Assessing Some Emerging Effects of Hexavalent Chromium on Leaf Physiological Performance in Sunflower (*Helianthus annuus* L.)

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Abstract— Sunflower plants of two cultivars (Hysun-33 & SF-5009) were treated with varying Cr^{6+}levels (mg kg-1) and leaf performance for photosynthetic rate (CO_2 exchange) and total chlorophyll contents was assessed. Post flowering data showed inhibitory effects of Cr application on photosynthetic rate and foliar chlorophyll contents. Limitations for intracellular CO_2 assimilation, transpiration rate and stomatal conductance found associated with inhibition of photosynthesis. Increased water use efficiency and CO_2 use potential of plants indicated more water and CO_2needto plants. Both factors predicted development of drought mechanism and stressed carbohydrate biosynthesis during photosynthesis in Cr affected plants, respectively. Cr load in roots was more than leaves due to root efficient absorption. Cr accumulation particularly in leaves interrupted chlorophyll photoactivity performance to inhibit photosynthesis. The rate of reduction increased from lower to higher Cr doses. Gradual response of plants towards low Cr doses was due to resistant mechanism of plants than at higher Cr doses. Cr application 50 mg/kg developed positive effects on leaf performance of both test varieties revealing phytoactive than phytotoxic. Sunflower variety SF-5009 was less affected by Cr than Hysun-33.

Index Terms— Hexavalent, Chromium, Sunflower, photosynthesis, chlorophyll, photoactivity, phytotoxic, phytoactive.

1 Introduction

HEAVY metals are the elements having density over 5g/cm3and pollute soil and water due to anthropogenic activities (Alloway, 1995; Rouphael et al., 2008). Rapid growth of human population and industrialization found to be a major cause of heavy metal pollution (Wahid et al., 2000). Burning of fossil fuel, mining, smelting of metalloids, fertilizers, pesticides, dust accumulation, leather & textile processes and waste water irrigation has increased this pollution on biosphere by interacting biotic and abiotic environmental components (Dube et al., 2001; Younis et al. 2013 a & b).

In nature, chromium occurs as chromite ($Fe_2Cr_2O_4$) and tarapacaite ($K_2Cr_2O_7$) and forms complexes with other metals i.e. $PbCrO_4$ (Babula et al., 2008). Cr has two stable oxidation states Cr^{6+} and Cr^{3+} where Cr^{6+} is considered more toxic, less water soluble than Cr^{3+}(Panda & Patra, 2000; Nieboer & Richardson,1980). In aquatic environment, human activities and weathering of Cr-enriched rocks causes oxidation, reduction, adsorption, dissolution and precipitation of Cr. At high pH Cr^{3+} precipitates and becomes soluble at acidic pH while Cr6+ forms are soluble at all pH conditions (Kimbrough et al., 1999). Cr^{3+} is up taken by passive means while Cr^{6+} is absorbed by active mechanism of plant membranes and competes with essential elements (Zayed & Terry, 2003; Kim et al., 2006).

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Soil and water contamination by Cr is of great concern which has negative effects on plant physiological processes like photosynthesis, mineral nutrition, water relations, respiration and turns land barren by loss of vegetation (Azmat & Khanum, 2005; Clijsters & Van Assche, 1985; Dube et al., 2003). Cr inhibited photosynthesis is confined to abnormal stomatal conductance, reduced intercellular spaces, chloroplast structural alteration and oxidative stress in plants that ultimately reduces growth and yield (Vazquez et al., 1987; Arun et al.,2005). Heavy metals enriched plants also possess health risks to consumers (Stobrawa et al., 2008).

Sunflower (*Helianthus annuus* L.) is an important seed oil crop in the world and in Pakistan. Its seeds contain 40% edible oil (PARC, 2007). As physiological attributes of leaf has strong relation with growth and yield of plant, so we focused these attributes in sunflower cultivars and various effects from Cr metal were assessed.

