and the concentration of H_2O_2 was measured from standard curve.

The scheme of Zhang et al. (2016) was used for calculation of superoxide radical ($O_2^{\bullet-}$) content. 2 g freshly prepared leaf samples centrifuged for 20–25 min at 10,000 × g, after its homogenization in 3 mL of 3% TCA. The reaction mixture consisting of 1 mL supernatant, 1 mL of 50 mM buffer solution of pH 7.0, and 1 mM hydroxylamine hydrochloride. The absorbance of supernatant material was recorded at 530 nm, and the $O_2^{\bullet-}$ content was measured with the help of calibrated curve.

The MDA indicates lipid peroxidation in plant tissues and was determined using a thiobarbituric acid reaction (TBAR) process (Heath and Packer, 1968). Homogenized leaf material (0.5 g) in 5 mL volume of 0.1% TCA and centrifuged at 10,000 × g for 10 min. for every 1 mL of the aliquot, 4 mL of 20% TCA containing 0.5% TBA were added, after swiftly cooling in an ice bath, the mixture was again centrifuged at 10,000 × g for 15 min. The supernatant material absorbance value was noted at 532 nm and 600 nm. The non-specific absorbance at 600 nm was deducted from the recorded absorbance at 532 nm. The obtained value with an extinction coefficient of 155 mM^{-1}cm^{-1} was used for the determination of MDA.

The method of Nayyar (2003) was employed for evaluating Electrolytic Leakage (EL). leaf discs prepared from five young and freshly obtained plant leaves from different treatments. Vials containing leaf discs in 10 mL distilled water were gestated at 25 °C for 24 h, and the initial electrical conductivity (EC1) was noted. The sample mixture was then autoclaved at 120 °C for 20 min for recording final electrical conductivity (EC2) at 25 °C and the EL was determined with the aid of equation.

$$EL(\%) = \frac{EC1}{EC2} \times 100 \qquad (2)$$

2.9. Analysis of protein and antioxidant enzyme assay

The same leaf tissues was used to measure photosynthesis and chlorophyll fluorescence (0.5 g) leaf material were homogenized with the help of 10 mL of 50 mM buffer solution (pH 7.8) previously labeled (Qadir et al., 2019) were used for the estimation of activity of antioxidant enzymes. The sample protein was determined by the method of Bradford (1976). Superoxidase dismutase (SOD) was assessed from 100 mM EDTA phosphate buffer solution (pH 7.8), by homogenizing fresh leaves, centrifuged for 15 min at 10,000 × g. SOD in the supernatant was resoluted following (Beauchamp and Fridovich, 1971) in 50 mM sodium–potassium phosphate buffer (pH 7.8), 20 μl enzyme extract, 2 μM riboflavin, 13 mM methionine, 75 μM NBT, and 100 mM EDTA. The mixture absorbance value was noted at 560 nm for 2 min.

APX enzyme activity was premeditated as per the method of Nakano and Asada (1981). A 3 mL reaction mixture solution was prepared from (0.1 mM EDTA-Na2, 100 mM phosphate buffer of pH (7.0), 1.0 mM H_2O_2, 0.3 mM ascorbic acid, and 100 µl enzyme extract) was thoroughly mixed. To carry out this reaction for 3 min hydrogen peroxide (H_2O_2) was added at a gap of 6 sec at 25 °C afterwards the absorbance value was read from 30 to 60 s at 290 nm.

The process of Hossain et al. (1984) was used for examination of Monodehydroascorbate reductase (MDHAR) and for dehydroascorbate reductase (DHAR) the procedure of Dalton et al. (1986), and the scheme of Rao (1992) was used for the evaluation of glutathione reductase (GR) activity and was evaluated by the oxidation rate of NADPH as dignified by a 1 min variation in absorbance at 340 nm.

2.10. Determination of Non-enzymatic antioxidants

The leaf tissue AsA content was calculated as per Zhang et al. (2016). The technique of Bates et al. (1973) was used for proline analysis in which, 0.5 g fresh leaves homogenized in 3% sulfosalicylic acid, the supernatant solution was mixed with an equal volume of glacial acetic acid and acidic ninhydrin. The mixture was heated at 100 °C for half an hour, to which 5 mL toluene was added further. The supernatant absorbance value was taken at 520 nm using a UV–vis spectrometer (Schmdzu Japan, 1800).

For determining the total glutathione (tGSH) pool the enzyme salvaging process put forth by Hossain et al. (2009) was employed. At 4 °C, 0.5 g fresh leaf material was homogenized by using 2 mL of 5% sulphosalicylic acid. The slurry was centrifuged at 10,000×g for 10 min, 0.5 mL supernatant was transferred to a pre-sterilized eppendorf tube to which 0.6 mL buffer and 40 µl of DTBN were added, after waiting for 2 min the absorbance value was read at 412 nm for calculating the reduced glutathione (GSH) concentration, this was followed by the addition of 50 µl of NADPH and 2 µl of GR for determining tGSH in the mixture solution. The oxidized (GSSG) glutathione was calculated by subtracting GSH from tGSH. This mixture was again made to settle for another half an hour at a normal temperature of 25 °C and the observed absorbance variation was noted at 412 nm.

2.11. AsA and GSH levels

The technique of Hossain et al. (2009) was employed for evaluating the AsA and GSH levels. The reduction of Fe^{3+} to Fe^{2+} occurring in an acidic solution by ascorbate and the reduced Fe^{2+} on reaction with a bipyridyl complex leads to the formation of a red-colored chelate that absorbs mostly at 525 nm. The observed difference between AsA and total AsA (tAsA) determines the amount of DHA in the solution. For calculating the total tGSH and GSSG levels the reductase salvaging technique was used.

2.12. Statistical analysis

The observed statistical differences among various plant attributes were compared by one-way analysis of variance (ANOVA) using SPSS 16.0. The least significant differences (LSD) were calculated using the Duncan's Multiple Range Test (DMRT) as post hoc. All differences were tested at $p \leq 0.05$ level of confidence for determining the statistical significance.

3. Results

The garden soil (GS) from JMI and FA samples collected from the Badarpur thermal power plant dumping site was slightly alkaline.

The FA CEC ($t = -4.027$, $p < 0.05$), OM content ($t = -14.882$, $P < 0.05$) were significantly higher than those of GS (Table 1).

The metal analyses of GS, FA, and FA-amended soils are depicted in Table 2. On analysis least metal clustering of Zn, Cu, Cr, and Cd were reported from control soil group (GS). However, the concentrations of these elements increase with increase in FA contents, thus were found in higher concentration in 100% FA. Accordingly, the concentrations of elements increased with higher FA content. The concentration of Cd was higher in GS (26.62 ± 0.25 µg g⁻¹ dw) compared to normal/permissible level of Cd in soil (0.1 µg g⁻¹ dw) and shale (0.3 µg g⁻¹ dw). The elemental clustering of Zn, Cu, and Cr were found to be within the soil permissible limits. The elemental concentration in different plant parts of tomato is displayed in Fig. 1(a-h).

The concentration of metals were found in increasing amounts in below (roots) and above-ground plant parts (shoots) with increase in the amount of FA. The concentration of all four metals were found to be higher in the roots compared to shoots. The order of element concentrations of metals in both plant parts was found to be Zn > Cu > Cr > Cd. The concentration of Zn increases by 4.75%, 15.86%, 35.64%, and 60.57%, and the concentration of Cu in the roots increased by 7.93%, 24.18%, 36.74%, and 48.11% respectively in 25%, 50%, 75% FA amended soils and 100% FA, while the concentration of Cr shows no variation under 25% FA amended soils but increased by 105.8%, 133.8%, and 179.8% respectively when supplemented with 50%, 75% FA and 100% FA alone, similarly Cd concentration increased by 10.73%, 303.38%, 350.8% and 361.01% from 25% FA amended soils to 100% FA alone respectively.

Likewise, in the shoots, the concentration of Zn increased from 33.52% in 25% FA amended soils to 47.33% and 139.28% in 50% and 75% FA amended soils, Cu increased from 21.61% to 71.61% while a maximum increase of 132.89% was observed in 75% FA supplemented soils, Cr increased from a minimum of 23.78% in 25% FA amended soils to a maximum of 323.24% reported in 100 %FA, similarly Cd fluctuated from a minimum of 28.12% in 25% FA amended soils to a maximum of 189.58% in 100% FA respectively. In the roots and shoots, higher concentrations of these elements were found in plants growing in FA in comparison to those growing in GS and other FA-amended substrates. Plants raised in 75% and 100% FA amended soils were found to concentrate metals in significantly ($p < 0.05$) higher amounts than in GS (Control). The main findings were that with augmentation in the concentration of FA in the substrate, the elemental concentration of all the four metals increased in below-ground parts (roots) and successively higher translocation was detected in aerial parts (shoots) of plants.

