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RESEARCH ARTICLE

Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress

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Abstract Chromium (Cr) is a biologically non-essential, carcinogenic and toxic heavy metal. The cultivation of Cr-tolerant genotypes seems the most favorable and environment friendly strategy for rehabilitation and remediation of Cr-contaminated soils. To prove this hypothesis and identify the Cr tolerance, the present study was performed to assess the physiological and biochemical response of sunflower genotypes to Cr stress. The seeds of six sunflower hybrids, namely FH-425, FH-600, FH-612, FH-614, FH-619, and FH-620, were grown in spiked soil for 12 weeks under increasing concentrations of Cr (0, 5, 10, and 20 mg kg^{-1}). A seed germination test was also run under different concentrations of Cr (0, 5, 10, 200 mM) in petri dishes. Plants were harvested after 12 weeks of germination. Different plant attributes such as growth; biomass; photosynthesis; gas exchange; activity of antioxidant enzymes, i.e., superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate (APX), and catalases (CAT); reactive oxygen species (ROS); lipid peroxidation; electrolyte leakage; and Cr concentration as well as accumulations in all plant parts were studied for the selection of the most Cr-tolerant genotype. Increasing concentration of Cr in soil triggered the reduction of all plant parameters in sunflower. Cr stress increased electrolyte leakage and production of reactive oxygen species which stimulated the activities of antioxidant enzymes and gas exchange attributes of sunflower. Chromium accumulation in the root and shoot increased gradually with increasing Cr treatments and caused reduction in overall plant growth. The accumulation of Cr was recorded in the order of FH-614 > FH-620 > FH-600 > FH-619 > FH-612 > FH-425. The differential uptake and accumulation of Cr by sunflower hybrids may be useful in selection and breeding for Cr-tolerant genotypes.

Keywords Chromium · Gas exchange parameters · Growth · Antioxidant enzymes · Sunflower hybrids · Reactive oxygen species

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Introduction

Anthropogenic activities, such as application of contaminated effluents, agrochemicals, phosphate fertilizers, industrial waste, sewage sludge, and pesticides, are the main cause of heavy metal contamination of soils (Adrees et al. 2015a; Rehman et al. 2015; Rizwan et al. 2016a). Along with anthropogenic activities, the natural sources like run off, forest fires, volcanic eruption, and production of sea salt aerosols are also a major cause of heavy metal release into the environment (Farid et al. 2015; Nagajyoti et al. 2010).

The wide industrial applications of chromium (Cr) make it a serious environmental pollutant. Chromium can be found in different states from Cr-I to Cr-VI while the most toxic form is Cr-VI, followed by Cr-III (Afshan et al. 2015). The extensive use of Cr^{6+} compounds in tanning, cooling water treatment, metal plating, and wood preservation industry is the main source of Cr contamination, and its bio-mobility and bioavailability in the environment (Ali et al. 2015).

In Pakistan, leather industry is the major source of Cr^{6+} release into the environment, which seriously affects the growth and development of living organisms (Ali et al. 2015). Different chemicals such as, chromium sulfate ($CrSO_4$), formic acid (CH_2O_2), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), magnesium sulfate ($MgSO_4$), calcium hydroxide ($Ca(OH)_2$), sulfuric acid (H_2SO_4), dyes, and fat liquors are being used during the tanning operation in leather industries (Ali et al. 2015).

Several studies have reported that Cr toxicity decreased the plant growth and biomass by generating reactive oxygen species at cellular and sub-cellular level (Atta et al. 2013; Bukhari et al. 2016a).

Under metal stress, a natural defense system has been evolved in plants with the involvement of both non-enzymatic and enzymatic antioxidants, synthesis of chelating agents, and production of osmolytes (Rizwan et al. 2015; Adrees et al. 2015a; Artiushenko et al. 2014). But the defense mechanism mainly depends upon the type of metal, plant species and genotype (Artiushenko et al. 2014).

Sunflower (*Helianthus annuus* L.) is a metal hyper-accumulator plant, capable of tolerating a certain concentration of Cr without showing toxic symptoms (Atta et al. 2013). But most of the times, Cr toxicity adversely affects growth and development of sunflower, that mainly depends upon metal concentration and growth medium (Fozia et al. 2008). The ability of sunflower to improve its biomass production helps the plants cope with Cr toxicity (Chae et al. 2014). Rizwan et al. (2016a, b) comprehensively reviewed the toxicity mechanisms in sunflower under other heavy metals like Pb, Cd, Cu, Ni, Zn, and Cr as well. Higher biomass production facilitates the accumulation of large amount of Cr from the growth media and its translocation towards the aerial plant parts (Atta et al. 2013; Cutright et al. 2010). The propensity of sunflower for the accumulation of metals, like Cr (Atta et al. 2013; Fozia et al. 2008), Cd (Júnior et al. 2015), Zn, Pb (Adesodun et al. 2010; Azad et al. 2011; Doncheva et al. 2013), Cu (Jiang et al. 2000; Kolbas et al. 2014), and As (Imran et al. 2013), has already been proved.

A significant enhancement was observed in metal extraction ability of a sunflower mutant which was 8.2, 9.2, and 7.5 times higher than that in control plants for Pb, Zn, and Cd, respectively, in aboveground plant parts (Nehnevajova et al. 2007). Furthermore, the biomass and oil of sunflower can be used for biodiesel, lubricants, and biogas production which should add an additional value and enhance the economic viability of phytoextraction (Harris et al. 2016; Del Gatto et al. 2015).

This suggests that existing varieties/cultivars of sunflower should be characterized for Cr uptake on the basis of their translocation ability. The present study was conducted to evaluate the Cr toxicity in terms of induced changes in morph-physiological and biochemical attributes of six sunflower hybrids and their potential to accumulation Cr from soil.

Materials and methods

Soil treatments, germination test, and growth conditions

A clay loam (25% sand, 22% silt, 53% clay) soil, collected at 0–15-cm depth from botanical garden of the Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan, was used in the present study. Prior to pot experiment, the soil was air-dried and passed through a 2-mm-diameter sieve to remove crop residues and stones and further characterized for chemical and physical properties (Table 1). Mature seeds of six sunflower genotypes (Faisalabad hybrid: FH-600, FH-612, FH-614, FH-619, FH-620, and FH-425) were collected from the Oilseed Research Institute, AARI, Faisalabad, Pakistan. To check the germination potential of these six hybrids, a test was carried out in petri dishes under different concentrations of Cr (0, 5, 10, 20 mM) in triplicates. Firstly, the seeds were washed with 10% H_2O_2 to remove germs and bacteria, followed by rinsing with distilled water. For germination test, ten seeds were placed in each petri dish and then incubated at 22 °C till germination in comparison to controls. Ten seeds

Table 1 Properties of soil used for the pot experiment

Physicochemical properties	
Texture	Clay loam
Sand (%)	25.0
Silt (%)	22.0
Clay (%)	53.0
pH (1/2.5 soil to water ratio)	6.75
EC_e (dS m^{-1})	2.90
SAR $(mmol^{-1})^{1/2}$	6.50
Organic matter (%)	0.35
Available P (mg kg^{-1})	2.20
HCO_3 (mmol L^{-1})	3.61
Cl^- (mmol L^{-1})	2.27
SO_4^{2-} (mmol L^{-1})	6.65
Ca^{2+} + Mg^{2+} (mmol L^{-1})	3.80
Na^{2+} (mmol L^{-1})	3.67
K^+ (mmol L^{-1})	0.05
Available Cu^{2+} (mg kg^{-1})	0.33
Available Zn^{2+} (mg kg^{-1})	0.81

were sown in each earthen pot containing 5 kg soil spiked with increasing Cr concentration (0, 5, 10, 20 mg kg^{-1} dry weight) under wire house conditions. After 15 days of germination, thinning was done to maintain five plants per pot and the pulled up plants were crushed carefully into the same pot. Each pot was fertilized with a 500-mL solution containing 2.19 g L^{-1} N (as $(NH_2)_2CO$), 0.5 g L^{-1} P (as $(NH_4)_2HPO_4$), and 2.14 g L^{-1} K (as K_2SO_4). The fertilizer solution was applied after 15 and 30 days of germination. All glass wares were thoroughly rinsed with 10% HNO_3 and then washed with distilled water to avoid contamination.