2 Materials and Methods

2.1 Experimental Trail

For two sunflower hybrids i.e. Hysun-33 (variety- A) andSF-5009 (variety-B), a pot experiment was conducted in Bio-Park of Bahauddin Zakariya University, Multan (Pakistan) during February-June, 2012 and data was observed at post flowering stage. A crystalline $K_2Cr_2O_7$ salt (Merck-Germany) was used as source chemical for hexavalent chromium. Various Cr^{6+} doses (50, 100, 150, 250, 350, 400 & 500 mg kg-1 of pot soil) were prepared and mixed with the pot soil. Salt weighing was executed with digital balance (MK-200B, Chyo-Japan). Each treatment including control was comprised with seven replicates and a total of 112 pots were arranged in a completely randomized block design (CRBD).Sandy loam, humified soil with pH 7.21, EC 1.73 ds/cm and organic matter

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7.12% was filled in well set earthen pots of 16 inch diameter. In each pot, six certified and uniform sized seeds were sown at equal distance and depth. Tap water was used to irrigate pots and thinning was performed at day 20 when seedlings attained an approximate size of 6 inch. For either variety two plants/pot were left as harvest.

2.2 Measuring Parameters

2.2.1 Rate of Photosynthesis

At day 90 (90-DAS) plant physiological analysis was carried out using a portable infrared gas analyzer (IRGA, ADC-LCA4/ Analytical Development Company, England). At each day, analysis conceded out during full sunny days from 9:00-11:00 (AM). Three young similar aged leaves were analyzed through leaf chamber and mean values were used to manipulate data. For physiological study; intracellularCO_2 assimilation (Ci = Vpm), rate of photosynthesis (A = µM m-2 s-1), transpiration rate (E = m. mol m-2 s-1), stomatal conductance (gs = mol m-2 s-1),water utilizing efficiency (WUE = A/E) and CO_2 use potential(CUP = Ci/A) of plants were measured. Leaf chamber was specified with temperature range 28-32.4ºC, ambient pressure 98 k Pa and PAR at leaf surface (Q_{leaf}) was kept 800-900 µmol m-2 s-1.

2.2.2 Total chlorophyll contents

Total chlorophyll contents were measured by using a chlorophyll meter SPAD-502 (Konica-Japan) having SPAD-unit accuracy ± 1. The selected plant leaf was inserted into leaf chamber followed by a gentle press and chlorophyll reading was noted on screen.

2.2.3 Cr metal uptake study

Cr heavy metal in roots and leaves was estimated by combustion &digestion method (Panichev et al., 2005). 1g oven dried homogenized plant sample was ashed in muffled electric furnace at 650 ºC, cooled in glass desiccator and dissolved in a mixture of dilute HNO_3 & HCl. The volume of solution was then increased up to 25ml by adding distilled water. At 357.9 nm, chromium metal was estimated using Perkin-Elmer atomic absorption spectrometer.

2.2.4 Statistical analysis of Data

The data was analyzed by one-way ANOVA, Duncan's Multiple Range Tests (Steel & Torrie, 1984) at α-0.05 using SPSS-17.0statistical software. Standard deviation was also applied.

3 Results

3.1Rate of photosynthesis

Table-1shows significant effects (P≤0.05) of Cr application on various leaf physiological aspects in sunflower plants from both test varieties. Intracellular CO_2 assimilation (Ci), rate of photosynthesis (A), transpiration rate (E) and stomatal conductance (gs) were strongly affected by Cr as compared to control. The reduction line was consistent at 100-150 mg/kg Cr dose i.e. 2.69-7.47%, 6.60-11.39%, 0.66-10.55% and 2.42-16.25%, respectively. At 250-500mg/kg Cr; leaf performance was rapidly declined by 13.28-23.68%, 21.14-40.34%, 27.72-43.14% and 25.25-58.09%,respectively.Cr dose 50 mg/kg exposed positive emerging effects on photosynthetic rate (A) and its associated attributes. Water use efficiency (WUE)and CO_2 use potential (CUP) of plants increased along increasing Cr concentration gradient up to 3.3&13.1%, showing significance behavior of Cr upon leaf physiological attributes in both varieties.

3.2 Total chlorophyll contents in leaves

Foliar chlorophyll contents decreased pretentiously (P≤0.05) along increasing Cr application. For both test varieties, rate of chlorophyll reduction was 6.99-11.44% and 18.80-30.88% at 100-150mg/kg & 250-500 mg/kg of Cr, respectively. The reduction line increased from low to high Cr dose level. At 50 mg/kg of Cr, both sunflower cultivars showed a positive response to hexavalent chromium where total chlorophyll contents were better than control (Table-2). Mean reduction values for physiological attributes also revealed Cr significant effects (Fig.1&2).