3.1. Effect of FA on Growth, Biomass, and metal tolerance index

The effects of FA amendments on growth-related parameters are given in Table 3. The root length, shoot length, total plant length, and total leaf area declined with increasing amounts of FA concerning the GS (control).

While examining the impacts of FA on the biomass of plant species, the biomass significantly increased in plant species ($P < 0.05$) when grown in 25% FA-treated soils in relation control soil group (Table 4). The observed increases in SFW, SDW, RFW, RDW, and

Table 1
Site-specific soil physicochemical properties.

Physicochemical properties	100% GS	100% FA
pH	7.76 ± 0.21	7.28 ± 0.19*
CEC (meq/100 mg soil)	4.47 ± 0.04	5.45 ± 0.54*
OM (%)	0.81 ± 0.08	3.89 ± 0.46*

$n = 10$ in each case; *$p < 0.05$ (Student's t test).

Table 2
Elemental Concentration ($\mu g \ g^{-1}$ dw) of studied heavy metals in FA and FA-treated soils.

Treatment	Zn	Cu	Cr	Cd
100% GS	7.56 ± 1.85[a]	26.19 ± 0.21[a]	12.78 ± 0.12[a]	26.62 ± 0.25[a]
FA25% + GS75%	86.93 ± 0.61[b]	31.07 ± 0.68[b]	16.73 ± 0.13[b]	28.86 ± 0.05[b]
FA50% + GS50%	91.05 ± 0.74[c]	32.52 ± 0.05[c]	22.96 ± 0.33[c]	31.61 ± 0.05[c]
FA75% + GS25%	95.02 ± 1.84[d]	41.05 ± 0.69[d]	31.61 ± 0.57[d]	34.81 ± 0.08[d]
FA 100%	123.29 ± 0.46[e]	52.97 ± 0.61[e]	35.20 ± 0.51[e]	37.91 ± 0.07[e]

Data represent the mean ± SD of five different replications. Different letters denote statistical significance at *P < 0.05*.

TPBM in 25% FA plants were 8.7%, 4.13%, 1.72%, 11.38%, and 17.48%, respectively, equated to plants raised in GS (Table 4). Higher concentrations of FA application drastically declined plant biomass when equated to the control group (Table 4). Major declines in biomass were reported in 75% and 100% FA treated soils; SFW decreased by 16.5% and 67.76%, SDW decreased by 15.60% and 68.34%, RFW decreased by 22.85% and 58.97%, RDW decreased by 16.26% and 30.89%, TPBM decreased by 42.64% and 76.97%, respectively.

A significant but positive association were observed between growth parameters and plant biomass. It was observed that root length with SFW ($r = 0.786$, $P < 0.01$), SDW ($r = 0.792$, $P < 0.01$), RFW ($r = 0.809$, $P < 0.01$), RDW ($r = 0.770$, $P < 0.01$) and TPBM ($r = 0.781$, $P < 0.01$); shoot length with SFW ($r = 0.838$, $P < 0.01$), SDW ($r = 0.856$, $P < 0.01$), RFW ($r = 0.864$, $P < 0.01$), RDW ($r = 0.912$, $P < 0.01$) and TPBM ($r = 0.934$, $P < 0.01$); plant length with SFW ($r = 0.702$, $P < 0.01$), SDW ($r = 0.706$, $P < 0.01$), RFW ($r = 0.759$, $P < 0.01$), RDW ($r = 0.810$, $P < 0.01$) and TPBM ($r = 0.794$, $P < 0.01$); total leaf area with SFW ($r = 0.678$, $P < 0.01$), SDW ($r = 0.704$, $P < 0.01$), RFW ($r = 0.737$, $P < 0.01$), RDW ($r = 0.760$, $P < 0.01$) and TPBM ($r = 0.765$, $P < 0.01$). The same trend was reported in MTI, which increased in the 25% FA treatment but decreased with higher levels of FA. MTI decreased by 26.79% and 42.85% in the 75% and 100% FA treatment, separately, in comparison to the control.

3.2. FA effect on photosynthetic and chlorophyll fluorescence parameters

The observed increase in photosynthetic pigments were noticed in soils amended with low-levels (25%) FA in 90-day-old plant leaves. The Chl. *a* was found highly concentrated than Chl. *b* and Carotenoids. The pigments like Chl. *a,* Chl. *b*, and T.Chl. were found to be present in significantly lower amounts ($P < 0.05$) in plants grown in soils with higher amounts of FA. Chl. *a* was reduced by 12.96% and 20.37% in 75% FA and 100% FA, respectively, compared to the control (Fig. 2a). Chl. *b* reduced by 9.63% in 75% FA-amended soil and 18.67% in 100% FA compared to the control (Fig. 2b). A notable reduction of 12.04% and 19.37% was observed in T.Chl pigment levels of plants grown in 75% and 100% FA soils, respectively, in comparison to 100% GS (Fig. 2c). A consistent increase in carotenoids from lower to higher levels of FA-amended soils was observed. Carotenoid levels increased by 16.07% and 35.74% in plants grown in 75% and 100% FA soils, respectively, in comparison to plants grown in 100% GS (Fig. 2d). The observed variation was found to be statistically significant (Chl. *a*, $F = 79.773$, $p < 0.01$; Chl. *b*, $F = 115.296$, $p < 0.01$; carotenoids, $F = 49.862$, $p < 0.01$; TChl., $F = 128.377$, $p < 0.01$).

3.3. Effects of FA on photosynthetic Rate, transpiration Rate, gas exchange Parameters, and SPAD index

In low-level FA treatment (25%) a slight increase in photosynthetic rate (*Pn*), transpiration rate (*E*), and SPAD chl. index (Fig. 2 **e-i)** was observed; however, the changes observed in FA treated

plants were found to be significant ($P < 0.05$) with respect to GS. Significant reductions in these parameters were observed with increasing concentrations of FA. The reduction in photosynthetic rate (*Pn*) was 22.75%, 37.80%, and 54.09% in plants grown in 50%, 75%, and 100% FA, respectively (Fig. 2e). Similarly, transpiration rate (*E*) decreased by 43.73% in 75% FA and 49.27% in 100% FA compared to 100% GS (Fig. 2f). Stomatal conductance (*gs*) decreased by 41.95% in 75% FA and 50.42% in 100% FA (Fig. 2g) compared to 100% GS. Intercellular CO_2 (*A*) and SPAD Chl. index decreased by 43.36% and 23.01%, respectively, in 100% FA in comparison to 100% GS (Fig. 2h &i). From the ANOVA results, the observed decrease in these parameters at higher FA concentrations were significant (*Pn*, $F = 14862.005$, $P < 0.01$; *E*, $F = 6292.184$, $P < 0.01$; *gs*, $F = 1012.157$, $P < 0.01$; intercellular CO_2, $F = 1586.469$, $P < 0.01$; SPAD Chl. index, $F = 815.128$, $P < 0.01$).

To study PS II activity in 90-day-old tomato leaves under different FA-amended soil conditions, Chlorophyll fluorescence parameters, such as F_o, F_m, F_v, F_v/F_m, Y (II), qP, and NPQ were measured. Low levels of FA (25%) did not cause any significant alteration in the PS II activity. The higher concentrations of FA-amended soils (50%, 75%, and 100%) obstructed activity of PS II, as evident from a substantial drop in the attributes of F_m, F_v, F_v/F_m, Y (II), qP and an increase in F_o (up to 50% FA amended soil) and NPQ (Table 5). The variations observed in these chlorophyll fluorescence parameters were statistically significant as revealed by ANOVA (F_o, $F = 124.992$; $P < 0.01$; F_m, $F = 73564.247$, $P < 0.01$; F_v, $F = 31724.758$, $P < 0.01$; F_v/F_m, $F = 1101.818$, $P < 0.01$; Y (II), $F = 97.121$, $P < 0.01$; qP, $F = 65.051$, $P < 0.01$; NPQ, $F = 85.986$, $P < 0.01$).