Plant sampling and analysis

Plants were harvested after 10 weeks of treatment. The roots were carefully detached from the soil. Deionized water was used to wash the plant samples. All plants were carefully separated into the roots, stems, and leaves. Then, growth parameters like plant height, root and stem length, leaves and flowers per plant, leaf area, and fresh and dry biomass of all plant parts including achene were measured. A simple electric balance was used for biomass measurements and leaf area was measured by leaf area meter (L1-2000, L1-COR, USA). The samples were then oven dried at 70 °C, until constant weight, for further analysis.

Pigment content assay

After 10 weeks of treatment, the second uppermost fully extended fresh leaves were used for chlorophyll (a, b, total chlorophylls) and carotenoids contents. The pigments were extracted in the dark with 85% (*v*/*v*, Sigma) aqueous acetone solution at 4 °C by insistent shaking until complete discoloration of the leaves. The extract was centrifuged at 4000 × rpm at 4 °C for 10 min, and the supernatant was used for light absorbance at 663, 644, and 452.5 nm with spectrophotometer (Halo DB-20/DB-20S, Dynamica Company, London, UK) (Metzner et al. 1965). The concentrations of chlorophylls and carotenoids were calculated according to the adjusted extinction coefficients and following equations given by Lichtenthaler (1987).

$$\text{Chlorophyll a}\left(\mu g/mL\right) = 10.3 \times E_{663} - E_{644}$$

$$\text{Chlorophyll b}\left(\mu g/mL\right) = 19.7 \times E_{644} - 3:87 \times E_{663}$$

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}$$

$$\text{Total carotenoids}\left(\mu g/mL\right) = 4.2 \times E_{452.5} - \{(0.0264 \times \text{chl a}) + (0:426 \times \text{chl b})\}$$

Chromium content analysis

Plant samples were dried at 70 °C and the known weight of plant root, stem, and leaves was placed in muffle furnace at 650 °C for 6 h. Plant samples turned into ash after 6 h. The ash was dissolved in a combination of 3 mL nitric acid (HNO_3) and 2-mL hydrochloric acid (HCl) solution. The extract were diluted to 50 mL with distilled water and analyzed for Cr content by using flame atomic absorption spectrometry (novA A400 Analytik Jena, Germany), following Ehsan et al. (2013).

Cr concentration was calculated by the following formula:

$$\text{Cr concentration}\left(\text{mgkg}^{-1}\right) = \text{metal reading of digested sample}\left(\text{mg L}^{-1}\right) \times \text{dilution factor},$$

where

$$\text{dilution factor} = \frac{\text{total volume of sample (mL)}}{\text{weight of plant material (g)}}.$$

Cr accumulation was calculated as follows:

$$\text{Cr accumulation}\left(\text{mg plant}^{-1}\right) = \text{Cr concentration in organ}\left(\text{mg kg}^{-1}\right) \times \text{dry weight of organ (kg)}.$$

The translocation factor (TF) was calculated as the ratio of shoot Cr concentration to root Cr concentration and shoot Cr concentration to leaf Cr concentration (Baker and Whiting 2002).

Determination of electrolyte leakage, MDA, and H_2O_2

The method described by Dionisio-Sese and Tobita (1998) was followed to measure electrolyte leakage (EL). After 8 weeks of treatment, the uppermost fully expanded leaves were cut into pieces of about 5-mm length and placed in test tubes filled with 8 mL of distilled water. The electrical conductivity of initial medium (EC1) was recorded after 2 h of incubation in a water bath at 32 °C. To measure electrical conductivity of second medium (EC2), the same samples were autoclaved for 20 min at 121 °C to release all the electrolytes into the solution. The samples were cooled to 25 °C and pH/conductivity meter (model 720, INCO-LAB Company, Kuwait) was used to record EC2. Finally, the EL was computed using the following equation:

$$EL = \left(EC1/EC2\right) \times 100.$$

The malondialdehyde (MDA), a product of lipid peroxidation in leaf and root tissues of plants, was measured by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968) with some minor amendments mentioned by Zhang and Kirham (1994) and Dhindsa et al. (1981). Leaf and root samples (0.25 mg) were homogenized in 5 mL of 0.1% trichloro acetic acid (TCA). The resultant was centrifuged for 5 min at 10,000×*g*. Four-milliliter 20% TCA containing 0.5% TBA was mixed in 1-mL aliquot of the supernatant. The mixture was suddenly cooled in ice bath after heating at 95 °C for 30 min. After centrifugation for 10 min at 10,000×*g*, the reading for nonspecific absorption at 600 nm was subtracted from the absorbance of supernatant recorded at 532 nm. An extinction coefficient of 155 mM^{-1} cm^{-1} was applied to calculate MDA content.

The hydrogen peroxide (H_2O_2) content was analyzed colorimetrically as presented by Jana and Choudhuri (1981). Leaf/root tissues (50 mg) were homogenized with 3 mL of phosphate buffer (50 mM, pH 6.5) to get H_2O_2 extract. The homogenate was centrifuged for 25 min at 6000×*g*. To measure H_2O_2 contents, 1 mL of 0.1% titanium sulfate in 20% (*v*/*v*) H_2SO_4 was mixed with 3 mL of extracted solution, and then mixture was centrifuged for 15 min at 6000×*g*. The supernatant was analyzed at 410 nm. The extinction coefficient of 0.28 μmol^{-1} cm^{-1} was applied to calculate H_2O_2 contents.

Gas exchange attributes and SPAD value

Eight weeks after Cr exposure, gas exchange parameters such as stomatal conductance (*G*s), photosynthetic rate (*A*), water use efficiency (*A*/*E*), and transpiration rate (*E*) were measured from the second upper most fully expanded leaf, using portable infrared gas analyzer (LCA-4 ADC, Analytical Development Company, Hoddesdon, England) (Shakoor et al. 2014; Farid et al. 2015). Readings were recorded between 11:00 to 13:00 by clamping the middle part of the leaf in the chamber of the instrument to keep temperature and photon flux density unchanged. Chlorophyll measurement, in terms of soil plant analysis development (SPAD) value, was performed on second upper most fully expanded leaf with the help of SPAD meter (SPAD-502).

Evaluation of antioxidant enzymes and protein content

Antioxidant enzymes including guaiacol peroxidase (POD), superoxide dismutase (SOD), ascorbate (APX), and catalase (CAT) in the leaves and roots were assessed spectrophotometrically. After 8 weeks of treatment, second fully expanded leaf and root samples were taken for enzymatic analysis. The samples (1.0 g) were quickly frozen in liquid nitrogen (N_2) and ground with precooled pestle and mortar. This ground samples were homogenized with 0.05 M phosphate buffer (pH 7.8) and filtered through four layers of muslin cloth and then centrifuged for 10 min at 12,000×*g*, at 4 °C. The final supernatant was used for the estimation of POD and SOD activities according to Zhang (1992). Soluble protein content was measured from same supernatant using Coomassie brilliant blue G-250 as a dye and albumin as a standard as reported by Bradford (Bradford 1976).