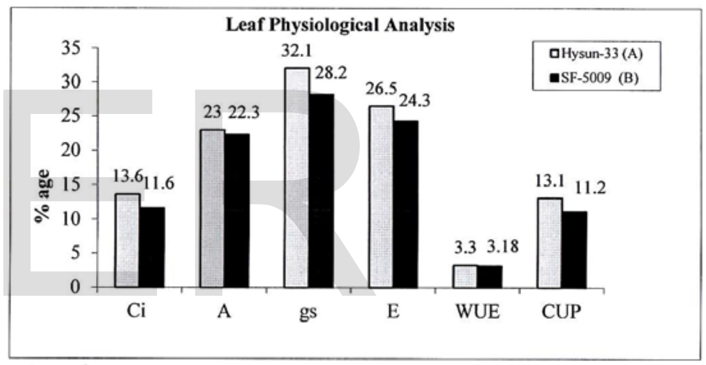

Fig. 1: Mean decrease in *Ci, A, gs, E* & increase *WUE, CUP* in Cr affected hybrids

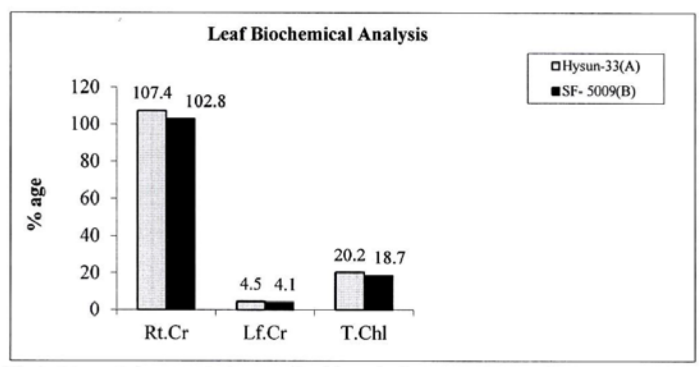

Fig.2: Mean Cr increase& decrease in chlorophyll contents in Cr affected hybrids

Table 1: Physiological analysis of two sunflower varieties treated with varying Cr^{6+} levels.

SOV	0	50	100	150	250	350	400	500	F-value
	Cr mg/kg of soil								
Ci(A)	335.8±6.87	336.9±4.25	327.6±4.18	310.7±3.42	293.9±5.93	281.2±4.86	276.2±1.9	250.6±6.8	223.2***
(B)	340.2±4.55	341.4±2.85	329.2±7.6	311.2±8.06	295±8.27	286.7±7.22	280.5±4.4	261.2±7.6	145.1***
A(A)	28.2±2.6	28.2±1.1	26.3±2	24.9±2.22	21.3±1.88	21±1.23	19.4±1.08	17.8±1.4	42.7***
(B)	28.5±3.1	28.6±2.9	26.14±1.43	26±2.48	22.1±2.05	21±1.88	19.5±1.4	17.8±1.2	25.9***
E(A)	8.95±0.52	9±0.67	8.3±0.64	8±0.48	6.4±0.72	6.1±0.45	5.5±0.43	5±0.21	60.2***
(B)	8.97±0.15	9.2±0.17	8.8±0.15	8.2±0.26	6.7±0.27	6.2±0.28	5.7±0.34	5.1±0.29	285.4***
gs (A)	4.06±0.29	4.1±0.1	3.9±0.34	3.3±0.21	2.7±0.23	2.7±0.29	2±0.61	1.7±0.16	115.3***
(B)	4.1±0.16	4.1±0.20	4±0.19	3.77±0.27	3.1±0.17	2.8±0.21	2.6±0.20	1.6±0.26	120***
WUE(A)	3.1±0.43	3.1±0.19	3.2±0.3	3.1±0.21	3.3±0.60	3.5±0.43	3.5±0.24	3.6±0.27	1.85*
(B)	3.1±0.31	3.1±0.29	2.9±0.19	3.1±0.37	3.3±0.41	3.4±0.35	3.4±0.27	3.5±0.29	2.09*
CUP(A)	11.91±1.11	11.95±0.50	12.45±1.18	12.47±0.84	13.79±1.41	13.68±0.95	14.39±0.74	14.58±0.94	40.2*
(B)	11.93±1.43	11.94±1.35	12.66±0.72	11.92±1.20	13.35±1.52	13.56±1.37	14.28±1.1	14.67±1.04	111.0*

(A), (B)= sunflower variety A&B, **±SD**= standard deviation, ***highly significant response,*less significant response

Table 2: Biochemical analysis for Cr translocation (roots, leaves & foliar chlorophyll contents) in sunflower plant.