To examine relationship between various photosynthetic and gas exchange parameters and total plant biomass, with different antioxidative enzymes, protein, proline, EL, and MDA levels in *Solanum lycopersicum* grown in FA-amended soils, Pearson's coefficient correlation were calculated. Statistically significant ($P < 0.05$) but negative correlations were observed between these parameters, while positive correlations were observed between various antioxidants and NPQ, except MDHAR, however, MDHAR and NPQ were found to be negative associated ($r = -0.861$, $P < 0.01$) in addition to this, protein content was observed to establish a positive correlation with gas exchange, biomass, and photosynthetic parameters (Table 6).

3.4. Effects of FA on ROS, Electrolytic Leakage, and lipid peroxidation

Superoxide radical ($O_2^{\bullet -}$) content improved with amplified levels of FA in the substrate. In comparison to GS the $O_2^{\bullet -}$ content showed an increase of 25.44%, 31.36%, and 38.46% respectively (Fig. 3a). Similarly, in comparison to the control (100% GS), H_2O_2 content was 41.93%, 48.38%, and 54.84% higher in 50%, 75%, and 100% FA, respectively (Fig. 3b).

Membrane permeability, as represented by EL, increased from lower to higher levels of FA-amended soils. The observed proliferation in EL was 13.89%, 21.68%, 30.95% and 39.88% in 25%, 50%, 75% and 100% FA amended soils in comparison to 100% GS respectively
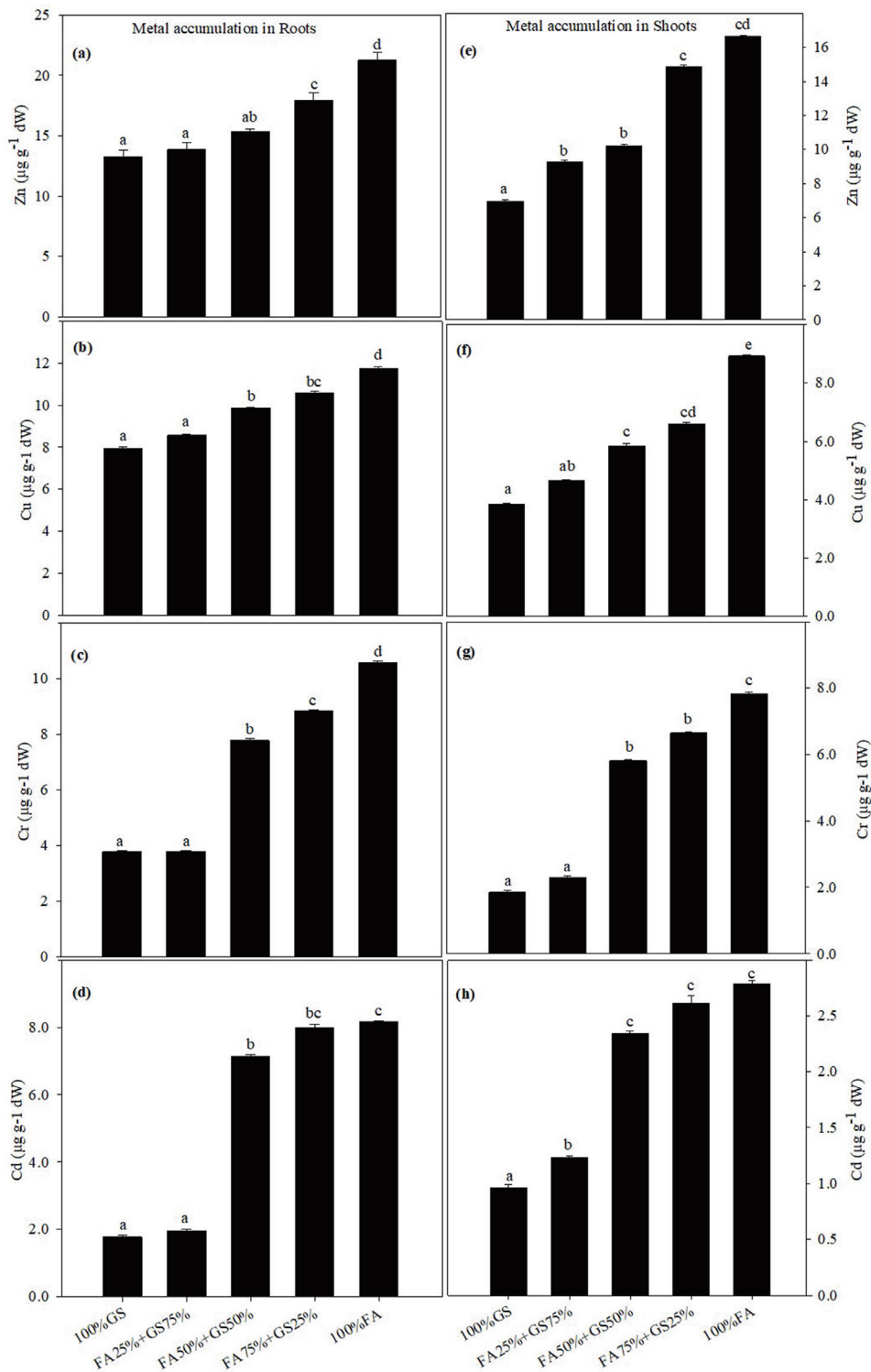
Fig. 1. Effect of different (FA) amendments on the accumulation of (A) Zn (μg g^{-1} DW), (B) Cu (μg g^{-1} DW), (C) Cr (μg g^{-1} DW) and (D) Cd (μg g^{-1} DW), in roots and shoots. Data represent the mean ± SD (n = 25 in each case). letters are statistically significant at *P < 0.05*.

(Fig. 3c). The MDA content in 90 days old plant leaves of tomato increased with higher concentrations of FA in the soils. In comparison to control the MDA content increased by 4.73%, 5.68% and 10.42% in 25%, 50% and 75% FA amended soils respectively. however, a maximum increase of 17.93% was observed in 100% FA compared to control (Fig. 3d).

3.5. Effects of FA on antioxidant Enzyme, Proline, and protein activity

APX activity increased with higher doses of FA amendment, the APX activity increased from 3.29 ± 0.03 U mg-^{1} protein to its highest value of 4.72 ± 0.06 U mg-^{1} protein and 5.21 ± 0.08 U mg-^{1} protein, reported at 75% and 100% FA, individually. The observed

Table 3

Effect of different FA amendments on growth parameters of tomato after 90 days of a time interval.

Treatment	Root Length (cm)	Shoot Length (cm)	Plant Length (cm)	Total leaf area (cm^2)
100 %GS	19.29 ± 0.53[d]	41.15 ± 0.92[e]	46.43 ± 0.50[c]	64.34 ± 0.26[e]
FA25%+GS75%	17.64 ± 0.55[c]	39.57 ± 0.71[d]	44.18 ± 0.57[b]	51.91 ± 0.26[d]
FA50%+GS50%	17.34 ± 0.49[c]	36.82 ± 0.12[c]	43.49 ± 0.50[b]	49.79 ± 0.12[c]
FA75%+GS25%	16.75 ± 0.19[b]	35.56 ± 0.38[b]	41.47 ± 1.10[a]	45.36 ± 0.47[b]
100 %FA	15.36 ± 0.41[a]	32.46 ± 0.33[a]	40.89 ± 0.48[a]	40.84 ± 0.11[a]

Data represented as mean ± SD of 5 readings. Letters are statistically significant at *P < 0.05*.

Table 4

Plant biomass parameters and Metal Tolerance Index (MTI) of Tomato under different concentrations of FA amended soils.