The activity of catalase (CAT, EC 1.11.1.6) was measured according to the method of Aebi (1984). The sample mixture consisted of 2.8 mL 50-mM phosphate buffer with 2 mM CA (pH 7.0), 100 μL enzyme extract, and 100 μL H_2O_2 (300 mM). The CAT activity was recorded by measuring the decrease in absorbance at 240 nm as a consequence of H_2O_2 disappearance ($\varepsilon = 39.4$ mM^{-1} cm^{-1}).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured according to the method of Nakano and Asada (1981). The sample mixture consisted of 2.7 mL 25-mM potassium phosphate buffer with 2 mM CA (pH 7.0), 100 μL H_2O_2 (300 mM, 100 μL ascorbate (7.5 mM), and 100 μL enzyme extract. The oxidation activity of ascorbate was recorded by measuring the change in wavelength at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}).

Statistical analysis

All values presented in this study are mean of three replicates. Analysis of variance (ANOVA) was done by using a statistical package, SPSS version 16.0 (SPSS, IBM, 2009) followed by Tukey's post hoc test between the means of treatments to determine the significant difference and standard deviation.

Results

Seed germination and growth traits

The variations in sunflower growth parameters including plant height, root length, number of leaves, number of flowers, and leaf area are shown in Fig. 1. Chromium application reduced seed germination (Table 2) and growth of all cultivars of sunflower as compare to control treatment. However, the reduction in germination and growth was Cr concentration and genotype-dependent. The germination and growth gradually decreased with increasing concentration of Cr. Maximum decline was observed in the plants grown under the highest Cr concentration (20 mg kg^{-1}) while FH-425 proved to be the most vulnerable genotype at the highest Cr concentration. The genotypic tolerance to increasing Cr concentration, regarding all growth parameters and germination, was in the order of FH-614 > FH-620 > FH-600 > FH-619 > FH-612 > FH-425.

Biomass

The difference in biomass accumulation among the six varieties of sunflower, exposed to different levels of Cr, is

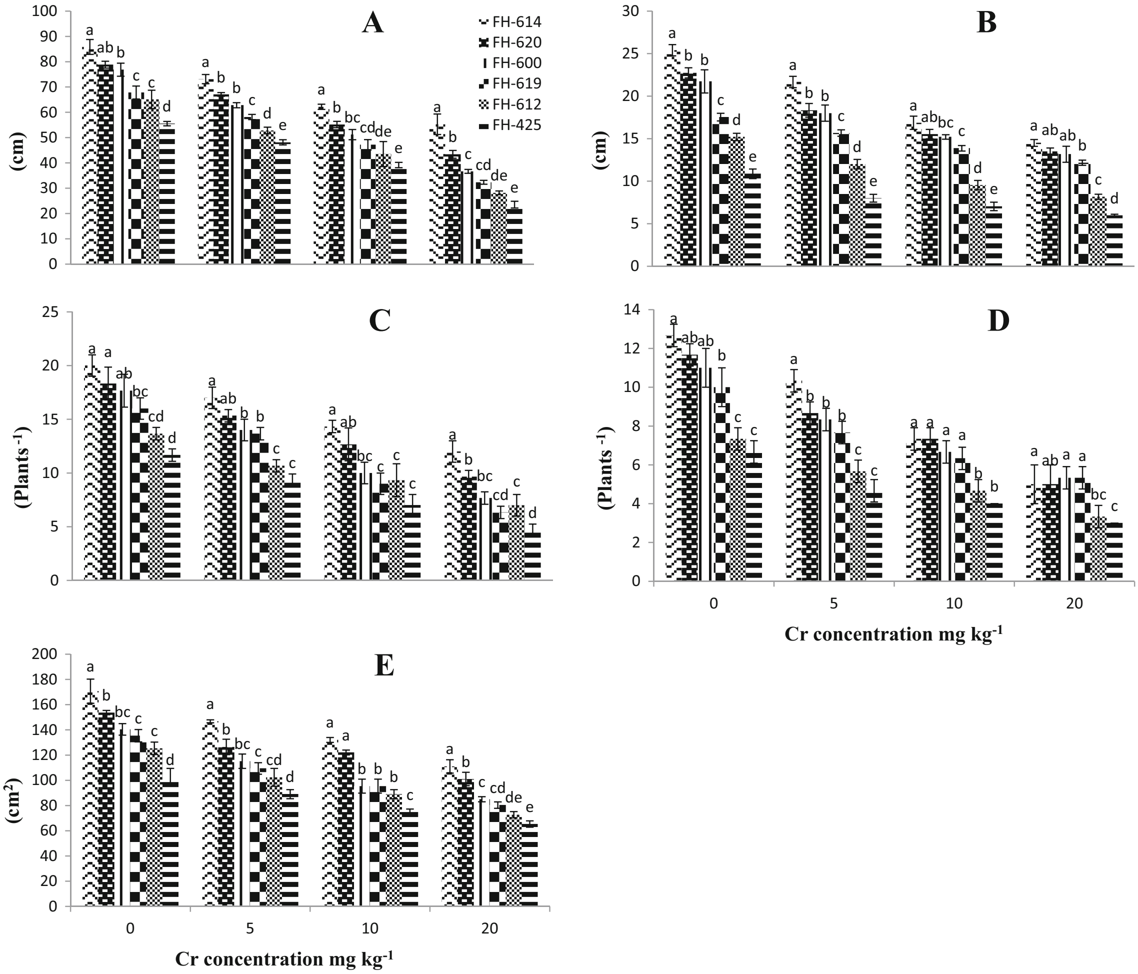

Fig. 1 Effect of Cr on plant height (**a**), root length (**b**), number of leaves (**c**), number of flower (**d**), and leaf area (**e**) in six different varieties of sunflower grown in soil with increasing Cr concentrations (0, 5, 10, and 20 mg kg^{-1}). Values are demonstrated as means of three replicates along with standard deviation. *Different letters* indicate that values are significantly different at $P < 0.05$

presented in Fig. 2. Increasing Cr concentration in growth media significantly decreased the biomass like leaf, stem, and root fresh and dry weight of plants. However, different varieties showed different trend for each parameter. For leaf fresh and dry weight as well as stem dry weight, the response of different varieties was as follow:

Table 2 The seed germination ability of six sunflower hybrids under Cr stress

Variety	Cr concentration in mM			
	Cr 0	Cr 5	Cr 10	Cr 20
FH-614	10.0 ± 0.0	9.67 ± 0.6	7.67 ± 0.6	6.33 ± 0.6
FH-620	10.0 ± 0.0	8.33 ± 0.6	7.33 ± 0.6	5.67 ± 0.6
FH-600	9.00 ± 0.0	8.00 ± 0.0	7.00 ± 0.0	5.33 ± 0.6
FH-619	8.67 ± 0.6	7.67 ± 0.6	6.33 ± 0.6	5.00 ± 0.0
FH-612	8.67 ± 0.6	7.00 ± 0.0	6.60 ± 0.0	4.67 ± 0.6
FH-425	8.00 ± 0.0	5.67 ± 0.6	5.00 ± 0.0	3.33 ± 0.6

$$\text{FH}{-}614 > \text{FH}{-}620 > \text{FH}{-}600 > \text{FH}{-}619 > \text{FH}{-}612 > \text{FH}{-}425.$$

For stem fresh weight, root fresh weight, and root dry weight, the results are presented in Fig. 2c, e, f. FH-614 proved to be the most tolerant genotype at all Cr levels. However, the overall genotypic response to Cr, regarding these traits, is presented below:

$$\text{FH}{-}614 > \text{FH}{-}600 > \text{FH}{-}620 > \text{FH}{-}619 > \text{FH}{-}612 > \text{FH}{-}425.$$

Chlorophyll content and carotenoids

Chlorophyll (a, b and total chlorophyll) and carotenoids concentrations significantly decreased in all the varieties of sunflower with increasing Cr concentrations in soil (0,

