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Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid

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ABSTRACT

Soil contamination with heavy metals is threatening the food security around the globe. Chromium (Cr) contamination results in poor quality and reduction in yield of crops. The present research was performed to figure out the Cr toxicity in sunflower and the ameliorative role of 5-aminolevulinic acid (ALA) as a plant growth regulator. The sunflower (FH-614) was grown under increasing concentration of Cr (0, 5, 10 and 20 mg kg^{-1}) alone and/or in combination with 5-ALA (0, 10 and 20 mg L^{-1}). Results showed that Cr suppressed the overall growth, biomass, gas exchange attributes and chlorophyll content of sunflower plants. Moreover, lower levels of Cr (5 and 10 mg kg^{-1}) increased the production of reactive oxygen species (ROS) and electrolyte leakage (EL) along with the activities of antioxidant enzymes i.e., superoxide dismutase (SOD), guaiacole peroxidase (POD), ascorbate (APX), catalase (CAT). But at higher concentration of Cr (20 mg kg^{-1}), the activities of these enzymes presented a declining trend. However, the addition of 5-ALA significantly alleviated the Cr-induced toxicity in sunflower plant and enhanced the plant growth and biomass parameters along with increased chlorophyll content, gas exchange attributes, soluble proteins and soil plant analysis development (SPAD) values by scavenging the ROS and lowering down the EL. The 5-ALA also enhanced the activities of antioxidant enzymes at all levels of Cr. The increase in Cr concentration in all plant parts such as leaf, root and stem was directly proportional to the Cr concentration in soil. The application of 5-ALA further enhanced the uptake of Cr and its concentration in the plants. To understand this variation in response of plants to 5-ALA, detailed studies are required on plant biochemistry and genetic modifications.

1. Introduction

Increasing population is the main cause of extensive agricultural practices around the world. Irrigation of crops and vegetables with municipal/industrial wastewater provides an easy route to various organic and inorganic pollutants to enter food chain. Among various inorganic pollutants, Heavy metals (HMs) are non-essential and carcinogenic in nature, which prevail in the environment due to their widespread application in tanning, steel, textile, painting, polishing and catalytic manufacturing units (Akram et al., 2012). Chromium (Cr) occurs in various oxidation states. The most stable states are Cr (III) and Cr (VI) (Farid et al., 2017). Chromium-VI is very toxic due its high stability and carcinogenic properties in addition to its ability to cause necrosis, bronchitis, asthma and dermatitis in humans (Farid et al., 2017). However, Cr-III is considered essential at trace level for the metabolism of glucose and lipids where it acts as a cofactor to enhance the insulin activity (Afshan et al., 2015). The use of industrial wastewater for irrigation and domestic purposes provides an opportunity for

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Cr (VI), due to its mobility and solubility, to enter the food chain (Afshan et al., 2015). All plants absorb Cr along with other micronutrients from the soil (Jabeen et al., 2016). The important food crops grown on HM contaminated soils faced growth inhabitation, yield reduction and deterioration in the quality of produce (Rizwan al, 2016a), which is an alarming situation to fulfill the food requirement of ever increasing population at global level.

Phytoremediation is effective and can be viewed as a relatively low cost, solar energy driven process for the management of contaminated soils (Farooq et al., 2016a, 2016b). It involves the extraction, translocation and accumulation of large quantity of contaminants in above-ground plant parts for safe disposal (Shakoor et al., 2014). But the heavy metal toxicity adversely affects the plant growth and development, ultimately leading to death under severe conditions (Bukhari et al., 2016a; Jabeen et al., 2016). Oilseed crops are being used worldwide as source of edible oil (Rizwan et al., 2016b). Sunflower is considered to be more heavy metal tolerant because of its higher biomass, fast growth, greater translocation factor and ability to store heavy metal in its fatty tissues (Shakoor et al., 2014). Sunflower has specific mechanisms to tolerate small concentration of HMs (Rizwan et al., 2016b) but during early growth stages, prolonged exposure of plants to higher level of HMs adversely affects their growth and development (Bukhari et al., 2015; Rizwan et al., 2016b). The continuous use of industrial and municipal waste water for the irrigation of crops is increasing the risk of land degradation with organic and inorganic pollutants. The sunflower can be used to remediate these soils by the accumulation of pollutants in above ground biomass (Farid et al., 2017). Many recent studies proved the suitability of sunflower plant for the extraction of heavy metals like Pb, Cr, Cu, Cd and Zn from the contaminated soils (Rizwan et al., 2016a, 2016b). Shaheen and Rinklebe (2015) reported that the sunflower showed higher efficiency to extract Zn, Ni, Pb and Cd as compared to Al, Cu, Mo and Se.