Treatment	SFW (g plant^{-1})	SDW (g plant^{-1})	RFW (g plant^{-1})	RDW (g plant^{-1})	TPBM (%)	MTI
100 %GS	8.84 ± 0.04[c]	2.18 ± 0.02[d]	4.07 ± 0.07[c]	2.46 ± 0.04[a]	4.69 ± 0.03[d]	1.12 ± 0.06[e]
FA25%+GS75%	9.61 ± 0.02[e]	2.27 ± 0.02[e]	4.14 ± 0.03[d]	2.74 ± 0.06[a]	5.51 ± 0.11[e]	1.22 ± 0.02[d]
FA50%+GS50%	9.16 ± 0.02[d]	2.12 ± 0.02[c]	4.10 ± 0.01 [cd]	2.59 ± 1.63[a]	3.24 ± 0.03[c]	0.92 ± 0.03[c]
FA75%+GS25%	7.24 ± 0.14[b]	1.84 ± 0.02[b]	3.14 ± 0.01[b]	2.06 ± 0.05[a]	2.69 ± 0.03[b]	0.82 ± 0.02[b]
100 %FA	2.85 ± 0.04[a]	0.69 ± 0.02[a]	1.67 ± 0.04[a]	1.70 ± 0.06[a]	1.08 ± 0.02[a]	0.64 ± 0.03[a]

Data represents as mean ± SD value of five replicates. Letters denote statistical significance at *P < 0.05*.

increase in APX activity was 43.46% and 58.35%, respectively, in 75% and 100% FA in comparison to the control (Fig. 3**e**). Similarly, the variation observed in DHAR activity in comparison to the control was 8.39%, 15.38%, 23.07% and 67.82%, respectively in 25%, 50%, 75% and 100% FA. However, a decrease of 18.35%, 38.47%, 51.36% and 58.20% was observed in MDHAR activity with increases in FA concentration in comparison to control in 25%, 50%,75% and 100% FA (Fig. 3**g**). The ANOVA revealed that the observed variations in antioxidant activities were statistically significant between treatments (SOD, F = 13.675, *P < 0.01*; APX, *F* = 905.551, *P < 0.01*; DHAR, *F* = 349.384, *P < 0.01*; MDHAR, *F* = 7.541, *P < 0.01*; GR, *F* = 17.744, *P < 0.01*).

In comparison to GS a significant increase in SOD activity was observed in all FA treatments. The SOD activity increased by 3.65%, 16.21%, 17.32% and 17.96% respectively in 25%, 50%, 75% and 100% FA (Fig. 3**h**). Similarly, GR activity fluctuated from 17.41% to 28.34% in 25% and 50 %FA amended soils, however, the greatest increase of 33% and 40.17% was reported from 75% FA amended soils and 100 %FA (Fig. 3**i**).

Nevertheless, significant decreases in the protein content of the leaves occurred with higher FA supplements. The protein content in the control was 22.51 ± 0.19 mg g^{-1} FW, 14.86 ± 0.17 mg g^{-1} FW in 75% FA, and 14.43 ± 0.45 mg g^{-1} FW in 100% FA, representing 57.22% and 35.89% decreases, respectively (Fig. 4**a**). Proline, an important osmoprotectant, showed an elevation from the control to higher doses of FA, the proline content increased by 143.12% and 201.25%, respectively, in 75% and 100% FA (Fig. 4**b**).

3.6. AsA and GSH pool

Under higher doses of FA stress conditions, the concentration of tAsA and AsA increased significantly in comparison to GS. The maximum increase of 9.96% in tAsA and 6.85% in AsA were observed in 75% FA-amended soil. Similarly, DHA followed a decreasing trend from lower to higher levels of FA. The observed variation reported in DHA was 4.76%, 28.57%, 23.80%, and 119.04%. Similarly, the variation observed in AsA/DHA ratio was 6.86%, 44.98%,37.80% and 49.74% in 25%, 50%, 75% and 100% FA amended soils in comparison to control (GS) (Fig. 5 **a-d**).

An increase in tGSH was observed in plants grown in higher FA concentrations. The tGSH, which was 42.58 ± 0.22 nmol g^{-1} FW in 100% GS, was 25.19%, 32.99%, and 47.79% higher in 50%, 75%, and 100% FA. The GSH also increased with increased FA in the soil.

The lowest value of GSH was reported in 100% GS, which increased to 30.18% and 44.35% in 75% and 100% FA-amended soil, separately. The GSSG value was 50.33% and 69.02% higher in 75% and 100% FA treatments, than control. While observing the result of different FA amendments on the ratio of GSH/GSSG, a declining trend was observed from lower to higher levels of FA. The GSH/GSSG ratio represents a decline of 11.83% and 14.42%, respectively, in the two highest levels (75% and 100%) of FA (Fig. 5 **e-h**). The observed variation in AsA and GSH was statistically significant as per the ANOVA result (tAsA, F = 376.088, *P < 0.01*; AsA, *F* = 16.756, *P < 0.01*; DHA, *F* = 6.834, *P < 0.01*; AsA/DHA, *F* = 4.914, *P < 0.05*; tGSH, *F* = 1399.325, *P < 0.01*; GSH, *F* = 308.373, *P < 0.01*; GSSG, *F* = 26.846, *P < 0.01*; GSH/GSSG, *F* = 2.643, *P < 0.05*).

4. Discussion

4.1. Physicochemical analysis

The FA collected from the Badarpur thermal power plant (BTTP) dumping site and garden soil from engineering campus JMI was moderately alkaline (Table 1). The FA alkalinity may be credited to presence of sulfur in smaller quantities and excessive hydroxides and carbonates of calcium and magnesium in coals of the Indian subcontinent (Love et al., 2013). The high level of OM in FA can be explained by the rhizospheric quality of the FA. The presence of plant cover over FA dumpsites adds to the propagation of (OM) content through the litter input, putrefaction, and mineralization of OM coupled with enzymatic activities of microbial fauna add to the buildup of nutrient cycling pool. Thus, the nutritional and biological properties of FA can be improved portentously depending on vegetation cover and efficacy over time (Pandey and Singh, 2012). Further, the slow rate of putrefaction and mineralization leads to the accumulation of OM in FA, and is mostly present as unburnt carbon (Pavlović et al., 2007; Mitrović et al., 2012; Gajić et al., 2016). This building up of OM is responsible for the high CEC values of soils (Técher et al., 2012; Love et al., 2013).

4.2. Heavy metals in GS and FA-Amended soils

During coal combustion in TPPs, metals like Mn, Zn, Ni, and Pb are released into the environment due to the destruction of OM during processes of coal combustion. Further, processes and
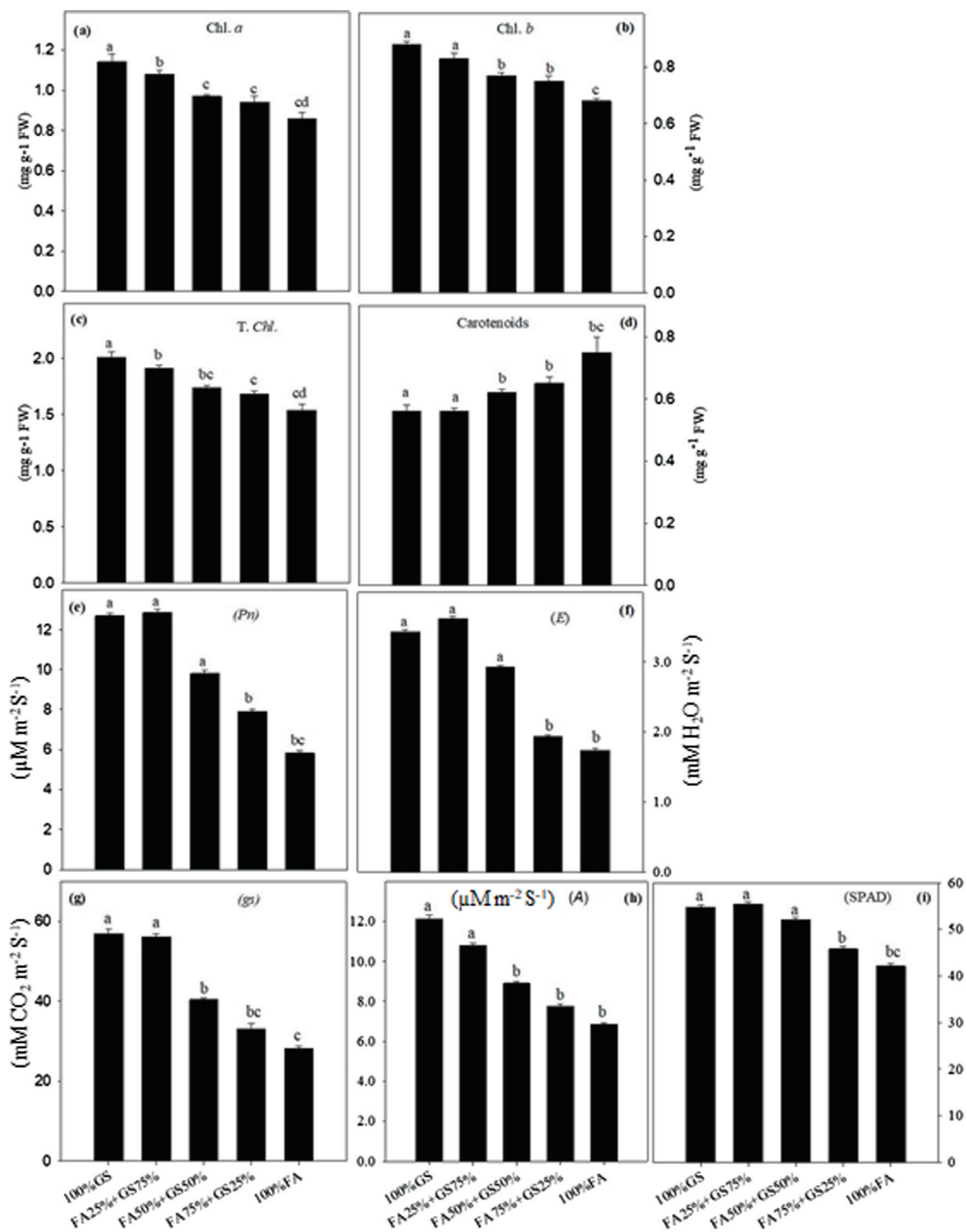
Fig. 2. Effect of different FA amended soils on (A) Chl. *a* (B) Chl. *b*, (C) total Chl. and (D) carotenoids (E) photosynthetic rate *(Pn)*, (F) transpiration rate *(E)*, (G) stomatal conductance *(gs)*, (H) CO_2 assimilation rate *(A)*, and (I) SPAD index in tomato leaves. Data represent the mean ± SD (n = 25 in each case). Different letters denote statistical significance at *P < 0.05*.