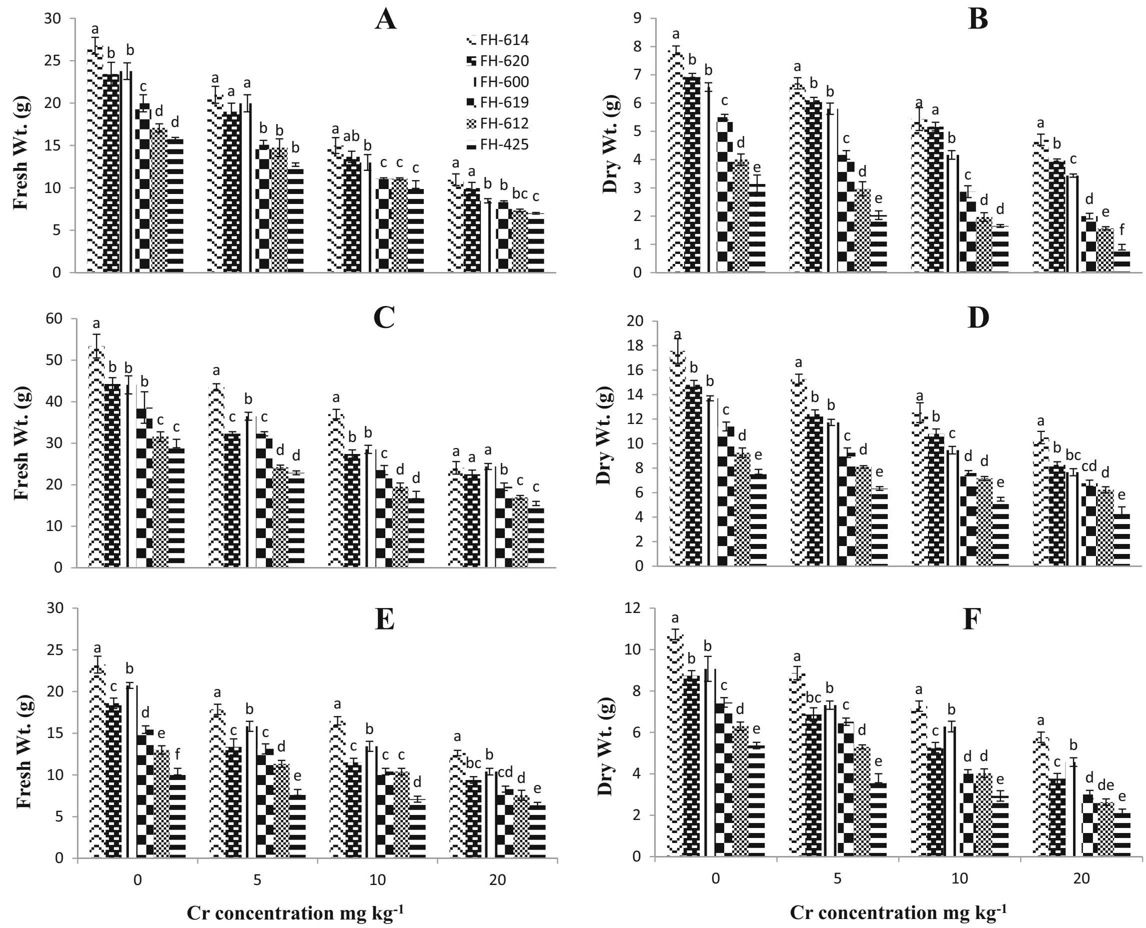

Fig. 2 Effect of Cr on leaf fresh weight (**a**), leaf dry weight (**b**), stem fresh weight (**c**), stem dry weight (**d**), root fresh weight (**e**), and root dry weight (**f**) in six different varieties of sunflower grown in soil with increasing Cr concentrations (0, 5, 10, and 20 mg kg^{-1}). Values are demonstrated as means of three replicates along with standard deviation. *Different small letters* indicate that values are significantly different at $P < 0.05$

5, 10, 20 mg kg^{-1}) as given in Fig. 3. Regarding genotypes, FH-614 was found to be the most tolerant and FH-425 the most susceptible variety at all the Cr levels. The general trend of chlorophyll (a, b, total chlorophyll) and carotenoids among the six varieties, as affected by Cr, is given below:

$$\text{FH}-614 > \text{FH}-620 > \text{FH}-600 > \text{FH}-619 > \text{FH}-612 > \text{FH}-425.$$

Gas exchange attributes

The Cr-induced effects on gas exchange attributes of sunflower varieties are given in Fig. 4. Generally, the increasing Cr concentration significantly decreased water use efficiency (Fig. 4a), transpiration rate (Fig. 4b), net photosynthetic rate (Fig. 4c), and stomatal conductance (Fig. 4d) of sunflower as compared to those in the control. However, the genotypic response of sunflower, to Cr stress, was variable. The lowest and highest gas exchange attributes were shown by FH-425 and FH-614, respectively, at each Cr treatment, as compare to the other varieties. The overall response of genotypes was in the order as follows:FH-614 > FH-620 > FH-600 > FH-619 > FH-612 > FH-425.

Soluble protein and SPAD value

Soluble protein (leaf and root) and SPAD value of all the varieties of sunflower showed significant reduction with increasing Cr concentrations, as compare to those in the control (Fig. 5a, b, c).

The FH-425 showed the lowest SPAD value in each treatment as compared to the other five varieties and the highest values were observed in FH-614, at all the Cr

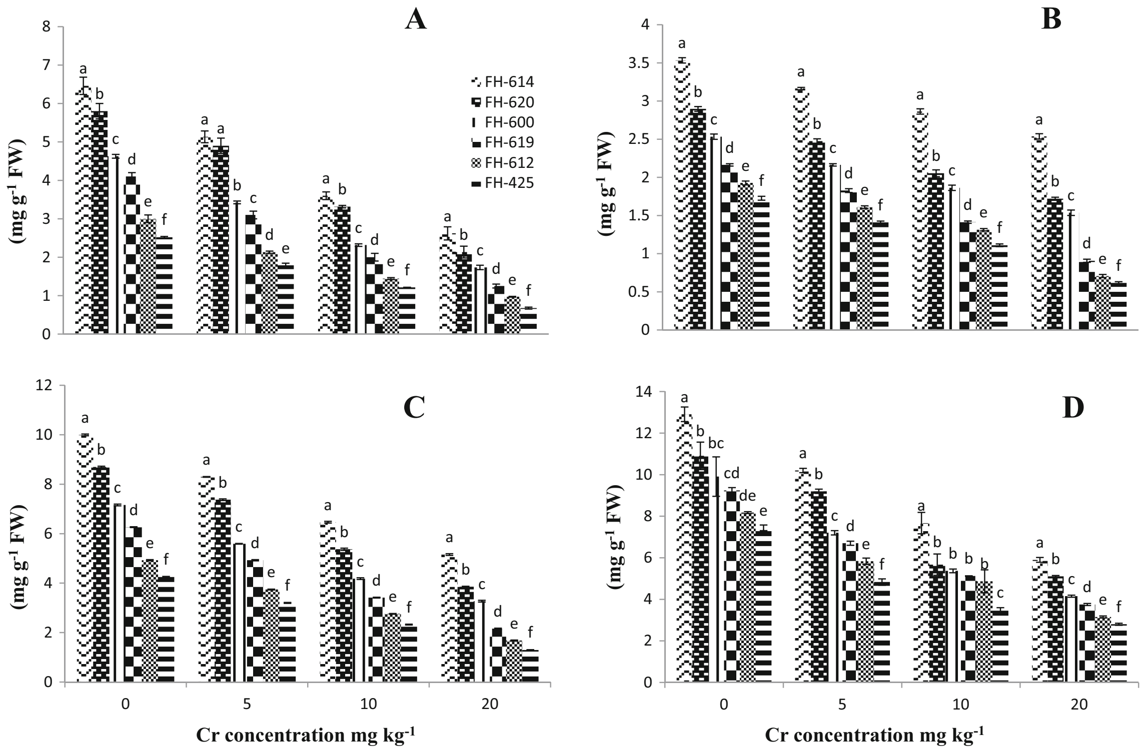

Fig. 3 Effect of Cr on chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**), and carotenoids (**d**) in six different varieties of sunflower grown in soil with increasing Cr concentrations (0, 5, 10, and 20 mg kg^{-1}). Values are demonstrated as means of three replicates along with standard deviation. *Different small letters* indicate that values are significantly different at $P < 0.05$