The use of phytoremediation with various soil amendments has attracted the researchers during recent years (Farooq et al., 2016a, 2016b). There are many examples of organic and inorganic acids which can be used to support the plant under heavy metal stress (Rizwan et al., 2016b). In addition, many chelators, stress alleviators and plant growth regulators (PGR) are being used to enhance the efficiency of plants in phytoremediation process (Bukhari et al., 2016b; Gill et al., 2015). Recent studies described the suitability of citric acid as chelating agent (Afshan et al., 2015), glycinebetain, mannitol, fulvic acid and silicon as stress alleviator (Farooq et al., 2016a, 2016b) and 5-ALA as PGR under HM, drought and salinity stress (Feng et al., 2015). Rizwan et al. (2016a) reported that the HM stress may decrease the endogenous secretion of PGR in plant tissues and the exogenously applied PGRs may facilitate the plants in stress tolerance. Gill et al. (2015) reported that 5-ALA is a useful candidate for *Brassica napus* under heavy metal stress like Cr and Cd. Similar results were reported by Akram et al. (2012) for sunflower, Beyzaei et al. (2014) for barely and Feng et al. (2015) for *Litchi chinensis* (Lychee). So, the present study was planned with the objectives (i) to identify the toxic effects of Cr on the morpho-physiological and biochemical processes of sunflower (ii) to assess the suitability and promoting role of 5-ALA in sunflower growth (iii) and the phytoextraction potential of sunflower for Cr under the application of 5-ALA.

2. Materials and methods

2.1. Soil and plant material

A loamy clay soil (24% sand, 21% silt, 55% clay), collected at 0–15 cm depth from botanical garden of Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan was used in the study. Before pot experiment, the soil was air-dried and passed through a 2 mm diameter sieve to remove stones and crop residues. Mature seeds of Sunflower (*Helianthus annuus* L.) genotype (Faisalabad Hybrid FH-614, Screened from first study, Farid et al., 2017) were taken from Oilseeds Research Institute, AARI, Faisalabad, Pakistan. The seeds were washed with 10% H_2O_2 and thoroughly rinsed with distilled water. Ten seeds 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 5 plants per pot and the removed plants were crushed and added to the same pot. Each soil 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 washed with distilled water to avoid contamination.

2.2. Treatments

After six weeks of germination, uniform plants (pots) were treated with increasing concentration of 5-Aminolevulinic Acid (5-ALA; 0, 10, 20 mg L^{-1}). In total there were twelve treatments, T_1: Cr (0 mg kg^{-1}) + 5-ALA (0 mM); T_2: Cr (5 mg kg^{-1}); T_3: Cr (10 mg kg^{-1}); T_4: Cr (20 mg kg^{-1}); T_5: 5-ALA (10 mg L^{-1}); T_6: 5-ALA (20 mg L^{-1}); T_7: Cr (5 mg kg^{-1}) + 5-ALA (10 mg L^{-1}); T_8: Cr (10 mg kg^{-1}) + 5-ALA (10 mg L^{-1}); T_9: Cr (20 mg kg^{-1}) + 5-ALA (10 mg L^{-1}), T_{10}: Cr (5 mg kg^{-1}) + 5-ALA (20 mg L^{-1}), T_{11}: Cr (10 mg kg^{-1}) + 5-ALA (20 mg L^{-1}), T_{12}: Cr (20 mg kg^{-1}) + 5-ALA (20 mg L^{-1}). Each treatment was replicated thrice and the experimental pots were rotated randomly in the wire house. The pots were free of weeds throughout the growing season. Soil was spiked with different concentrations of Cr (0, 5, 10, 20 mg kg^{-1} dry weight) while 5-ALA was sprayed to completely wet both sides of leaves after every 4 days for next 8 weeks.

2.3. Plant sampling and analysis

Plants were harvested after eight weeks of treatments. Plant samples were washed thoroughly and tissue papers were used to clean them. All plants were carefully separated into roots, stems and leaves. Growth parameters including plant height, root and stem length, number of leaves and flowers per plant, area of leaves, 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.

2.4. Pigment content assay

After eight weeks of treatments, the second uppermost fully extended fresh leaf of Sunflower plants was used for measuring chlorophyll (a, b, total chlorophylls) and carotenoids. The pigments were removed in the dark with 85 percent (v/v, Sigma) aqueous acetone solution at 4 °C by insistent shaking until the leaves become colorless. Then, the mixture was centrifuged at 4000 × rpm and 4 °C for 10 min. The supernatant was used for light absorbance by spectrophotometer (Halo DB-20/ DB-20S, Dynamica Company, London, UK) at 663, 644 and 452.5 nm (Metzner et al., 1965). The chlorophylls and carotenoids were calculated according to the adjusted extinction coefficients and equations given by Lichtenthaler (1987).

$$\text{Chlorophyll a } (\mu g/mL) = 10.3 \times E_{663} - 0.98 \times E_{644}$$

$$\text{Chlorophyll b } (\mu g/mL) = 19.7 \times E_{644} - 3.87 \times E_{663}$$

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

$$\text{Total carotenoids } (\mu g/mL) = 4.2 \times E_{452.5} - \{(0.0264 \times \text{chl a}) + (0.426 \times \text{chl b})\}$$

At the end, these pigment fractions were calculated as mg g^{-1} fresh