Table 5
Changes in chlorophyll fluorescence parameters in tomato under different treatments of FA after a time interval of 90 days.

Treatment	Fo	F_m	F_V	F_v/F_m	YII	qP	NPQ
100 %GS	224.84 ± 1.02[a]	1220.52 ± 1.70[d]	995.68 ± 1.02[d]	0.82 ± 0.00[d]	0.78 ± 0.01[d]	0.82 ± 0.01[c]	0.07 ± 0.01[b]
FA25%+GS75%	228.69 ± 4.71[a]	1231.59 ± 2.18[e]	1002.91 ± 4.56[e]	0.81 ± 0.00[d]	0.75 ± 0.03[c]	0.89 ± 0.01[d]	0.05 ± 0.01[a]
FA50%+GS50%	264.24 ± 4.11[d]	962.00 ± 2.45[c]	697.76 ± 4.53[c]	0.72 ± 0.01[c]	0.71 ± 0.01[b]	0.82 ± 0.02[c]	0.11 ± 0.01[c]
FA75%+GS25%	250.63 ± 2.22[c]	797.62 ± 0.52[b]	546.99 ± 1.96[b]	0.69 ± 0.00[b]	0.69 ± 0.02[b]	0.78 ± 0.02[b]	0.15 ± 0.02[d]
100 %FA	241.78 ± 2.65[b]	721.80 ± 2.21[a]	480.02 ± 1.12[a]	0.67 ± 0.00[a]	0.58 ± 0.02[a]	0.71 ± 0.02[a]	0.17 ± 0.01[e]

Data represents as mean ± SD of five replicates. Different letters are statistically significant at *P < 0.05*.

Table 6

Correlation co-efficient between photosynthetic, gas exchange parameters and total plant biomass, with different antioxidative enzymes, protein, proline, EL, and MDA levels in *Solanum lycopersicum* grown in different FA amended soils.

	Pn	*E*	*gs*	*A*	TPBM	SPAD	F_v/F_m	YII	qP	NPQ
SOD	−0.774**	−0.733**	−0.807**	−0.814**	−0.743**	−0.671**	−0.840**	−0.645**	−0.474*	**0.767**
APX	−0.986**	−0.954**	−0.983**	−0.987**	−0.945**	−0.957**	−0.975**	−0.918**	−0.804**	**0.923**
DHAR	−0.891**	−0.819**	−0.821**	−0.837**	−0.882**	−0.893**	−0.801**	−0.968**	−0.818**	**0.808**
MDHAR	0.937**	0.904**	0.963**	0.992**	0.873**	0.887**	0.965**	0.858**	0.675**	−0.861**
GR	−0.793**	−0.739**	−0.804**	−0.877**	−0.731**	−0.746**	−0.822**	−0.813**	−0.534**	**0.739**
tAsA	−0.920**	−0.853**	−0.876**	−0.906**	−0.887**	−0.908**	−0.858**	−0.966**	−0.798**	**0.827**
AsA	−0.829**	−0.790**	−0.844**	−0.871**	−0.765**	−0.774**	−0.844**	−0.800**	−0.600**	**0.751**
DHA	−0.446*	**−0.383** ns	**−0.348** ns	**−0.362** ns	−0.479*	−0.503*	**−0.316** ns	−0.567**	−0.555**	**0.393** ns
tGSH	−0.903**	−0.844**	−0.897**	−0.958**	−0.832**	−0.872**	−0.889**	−0.916**	−0.661**	**0.799**
GSH	−0.892**	−0.829**	−0.885**	−0.947**	−0.822**	−0.856**	−0.879**	−0.924**	−0.641**	**0.791**
GSSG	−0.846**	−0.809**	−0.845**	−0.894**	−0.777**	−0.838**	−0.829**	−0.793**	−0.664**	**0.742**
EL	−0.930**	−0.889**	−0.932**	−0.976**	−0.860**	−0.903**	−0.931**	−0.892**	−0.690**	**0.849**
MDA	−0.903**	−0.860**	−0.864**	−0.893**	−0.854**	−0.908**	−0.847**	−0.917**	−0.774**	**0.835**
Protein	**0.921**	**0.883**	**0.962**	**0.964**	**0.882**	**0.843**	**0.975**	**0.784**	**0.660**	−0.871**
Proline	−0.922**	−0.878**	−0.903**	−0.935**	−0.873**	−0.891**	−0.904**	−0.889**	−0.706**	**0.886**

**. (*P < 0.01*) level. *. (*P < 0.05*) level. ns non-significant.

properties such as weathering, combustion, mineralogy, temperature, distribution of particle size, and FA enrichment lead to the proliferation of toxic elements (Gajic et al., 2013; Verma et al., 2016). The higher levels of toxic metals Cu, Zn, Cd, and Cr were present in FA compared to GS, even after the process of natural weathering at FA dumping site. These findings are consistent with previous reports (Gupta et al., 2007; Pandey et al., 2010; Singh et al., 2010b).

Availability, non-availability, and metal bioaccumulation in plants are governed by several ecological factors, including pH, metal solubility, soil texture and mineralogy, salinity, the occurrence of metal chelators, and other humic substances, and the presence or absence of other elements. The observed difference in metal accumulation between roots and shoots in several plants suggests that diverse cellular bioaccumulation mechanisms may control partitioning and metal translocation (Pandey et al., 2010; Singh et al., 2010a). In the ongoing study, plant roots of tomato accumulated more metals than the shoots (Fig. 1). The roots may act as barriers against the translocation of metals, which could be the possible mechanism of tolerance operative in the root systems of plants (Singh et al., 2010a). Heavy metal sequestration in root vacuoles explains the poor metal translocation to aerial plant parts (shoots), a normal toxicity response mechanism (Pandey et al., 2010). Cytogenetic makeup as well as physiological, biochemical, and anatomical features are responsible factors for metal amassing and distribution in aerial plant parts (Singh et al., 2010a).

4.3. Growth, Biomass, and metal tolerance

A significant decrease was observed in shoot length, root length, plant length, and total leaf area with the application of higher FA doses; the greatest decline in these parameters was observed in 100% FA (Table 3). Similar results were observed in chickpea grown on FA-amended soils (Pandey et al., 2010). An increase in above and below ground biomass of *Oryza sativa* was observed when grown in 20 t ha^{-1} FA application (Sarangi et al., 2001). Our results showed that low levels of FA (25%) had encouraging effects on SFW, SDW, RFW, RDW, and TPBM on 90-day-old tomato plants, but higher concentrations of FA suppressed growth and biomass in plants (Table 4). Analogous outcomes were observed in lemongrass (Panda et al., 2018) and other plants under different FA amendments (Bisoi et al., 2017). In low-level FA-amended soils, the physicochemical properties of FA promote plant growth and development (Ram and Masto, 2014; Verma et al., 2014), by causing no or less metal toxicity, proper moisture retention, water uptake, and nutrient transport.