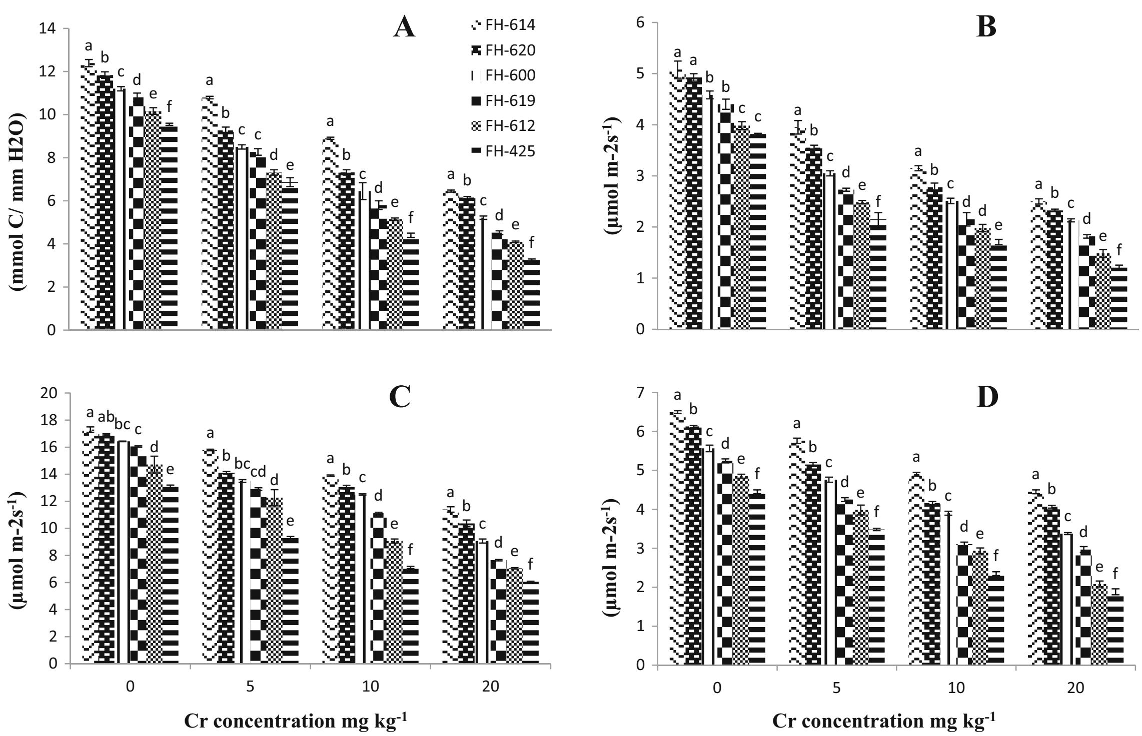

Fig. 4 Effect of Cr on water use efficiency (**a**), transpiration rate (**b**), net photosynthetic rate (**c**), and stomatal conductance (**d**) in six different varieties of sunflower grown in soil with increasing Cr concentrations (0, 5, 10, and 20 mg kg^{-1}). Values are demonstrated as means of three replicates along with standard deviation. *Different letters* indicate that values are significantly different at $P < 0.05$

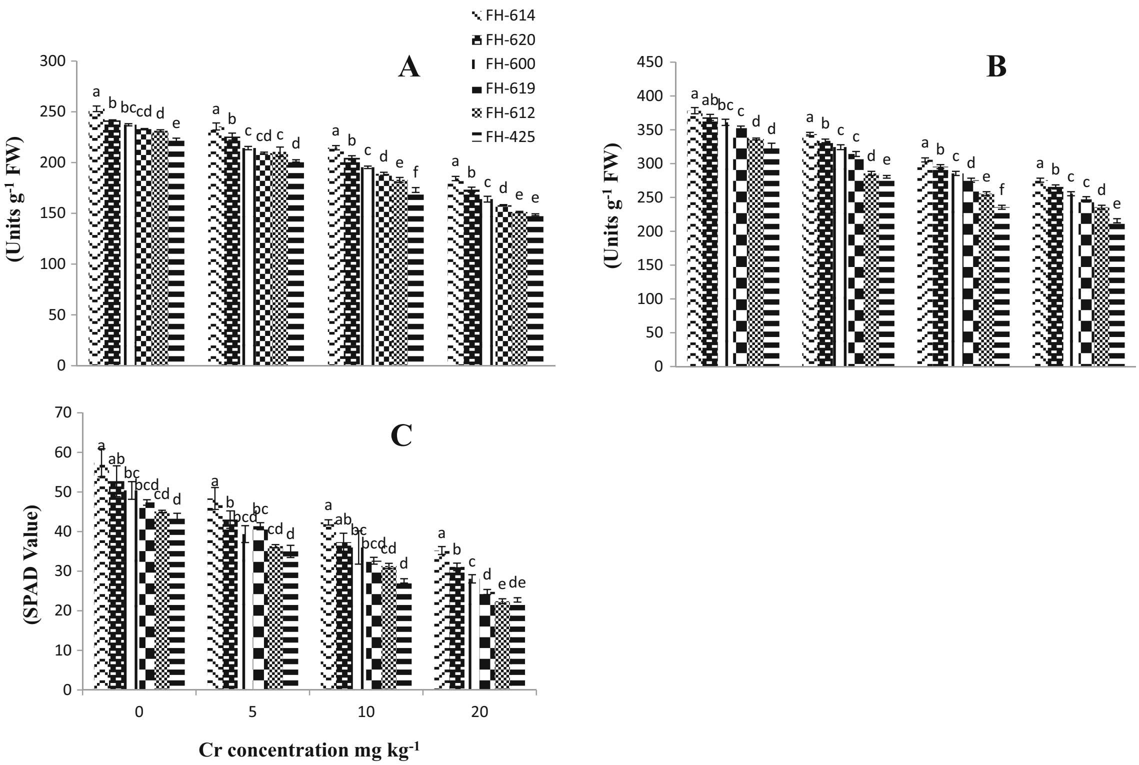

Fig. 5 Effect of on soluble protein in the root (**a**), soluble protein in the leaves (**b**), and SPAD value (**c**) in six different varieties of sunflower grown in soil with increasing Cr concentrations (0, 5, 10, and 20 mg kg^{-1}). Values are demonstrated as means of three replicates along with standard deviation. *Different letters* indicate that values are significantly different at $P < 0.05$

concentrations. Similar pattern was found in soluble protein content of the roots and leaves. The sequence of SPAD and soluble protein content in all the varieties is given below:

$$\text{FH−614} > \text{FH−620} > \text{FH−600} > \text{FH−619} > \text{FH−612} > \text{FH−425}.$$