The decline in plant biomass observed in metal-contaminated soils is associated with the root system and concentration of toxic metal interactions. In our study, the root systems of tomato plants were found to be more affected than shoot systems when grown in higher levels of FA, which gradually exposed the plants to the elevated concentration of these toxic metals. A poorly developed root system results in decreased water uptake and nutrient transport, thereby negatively affecting plant growth and total biomass (Boonyapookana et al., 2005; Jana et al., 2017; Panda et al., 2018).

MTI is one of the most common parameters for determination of metal tolerance in different plant species and one of the potential indicators of phytoremediation process (Zacchini et al., 2009; Panda et al., 2018). Increased MTI under low FA amendments and decreased MTI under higher FA applications (Table 4) demonstrate that, the tomato plants can tolerate metal contaminated soils to a certain extent, it has been observed that lower levels of FA increases the plant growth and biomass but higher levels were found to cause inhibitory effects as has been reported earlier in lemongrass (Panda et al., 2018), red mud (Gautam et al., 2017) and *Brassica* (Jana et al., 2017).

4.4. Chlorophyll pigments and fluorescence parameters

The observed reduction in chlorophyll pigments (Chl *a,* Chl *b,* and TChl) of plants (Fig. 2) grown in higher levels of FA represents the expression of diverse metallic ions or by metal spawned free radical destruction of chlorophyll molecules and replacement of Mg^{2+} ions by metal ions of Cu^{2+}, Zn^{2+}, Cd^{2+} in chlorophyll molecules leads to the breakdown of photosynthetic pigments (Jana et al., 2017). Metals tend to alter the photosynthetic rate by changing the composition of fatty acids, which upsets chloroplast assembly (Pandey et al., 2010). The decline in chlorophyll pigments (Chl *a,* Chl *b,* TChl) was previously witnessed in *E. dodonaei* and *C. occidentalis* plants when developed on substrates like mine and FA wastes (Love et al., 2013; Randelovic et al., 2016). A similar decrease in the concentration of chlorophyll pigments was also reported in *Azadirachta indica*, *Dalbergia sisoo* and *Polyalthia longifolia* grown on FA affected soils (Qadir and Siddiqui, 2014; Qadir et al., 2016b).

Carotenoids protect the chlorophyll molecules by serving as adjunct pigments under stressful conditions. Carotenoids adapt through numerous means such as replacing peroxidation, quenching the photo-dynamic reactions, and collapsing the chloroplast membrane. In this study, the carotenoid pigment levels in tomato plants increased with higher FA concentration (Fig. 2d), probably due to the ability of plant species to stabilize the deleterious impacts of metal-spawned reactive oxygen radicals under FA
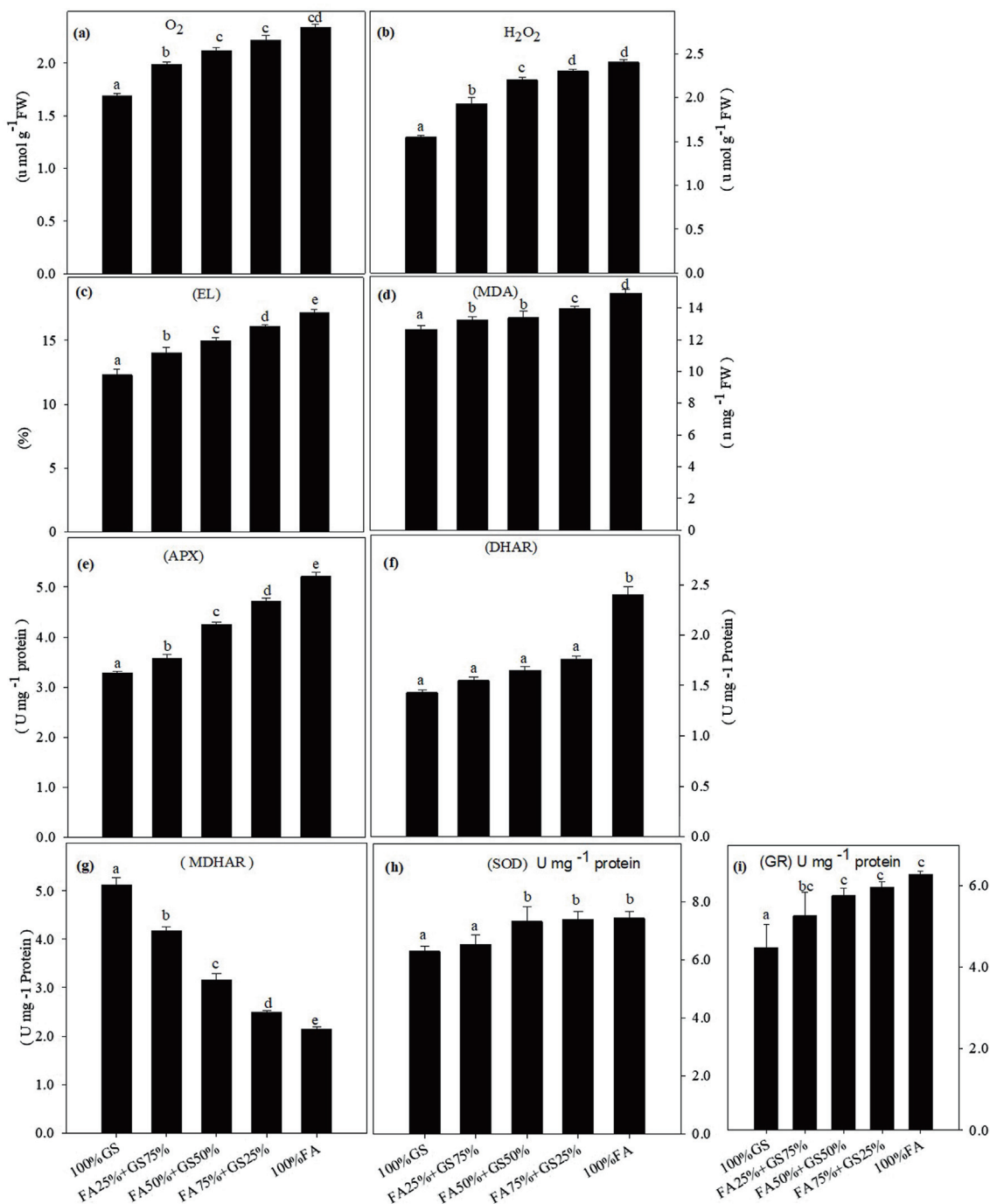
Fig. 3. Effect of different FA amended soils on (A&B) ROS generation, (C) EL and (D) MDA content, (E,F,G) activity of APX, DHAR, and MDHAR, and (H&I) activity of SOD and GR respectively in tomato leaves. Data represent the mean ± SD (n = 25 in each case). Letters indicate statistical significance at $P < 0.05$.

stress. These outcomes are compatible with the conclusions in *Sesbania cannabina* (Pandey et al., 2016) Sinha and Gupta (2005); Pandey et al. (2016) in chickpea who also stated an intensification in carotenoid contents under different FA amendments.