Electrolyte leakage, MDA. and H_2O_2

Oxidative stress in the different varieties of sunflower was evaluated by measuring the concentration of electrolyte leakage (EL), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) in the leaves and root of sunflower (Supplementary Fig. 1). Increasing concentration of Cr significantly increased the EL, H_2O_2, and MDA concentration in all the varieties of sunflower. FH-614 showed tolerance to Cr, as compared to the rest of the varieties at all the levels of Cr concentration (5, 10, 20 mg kg^{-1}) while the maximum concentrations of EL, H_2O_2, and MDA were measured in FH-425. The increased concentrations of EL, H_2O_2, and MDA within the six FH varieties were as follows:

$$\text{FH−425} > \text{FH−612} > \text{FH−619} > \text{FH−600} > \text{FH−620} > \text{FH−614}.$$

Antioxidant enzyme activity

The activity of different antioxidant enzymes SOD, POD, CAT, and APX in the leaves and roots was measured to investigate the effects of Cr on antioxidant capacity of sunflower (Supplementary Fig. 2). Increasing concentration of Cr in soil affected the normal functioning of antioxidant defense system of sunflower plant in all the varieties. It was interesting that at initial concentrations of Cr (5 and 10 mg kg^{-1}), the activity of antioxidant enzymes (SOD, POD, APX, CAT) was increased, as compared to that in the control. At higher Cr concentration (20 mg kg^{-1}), the activity of these enzymes exhibited a declining trend. Enzymatic activity trend at different treatments of Cr was as follows:

$$\text{Cr 10 mg kg}^{-1} > \text{CR 20 mg kg}^{-1} > \text{Cr 5 mg kg}^{-1} > \text{Cr 0 mg kg}^{-1}.$$

According to the results, the comparison among the varieties for enzymatic activity in the roots and leaves, at different Cr concentrations, is presented below:

FH−614 > FH−620 > FH−600 > FH−619 > FH−612 > FH−425.

Chromium concentration, accumulation, and translocation factor

Cr uptake in the different cultivars of sunflower was significantly increased when plants were grown in spiked soil with gradually increasing Cr concentration, i.e., Cr 5, 10, and 20 mg kg^{-1} as compared to that in the control plants (Table 3). The translocation factor of Cr from the root to the shoot and then from the shoot to the leaf is given in (Table 4). The maximum and minimum concentration and accumulation of Cr at all the stress levels were found in FH-614 and FH-425, respectively. At each Cr treatment, the uptake and accumulation trend was as follows:

FH−614 > FH−620 > FH−600 > FH−619 > FH−612 > FH−425.

Discussion

Increasing heavy metal (HM) concentration in water and soil is becoming a major health issue around the globe due to its direct effects on biota via food chain (Shakoor et al. 2014; Shaheen and Rinklebe 2015). Previous studies widely

Table 3 The Cr concentration in all parts of plant (achene, leaf, stem, root) and accumulation per plant in six hybrids of sunflower under increasing concentration of Cr (0, 5, 10, 20 mg kg^{-1}). Values are means of three replicates ± SD. Different small letters depicts that values are significantly different at ≤0.05

Varity	Cr concentration (mg kg^{-1})				Cr accumulation (μg plant^{-1})			
	Cr 0	Cr 5	Cr 10	Cr 20	Cr 0	Cr 5	Cr 10	Cr 20
	Achene				Achene			
FH-614	0.085 ± 0.005a	2.20 ± 0.10a	6.30 ± 0.10a	13.40 ± 0.20a	0.24 ± 0.00a	4.91 ± 0.01a	10.08 ± 0.41a	16.30 ± 0.02a
FH-620	0.075 ± 0.005b	1.95 ± 0.05b	5.85 ± 0.05b	12.90 ± 0.10a	0.18 ± 0.01b	4.16 ± 0.00ab	8.28 ± 0.62b	14.83 ± 0.03a
FH-600	0.061 ± 0.001c	1.81 ± 0.03b	5.53 ± 0.03c	12.03 ± 0.20b	0.13 ± 0.00c	3.65 ± 0.02bc	6.66 ± 0.05bc	12.63 ± 0.01ab
FH-619	0.057 ± 0.002c	1.65 ± 0.05c	5.10 ± 0.05d	10.93 ± 0.25c	0.11 ± 0.01c	2.91 ± 0.02 cd	5.89 ± 0.02c	9.11 ± 0.03bc
FH-612	0.047 ± 0.001d	1.45 ± 0.05d	4.58 ± 0.07e	9.86 ± 0.20d	0.09 ± 0.00d	2.24 ± 0.00de	5.04 ± 0.41 cd	6.90 ± 0.02 cd
FH-425	0.040 ± 0.01d	1.32 ± 0.01d	3.81 ± 0.02f	8.30 ± 0.10e	0.06 ± 0.01d	1.716 ± 0.00e	3.76 ± 0.12d	5.25 ± 0.02d
	Leaf				Leaf			
FH-614	0.64 ± 0.03a	15.26 ± 0.25a	26.13 ± 1.05a	52.40 ± 2.56a	5.03 ± 0.28a	102.32 ± 4.72a	141.84 ± 9.31a	246.62 ± 22.46a
FH-620	0.52 ± 0.01b	13.76 ± 0.25b	23.13 ± 0.90ab	47.40 ± 1.57b	3.60 ± 0.03b	83.99 ± 2.90b	119.43 ± 1.16b	187.96 ± 3.76b
FH-600	0.45 ± 0.02c	12.86 ± 0.20bc	20.13 ± 0.96bc	42.06 ± 1.00c	2.97 ± 0.09b	74.65 ± 3.75c	83.96 ± 6.56c	144.42 ± 4.06c
FH-619	0.40 ± 0.01d	12.26 ± 0.83c	20.13 ± 2.65bc	38.40 ± 1.47c	2.19 ± 0.04c	51.10 ± 3.79d	57.35 ± 3.76d	76.70 ± 1.04d
FH-612	0.33 ± 0.01e	10.33 ± 0.15d	18.66 ± 0.47c	34.13 ± 0.90d	1.34 ± 0.12d	30.67 ± 2.89e	36.74 ± 3.51e	53.44 ± 1.14de
FH-425	0.30 ± 0.01e	9.10 ± 0.10e	17.20 ± 0.10c	30.13 ± 1.00d	0.94 ± 0.08e	18.49 ± 1.18f	28.38 ± 0.95e	27.146 ± 3.41e
	Stem				Stem			
FH-614	0.61 ± 0.01a	24.26 ± 0.92a	45.13 ± 0.90a	73.40 ± 1.47a	10.71 ± 0.70a	370.72 ± 24.08a	565.43 ± 32.64a	770.53 ± 34.35a
FH-620	0.51 ± 0.01b	21.73 ± 0.75b	41.60 ± 0.65b	67.56 ± 0.50b	7.54 ± 0.30b	269.36 ± 6.56b	449.45 ± 23.70b	558.49 ± 14.54b
FH-600	0.45 ± 0.00c	18.50 ± 0.50c	37.13 ± 0.56c	59.56 ± 1.74c	6.18 ± 0.05c	217.15 ± 10.51c	351.43 ± 7.47c	456.43 ± 11.55c
FH-619	0.39 ± 0.00d	16.50 ± 0.50c	33.03 ± 0.55d	52.23 ± 0.80d	4.50 ± 0.18d	152.68 ± 6.29d	251.01 ± 5.59d	353.51 ± 16.34d
FH-612	0.33 ± 0.02e	13.16 ± 1.04d	25.80 ± 1.13e	45.23 ± 1.20e	3.05 ± 0.33e	106.71 ± 9.67e	185.013 ± 11.9e4	282.09 ± 17.51e
FH-425	0.26 ± 0.01f	11.53 ± 0.50d	20.46 ± 1.05f	39.23 ± 0.80f	2.00 ± 0.12f	73.06 ± 4.21f	111.94 ± 7.87f	173.71 ± 12.35f
	Root				Root			
FH-614	0.90 ± 0.04a	36.10 ± 1.15a	58.46 ± 0.72a	100.01 ± 4.58a	9.72 ± 0.21a	319.88 ± 6.76a	424.75 ± 10.39a	577.43 ± 51.26a
FH-620	0.79 ± 0.10b	32.43 ± 0.51ab	53.13 ± 0.85ab	92.33 ± 2.51b	6.96 ± 0.33b	222.75 ± 12.22b	279.91 ± 16.21b	348.23 ± 28.79b
FH-600	0.70 ± 0.02c	29.66 ± 1.52bc	48.80 ± 1.99bc	86.05 ± 1.02bc	6.35 ± 0.51b	216.96 ± 9.97b	306.60 ± 17.02b	392.86 ± 22.38b
FH-619	0.61 ± 0.01d	29.33 ± 2.51bc	43.13 ± 1.10 cd	80.66 ± 1.52c	4.558 ± 0.19c	191.45 ± 21.63b	172.59 ± 11.05c	242.13 ± 19.29c
FH-612	0.55 ± 0.01de	26.00 ± 1.00 cd	39.13 ± 0.96de	74.01 ± 1.01d	3.46 ± 0.15d	137.83 ± 7.03c	157.97 ± 11.92c	194.76 ± 8.69 cd
FH-425	0.50 ± 0.03e	23.03 ± 1.01d	31.80 ± 6.7e	68.33 ± 1.52d	2.722 ± 0.23d	87.02 ± 4.73d	94.24 ± 26.30d	143.56 ± 14.85d