In the present study, an observed proliferation in fluorescence parameters under low and decline under higher levels of FA. F_o increased under higher doses of FA, the effect of FA stress on photosynthetic effectiveness and chain of electron transport, the noted difference in different fluorescence parameters of Chl. are the outcome of embarrassment of electron transport system or impairment to PS II inhibitor side. Reduction in Fv/Fm, and intensification in extreme

heat energy and fluorescence due to the buildup of sedentary PS II reaction hubs and poorer qP of PS II photochemistry in tomato plants (Calatayud and Barreno, 2001). The F_V/F_m, Y(II), and qP are strong indicators of stress that results in damage to PSII (Murchie and Lawson, 2013; Panda et al., 2018). Decreased PSII activity under high FA amendments due to metal toxicity has also been reported by (Raja et al., 2014; Panda et al., 2018). Increased NPQ under higher FA amendments in tomato plants is an indication of non-photochemical reactions, which may be caused by increased heat dissipation or a decline in the rate of initial charge separation (Feng et al., 2019; Wimalasekera, 2020).
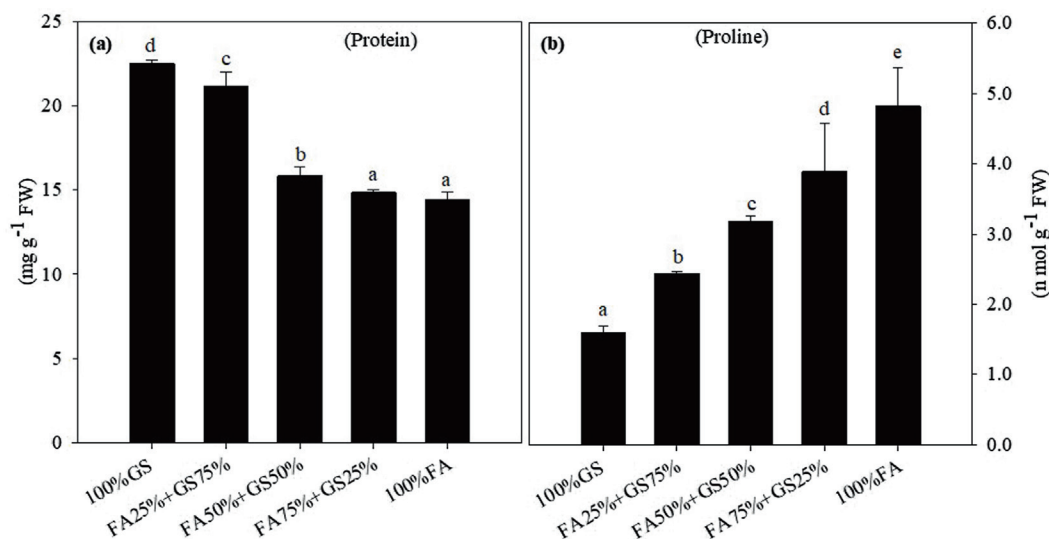
Fig. 4. Effect of different FA amended soils on (A) Protein (B) proline content in tomato leaves. Data signifies the mean ± SD (n = 25 in each case). Letters denote statistical significance at $P < 0.05$.

4.5. Photosynthetic Rate, Transpiration, stomatal Conductance, intercellular CO$_2$, and SPAD index

During present study, gas exchange parameters decreased with increased FA stress (Fig. 2e). Photosynthetic rate (Pn) is one of the best indicators of growth and development that is also sensitive to metal stress and FA in plants (Gupta et al., 2002; Raja et al., 2014; Verma et al., 2014). The decline in Pn under higher FA amendments may be due to structural modifications in the photosynthetic machinery, apparent from the decreased SPAD index values and functional alteration of stomata (Panda et al., 2018). Any impairment in stomatal behavior disturbs the gaseous exchange, thereby reducing photosynthesis water exchange and CO$_2$ assimilation (A) (Gupta et al., 2002; Singh et al., 2020). In plants FA stress leads to a decrease in gs, thereby influencing transpiration rate (E) and ultimately leads to modifications in adaptive mechanism in plant species (Singh et al., 2020). While studying the impacts of FA on growth, yield, and photosynthesis, Raja et al. (2014) testified a decline in the rate of photosynthesis and gas exchange parameters. The functional alteration in gs and SPAD index may also be due to the high metal concentration in FA, which causes dilapidation of chlorophyll molecules through free radical generation (Pandey et al., 2010; Bisoi et al., 2017; Panda et al., 2018).

4.6. Reactive oxygen Species, Electrolytic Leakage, lipid Peroxidation, Protein, and proline

Higher metal concentrations (Table 2) lead to metal toxicity, triggering ROS production, which in turn causes oxidative damage either by the transfer of electrons or through metal-induced inhibition of metabolic reactions (Pandey et al., 2010; Ahmad et al., 2010; Kaya et al., 2019, 2020). In plants metal-mediated oxidative stress is of paramount importance in the destruction of membrane integrity of lipids, proteins, nucleic acids, and pigments, thereby disrupting the fundamental physiological processes (Mittler, 2002; Pandey et al., 2010; Randelovic et al., 2016). Peroxidation of membrane lipids favors the generation of MDA as an end product, and its presence is largely considered as a signal of oxidative stress (Conrad et al., 2018; Farouk and Al-amri, 2019; Jan et al., 2018, 2020; Handa et al., 2019). Additionally, ROS production under FA stress enhances MDA production, perturbing the membrane fluidity and integrity (El-Kafafi et al., 2017; Jaiswal et al.,

2020), interfering with protein channeling (Bilal et al., 2019; Dumont and Rivoal, 2019), enhancing electrolyte leakage (Alyemeni et al., 2017), and inhibiting enzyme activities (Garg and Manchanda, 2009). The observed escalation in ROS, MDA, and EL levels at higher doses of FA represents the level of oxidative stress (Fig. 3a-b). A similar upsurge in ROS, MDA, and EL was also witnessed in chickpea and *Sesbania cannabina* grown in FA-amended soils (Sinha and Gupta, 2005; Pandey et al., 2010; Kumari et al., 2013). Metal mediated increase in MDA and EL levels such as arsenic (Talukdar, 2013), chromium (Shanker et al., 2004; Afshan et al., 2015) copper, and salt stress (Fatma et al., 2014; Singh et al., 2014) has reported from time to time.

The observed decrease in the contents of protein in tomato plants (Fig. 4a) due to increased FA amendments is an indication of FA-induced proteolysis. Soluble protein levels are chief indicators of alterations in plant metabolism as a reaction to a wide range of natural and xenobiotic stressors (Jana et al., 2017). Similarly, a decline in protein content under high doses (60%) of FA was also reported in *Cymbopogon citratus* and *Sesbania cannabina* plants (Sinha and Gupta, 2005; Gautam et al., 2012). Tomato plants accumulated significant levels of proline with FA treatment (Fig. 4b). Increased levels of proline indicate a defensive response against FA-induced stress. Under FA stress, higher levels of proline were also described in *Medicago sativa, Cicer arientum,* and *B. juncea* (Junaid et al., 2013).

4.7. Plant antioxidants

Plants are armed with vibrant enzymatic and non-enzymatic antioxidant structures that normalize the production and regulation of ROS under different environmental conditions (Hossain et al., 2012). Plant species with extraordinary antioxidant capacity are tolerant to toxicity caused by different metal species (Randelovic et al., 2016). The present result showed a significant increase in different antioxidants with higher doses of FA treatment (Fig. 3c-d). An increase in different levels of SOD, APX, MDHAR, DHAR, and GR was witnessed in tomato plants grown in soil with high FA amendments. This upsurge in enzymatic antioxidant activities triggers cellular protective mechanisms that alleviate the harmful effects caused by FA stress (Panda et al., 2018). Several researchers have deliberated on the varied aspects of antioxidant enzymes, which perform crucial roles in the biosynthe-
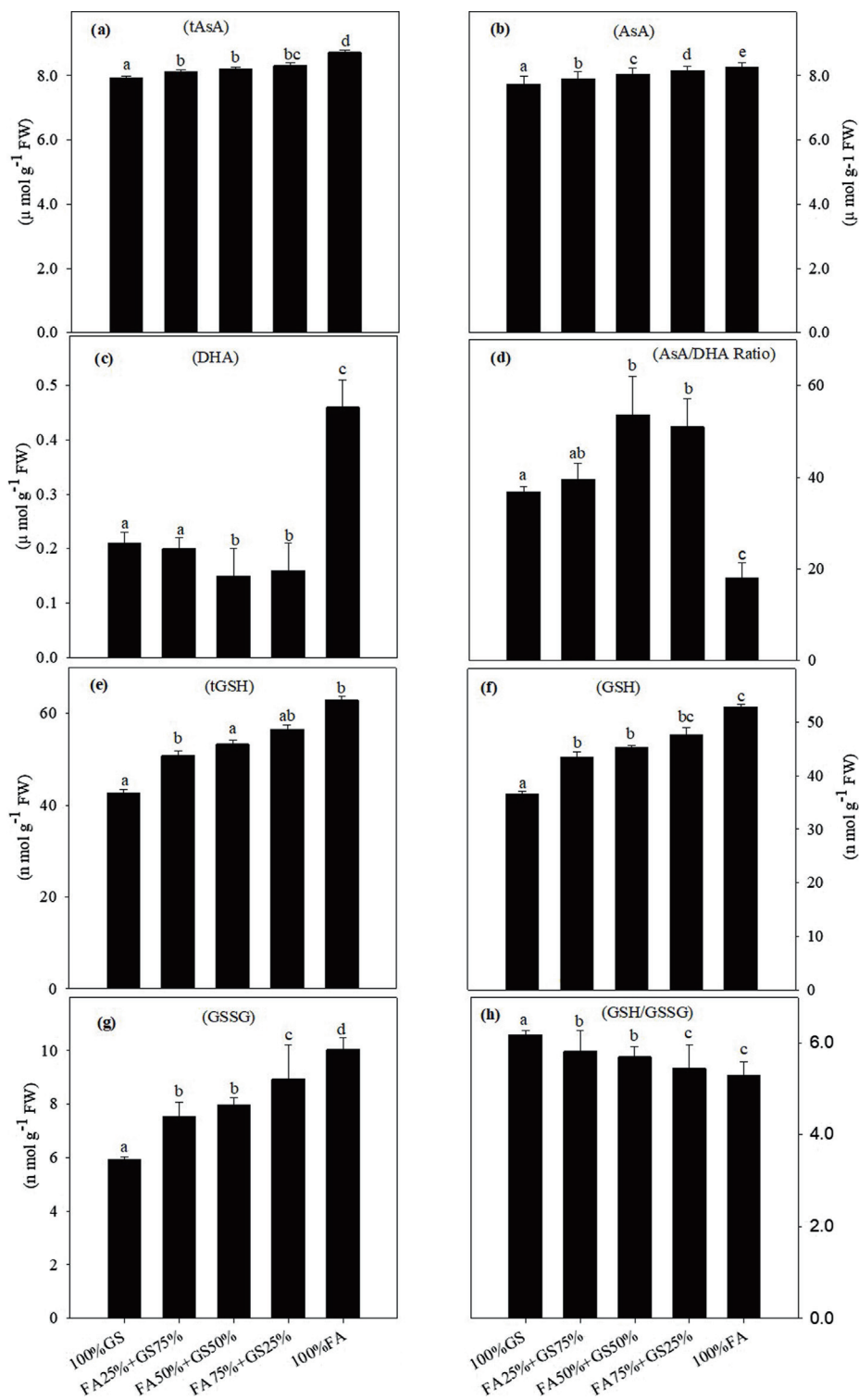
Fig. 5. Effect of different FA amended soils on AsA-GSH pool (A) tASA, (B) AsA, (C) DHA, (D)AsA/DHA (E) tGSH (F) GSH, (G) GSSG and (H) GSH/GSSG ratio in tomato plants. Data represent the mean ± SD (n = 25 in each case). Different letters represent statistical significance at *P < 0.05*.