Table 4 Translocation factor (TF) of Cr from the root to the shoot and the shoot to the leaf

Varity	Cr Concentration (mg kg^{-1})							
	TF shoot to root				TF leaf to shoot			
	Cr 0	Cr 5	Cr 10	Cr 20	Cr 0	Cr 5	Cr 10	Cr 20
FH-614	0.67 ± 0.02	0.67 ± 0.03	0.77 ± 0.02	0.73 ± 0.03	1.04 ± 0.03	0.62 ± 0.22	0.57 ± 0.2	0.71 ± 0.25
FH-620	0.64 ± 0.02	0.67 ± 0.03	0.78 ± 0.02	0.73 ± 0.01	1.01 ± 0.01	0.63 ± 0.21	0.55 ± 0.1	0.70 ± 0.15
FH-600	0.64 ± 0.01	0.62 ± 0.04	0.76 ± 0.02	0.69 ± 0.01	1.00 ± 0.02	0.69 ± 0.30	0.54 ± 0.3	0.70 ± 0.10
FH-619	0.64 ± 0.01	0.56 ± 0.04	0.76 ± 0.01	0.64 ± 0.02	1.01 ± 0.01	0.74 ± 0.30	0.60 ± 0.07	0.73 ± 0.07
FH-612	0.60 ± 0.05	0.50 ± 0.04	0.65 ± 0.01	0.61 ± 0.00	1.00 ± 0.01	0.78 ± 0.14	0.72 ± 0.05	0.75 ± 0.09
FH-425	0.51 ± 0.01	0.50 ± 0.03	0.66 ± 0.01	0.57 ± 0.02	1.16 ± 0.04	0.78 ± 0.17	0.84 ± −.04	0.76 ± 0.10

Values are means of three replicates ± SD

reported a sever reduction in seed germination, plant growth, biomass, and yield under HM stress (Farid et al. 2015; Adrees et al. 2015a; Ramzani et al. 2016). Similarly, in present study, the increasing concentration of Cr in the soil reduced the germination potential, growth, and biomass of the six hybrids of sunflower (Figs. 1 and 2, Table 2). It has been observed that the smaller concentrations of HMs are beneficial for sunflower seeds as described by Imran et al. (2013). Under lower concentration (2–4 mg L^{-1}) of arsenic (As), an increase was observed in radicle and plumule length of seedlings while the same was not detected under Cr stress. Similar to our results, Cd decreased the seed germination rate and imposed seed dormancy (Junior et al. 2016), while sunflower seeds have higher seed germination rate as compared to soybean and brassica under lead (Pb) and cadmium (Cd) stress (Lee et al. 2013). The similar results were also observed in different varieties of maize (Akhtar et al. 2017), sunflower (Saleem et al. 2015; Atta et al. 2013), *Brassica napus* (Gill et al. 2016a), and mung bean (Jabeen et al. 2016) under Cd and Cr stress. Different varieties showed variable behavior under increasing Cr concentration (Figs. 1 and 2). Higher concentrations of Cr (10 and 20 mg kg^{-1}) reduced leaf area, plant height, root length, and biomass but the number of leaves and flowers remained unaffected. The similar results were found in sunflower under Cd (Cornuet et al. 2016), Pb (Azad et al. 2011; Kastori et al. 1998), Cu (Jiang et al. 2000; Kolbas et al. 2014, 2015), Co, Ni, and Cr (Gopal and Khurana 2011) stress. The decline in plant's physiological parameters was due to the rapid uptake of Cr, and the results were clear in the form of toxic symptoms on plant leaves (chlorosis and necrosis), lower plant height and reduced number of leaves (Júnior et al. 2014). Presence of heavy metals in growth media hampers the uptake of other essential minerals, which are required for normal plant growth and development. So, the development of toxic symptoms might be due to the deficiency of essential minerals owing to Cr stress (Kötschau et al. 2013; Rivelli et al. 2014).

Photosynthetic pigments are very sensitive to HM stress and therefore can be trustworthy indicators to monitor HM toxicity (Anjum et al. 2015). Significant reduction in chlorophyll (a, b and total chlorophyll) and carotenoids, with increasing Cr concentration (5, 10, 20 mg kg^{-1}), was attributed to inhibited uptake and translocation of mineral nutrients (Atta et al. 2013; Kötschau et al. 2013). FH-614 proved to be the most tolerant sunflower hybrid to Cr stress and showed very little reduction in photosynthetic pigments, as compared to the other varieties. Sunflower showed similar results under Cr (Saleem et al. 2015), Cd (De Maria et al. 2013), and Pb (Azad et al. 2011; Kastori et al. 1998) application. Similar trend was observed in *Brassica napus*, mung bean, and tobacco under Cr stress by Gill et al. (2016b), Jabeen et al. (2016), and Bukhari et al. (2016b), respectively. This phenomenon also supported by the reduction in chlorophylls and carotenoids in *Brassica napus* under Cd (Ehsan et al. 2014; Farid et al. 2015), Pb (Shakoor et al. 2014; Kanwal et al. 2014), and Cu (Habiba et al. 2015), and in cotton under Ni (Zaheer et al. 2015) and Pb (Anwaar et al. 2015) stress.

In present study, a significant variation was observed in the gas exchange attributes of all the sunflower varieties which might be the consequence of decreased chlorophylls, carotenoid content, and activities of CO_2-fixating enzymes (Rizwan et al. 2017). Furthermore, the HMs interrupt metabolic activities in plant organelles and tissues, particularly in the chloroplast and leaves, which subsequently change the structure and functions of photosystem II by disturbing electron transport chain (Qadir et al. 2004; Di Cagno et al. 2001). Many studies have reported the alteration in water status of plants under HM stress (Barceló and Poschenrieder 1990; Perfus-Barbeoch et al. 2002). Atta et al. (2013) stated that higher concentration of Cr reduced the water use efficiency, transpiration, photosynthesis, and stomatal conductance in the leaves of sunflower hybrids, and reduction rate was directly proportional to the Cr concentration. Our results are in accordance with recent studies under different heavy metals such as Cr on mung bean and *Brassica* (Jabeen et al. 2016; Afshan et al. 2015), As + Cd and Cr + Al on maize (Anjum et al. 2015, 2016a), and Pb + Cd + Cu + Ni on *Brassica napus* (Shakoor et al. 2014; Ehsan et al.

2014; Habiba et al. 2015; Kanwal et al. 2014; Farid et al. 2015).

Soil plant analysis development (SPAD) and soluble proteins content significantly decreased in all the six hybrid varieties of sunflower under increasing Cr concentration (Fig. 5). Higher concentration of heavy metals in the rhizosphere and plant tissues inhibits the nutrient and water uptake and their translocation from the roots to the aboveground parts of plant as observed by Farooq et al. (2016) and Rizwan et al. (2017). Meanwhile, the production of ROS ($O_2^{\cdot-}$, ·OH) and EL also decreased the soluble proteins in plant tissues (Ehsan et al. 2014). Similar results were found under Cr (Gill et al. 2015), Cd (Wahid et al. 2008), Cu (Adrees et al. 2015a), Ni (Kanwal et al. 2014), and Pb (Shakoor et al. 2014). Gupta et al. (2009) reported that the decrease in soluble protein content in *Zea mays*, grown hydroponically, was due to the oxidative damage caused by the Pb. Park et al. (2012) and Ghani (2011) reported the similar results in *Brassica*. The results of the present investigation are in line with previous findings that the excess Cr concentration decreased the leaf greenness (pigment content), an indicator of higher SPAD value (Gill et al. 2015; Singh et al. 2013). This decrease in SPAD value might be due to the anatomical deformation of chloroplast under metal stress (Najeeb et al. 2011).

Oxidative stress and electrolyte leakage in plants under heavy metal stress have been widely reported in recent studies (Gallego et al. 2005. In the present study, similar results have been found under Cr stress in sunflower hybrids regarding oxidative damage and electrolyte production (Supplementary Fig. 1). Increase in ROS and EL under escalating Cr concentration in all the hybrids showed different effects. The FH-614 showed tolerance to ROS and EL production by activating the ROS scavenging antioxidant defense system as described by Shakoor et al. (2014) and Farid et al. (2015). The increasing concentration of Cr in the plant cells and tissues causes K^+ efflux, which is abundant in plant cells mediated by two ion channel groups (Demidchik et al. 2014). Similarly, the ROS are generated by the reaction associated with K^+ leakage in plants under metal stress. In most cases, the biosynthesis of ROS, redox regulation, and oxidative stress is started in plants by the production of O_2^- via single-electron reduction from triplet oxygen (O_2) (Demidchik 2012). Further, the sequential formation of H_2O_2 and OH^- is done by the addition of two electrons, respectively (Halliwell and Gutteridge 1999). Many studies reported that plants produced H_2O_2, O_2^-, and OH, and caused EL in response to heavy metals (Wang et al. 2008; Ehsan et al. 2014), salinity (Arshad et al. 2016; Di Cagno et al. 1998), pathogens (Giovanini et al. 2006), drought (Noman et al. 2015), hyperthermia (Dong et al. 2009), herbicides (Song et al. 2007), hypothermia (Edreva et al. 1998), and other stresses. Our results are in accordance with many studies performed under different heavy metals such as *Brassica napus* and *Alternanthera bettzickiana* under Cr, Cd, Pb, Cu, and Ni (Afshan et al. 2015; Ehsan et al. 2014; Shakoor et al. 2014; Habiba et al. 2015; Kanwal et al. 2014; Tauqeer et al. 2016); mung bean and wheat under Cr (Jabeen et al. 2016; Adrees et al. 2015b; Ali et al. 2015); and cotton under Pb, Cd, (Farooq et al. 2013), Zn (Anwaar et al. 2015), and Ni (Khaliq et al. 2016).

Naturally, plants have developed an antioxidant defense system consisting of some specific metabolites and antioxidant enzymes which help plants survive and adapt under HM stress (Shakoor et al. 2014; Adrees et al. 2015b). The oxidative stress, generated by ROS, alters the activities of antioxidant enzymes (Supplementary Fig. 2). This vital approach helps the plants reduce HM stress (Shamsi et al. 2014), while increasing concentration of ROS, coupled with growth inhibition, further deteriorates the activities of antioxidant enzymes (Schützendübel et al. 2001; Shakoor et al. 2014). At lower concentration of Cr (5 and 10 mg kg^{-1}), the activities of all the enzymes SOD, POD, CAT, and APX were increased, as compared to those in the controls, followed by a decline at higher Cr concentration (20 mg kg^{-1}). The sunflower hybrids responded differently to applied Cr stress. FH-614 developed a good oxidative defense system and increased its enzymatic activities to reduce the oxidative damage. On the other hand, FH-425 could not enhance enzymatic activities to counter act the oxidative stress and hence showed more toxic effects, as compared to FH-614. Chen and Murata (2011) concluded that under higher metal stress, the antioxidant enzyme activities were reduced due to overproduction of ROS. These observations indicate the ability of plants to withstand Cr stress, by stimulating antioxidant enzyme activities, up to a certain extent. But at higher metal concentration, plant loses its capability to cope with the deleterious effects of Cr (Belhaj et al. 2016).

The present study revealed a variable response of different plant parts to Cr stress in terms of antioxidant enzyme activities. Higher activity of CAT and SOD was observed in the roots as compared with the leaves. However, POD and APX behaved opposite to CAT and SOD (Supplementary Fig. 2. Previously, it has been reported that the activities of POD were higher in *Brassica napus* leaves as compared to those in the roots under Cu stress (Habiba et al. 2015), while CAT and SOD showed an opposite response to Cd stress in hydroponics (Ehsan et al. 2014). The fluctuations in the activities of antioxidant enzymes might be due to the difference in plant species, type and concentration of metal stress as well as growth conditions (Gallego et al. 2005).

During active growth stages, plants rapidly uptake mineral elements and also absorb organic and inorganic contaminants attached with them, from the growth medium. These contaminants are then translocated to aboveground parts of the plants (Rizwan et al. 2017; Ali et al. 2015). The contaminants cause toxicity in plants by destroying plant tissues and cells (Adrees et al. 2016). The plants can tolerate a certain level of heavy metal stress which varies with plant species, genotype, and concentration of metal in the growth media (Arshad et al. 2016; Bukhari et al. 2015). The present study revealed a variable response of different sunflower hybrids to the same concentration of Cr (Table 2). FH-614 contained higher amount of Cr in all the plant parts with overall better growth and biomass production, as compared to the FH-425. The difference in Cr concentration and plant growth attributes shows the effectiveness of antioxidant defense system to counteract the Cr toxicity (Belhaj et al. 2016; Meers et al. 2005a). Oil seed crops are considered to be the hyper-accumulator of heavy metals due to their higher biomass, rapid uptake, and greater translocation factor from the roots to the shoots (Rizwan et al. 2017). Increased Cr concentration was inversely proportional to the plants' physiological and biochemical formations while it was directly proportional to the ROS and EL, which reduced the overall plant growth (Adrees et al. 2015a; Ali et al. 2013). The similar trend of Cr toxicity and accumulation was found in sunflower, *Brassica napus*, mung bean, castor bean, wheat, and barley (Fozia et al. 2008; Afshan et al. 2015; Ali et al. 2013).

Conclusions

The present study concluded that the increasing concentration of Cr caused a significant decline in growth, biomass, photosynthesis, gas exchange attributes, and antioxidant enzyme activities of sunflower hybrids, by elevating ROS production and electrolyte leakage, in genotype-dependent manner. FH-614 accumulated the highest amount of Cr and proved to be the most tolerant sunflower hybrid with least toxic effects, as compared with the other genotypes, by virtue of strong antioxidant defense system. However, FH-425 was found to be the most vulnerable to Cr toxicity. The FH-614 could be used for further studies to uncover the molecular basis of underlying Cr tolerance mechanism. The present study also indicates the possibility of employing sunflower for the phytomanagement of heavy metal.

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