sis of significant biomolecules, in transportation systems, and above all in stress tolerance. Improved pursuit of SOD, CAT, and APX was also reported in *Pithocellobium dulce* and *A. indica* growing under FA stress (Qadir et al., 2019). Similarly, enhanced antioxidant enzyme (CAT, SOD, and GR) accomplishments were also reported in shoots, roots, and leaves of chickpea grown on FA substrates (Pandey et al., 2010). High activities of APX in reaction to FA

stress suggest an effective H_2O_2 scavenging capability. Furthermore, SOD is among the pivotal enzyme of the antioxidant system, providing the former level of protection in scavenging of harmful reactive oxygen radicals. SOD enhances the $O_2^{\bullet-}$ dismutation thus concentrating H_2O_2 as a byproduct during the chemical reaction, which is then eliminated by the activities of APX and CAT respectively (Foyer and Noctor, 2011). Augmented APX activities were

also reported in *A. indica* leaves when subjected to FA and combined stress (Qadir et al., 2016b; Zandalinas et al., 2017). The MDHAR isoenzymes amplified activities have also been pronounced in different salt-tolerant transgenic plant species (Sharma et al., 2012). In the presence of condensed GSH, the reduction of DHA to AsA, mediated by DHAR, thereby keeping AsA in its reduced form (Sharma et al., 2012). Increased DHAR activity is directly associated with enriched tolerance to stress like salinity, drought, and enhanced temperatures (Ahmad et al., 2010, 2019).

SOD and GR activities increased considerably in tomato plants budding under FA stress. These results are in conformity with (Pandey et al., 2010) who also observed an intensification in the activities of SOD and GR levels in Chickpea plants growing in different FA amended soils. Increased GR activity in plants also triggers GSH accumulation, which confers stress tolerance to plants. The AsA-GSH corridor operates in several plant compartments comprising of AsA, GSH, and associated enzymes (APX, DHAR, MDHAR, and GR), the AsA and GSH recycling pathway has an imperative part in the detoxification of ROS species. Apart from ROS scavenging, the pathway provides oxidative stress tolerance in plants (Sharma et al., 2012; Hasanuzzaman et al., 2019).

Present observations revealed augmented levels of leaf tAsA, AsA, and DHA in 90-day-old tomato plants growing in FA-amended soils. AsA accumulation and augmented ratios of AsA/DHA could be linked to a vigorous stress pressure (Fig. 5 **a-d**). GSH cycle modulation is convoluted in maintaining the ratio of GSH/GSSG, indispensable for cellular redox. In this fashion, the GR bustle could salvage GSH at disbursement of NADPH (Foyer and Noctor, 2011).

In the present study, a significant intensification in levels of tGSH, GSH, and GSSG activity was witnessed in tomato plants grown in soils mixed with FA in comparison to control (Fig. 5 **e-h**). Further, a slight reduction was observed in GSH/GSSG ratio. The noticed proliferation in GR activity in reaction to FA stress (Fig. 3**d**) signifies the maintenance of GR activity in plants despite enhanced levels of tGSH, GSH, and GSSG. These results are also indicative of increased recycling of GSH, in response to oxidative damage. Under stressful environmental conditions amplified GSH and AsA content in addition to the ratios of GSH/GSSG and AsA/DHA in two citrus genotypes, reducing oxidative damage and thereby providing tolerance to combined stress (Zandalinas et al., 2017). The result is indicative of a superior ROS non-enzymatic purification organization and active recycling of GSH in plants under FA stress. Previously, it has been argued that more GSSG accumulation could be associated with ROS build-up in plant cells (Foyer and Noctor, 2011). Present findings are reliable with this account; accumulation of MDA in tomato leaves higher FA amendments (Fig. 3**b**). Furthermore, the intensified introduction of GR in plant leaves under FA stress might be adequate to maintain a suitable AsA/DHA ratio, providing a greater volume for scavenging of ROS (Zandalinas et al., 2017). Under stressful environmental conditions higher AsA/DHA and GSH/GSSG ratios were also described in tobacco and maize plants (Chen et al., 2003). Thus, it seems crucial for plant cells to sense oxidative stress and react by changing ratios of AsA to DHA and GSH to GSSG.

The negative correlation between antioxidants with PS II activity in tomato plants under different treatments of FA suggests that they are unable to successfully resist ROS generation caused by heavy metals. Thus, plants are unable to maintain growth and photosynthesis under higher levels of FA application (Table 6).

5. Conclusions

We concluded from the present study that FA amendment in lower concentrations could be advantageous for proper growth and development of *Solanum lycopersicum* L. The plants species maintained PS II activity and achieved highest biomass under 25% FA-amended soil, providing evidence that this is the most appropriate treatment for the growth of this plant species. Thus, *S. lycopersicum* L. can tolerate up to 25% FA and is a metal tolerant, as evidenced by the MTI, which was more than 100%. At higher levels of FA amendment, adverse results were observed in the CO_2 photosynthetic rate, structural and functional modifications of PS II. We observed declines in chlorophyll, protein, growth parameters, and chlorophyll fluorescence with higher FA-amended soil. Further, the induction of antioxidants (SOD, proline, AsA, MDA, APX) under elevated levels of FA treatment helps in ROS detoxification and provides metal tolerance to the plant species. The elevated levels of enzyme and non-enzyme antioxidants and augmented AsA/DHA ratio also mimic in overcoming oxidative damage induced by FA stress. By accumulating metals *Solanum lycopersicum* L. can tolerate FA stress through shifts in photosynthetic, morphological, and biochemical traits under low levels of FA-amended soils.

6. Ethics approval

Not applicable

7. Consent to participate

All authors consent to participate in the manuscript publication

8. Consent for publication

All authors approved the manuscript to be published

9. Availability of data and material

All data and materials as well as software application or custom code support our claims and comply with field standards. All data generated or analyzed during this study are included in this published article.

Author contributions

S.U.Q and WAS conceived the original research plan. V.R, TS, SA and MES analyzed the data. All authors contributed in writing and revision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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