Cr^{6+} in soil (mg/kg)	Hysun-33(A)		SF-5009(B)			
	Cr uptake (ppm)					
	Roots ⟶	Leaves	Roots ⟶	Leaves	T. Chl (A)	T. Chl (B)
00	-	-	-	-	47.1±0.63	47.6±1.45
50	26.46±1.46	1.51±0.5	25.14±2.14	1.46±0.23	47.3±0.54	47.7±1.22
100	54.05±2	2.23±0.23	51.47±1.5	2.10±0.21	43.0±0.62	44.3±1.69
150	71.13±3.06	3.31±0.6	65.91±2.46	3.00±1.32	41.7±0.26	42.3±1.90
250	106.74±6.4	5.54±0.5	103.5±5.41	4.91±0.72	37.6±1.04	38.7±1.23
350	143.3±9.24	6.02±0.52	136.7±14.4	5.47±0.41	36.5±0.38	37.1±1.68
400	161.43±6.5	6.24±0.23	159.3±16.5	6.00±2	34.2±1.02	36.5±0.87
500	189.61±4.3	6.97±1.25	182.2±16.9	6.23±0.61	32.5±0.85	33.2±0.67
F-value	537.8***	58.7***	128.24***	18.183***	433.2***	104.1***

3.3 Chromium uptake study

At Cr dose 50-500mg/kg of soil, Cr contents increased in roots from 25.14-189.61 ppm. In leaves, Cr contents were 1.46-6.97 ppm. Increase in Cr contents was directly related to its application in soil. Fig.2. Rate of Cr accumulation was more in Hysun-33(A) than SF-5009(B).

4 Discussion

At 100 & 400 µM Cr^{6+}, spinach (Spinaceae oleracea L.) has showed lower leaf water potential, transpiration rate and alteration in plant water use efficiency (Gopal, 2009). In rice plants, Cr^{6+} affects 62% rate of photosynthesis and 66% stomatal conductance (Ahmad et al., 2011). Net photosynthetic rate, transpiration rate, stomatal conductance and internal CO_2 concentration changed significantly as compared to control at Cr^{6+} doses 10-6 M and 10-4 M (Liu et al., 2008). Low rate of photosynthesis due to inhibition of electron transport systems, abnormal photochemical process, low chlorophyll contents which resulted in stunted plant growth is due to accelerated Cr accumulation in plants (Van Assche & Clijsters, 1983; Singh & Agarwal, 2007; Tang et al., 2012). Photosynthetic pigments are strongly damaged by Cr induced O_2 radicals having redox potential of 1.38 eV (Pinto et al., 2000; Vernay et al., 2007).

Our investigation from present study also agreed with these result lines as overall leaf physiology altered by Cr^{6+} application. A minimal effect of Cr was observed at lower Cr doses (100-150 mg/kg) than higher doses 250-500mg/kg). Leaf physiological performance for various attributes reduced remarkably up to 60%.Water use efficiency and carbon use potential of plants increased by 3.3 & 13.1%, respectively. Results obtained at 50 mg/kg Cr revealed leaf physiology and chlorophyll contents improved likewise control and this Cr dose found to be beneficial for test varieties remained unreported by earlier researchers. Increased water use efficiency indicated more water requirement to Cr stressed plants found associated with reduced transpiration rate and less rate of stomatal conductance following less CO_2 intake and water uptake to inhibit rate of photosynthesis. Increased values of carbon use potential were due to less CO_2 assimilation during photosynthesis predicted inefficiency of carbon fixing enzymes and carbohydrate biosynthesis (Table-1).

Alteration in foliar chlorophyll contents increased with increasing Cr application and pretentiously reflected metal toxicity in treated plants (Table-2).

Chromium destroys animo-levulinic acid dehydratase (ALAD), a precursor enzyme for chlorophyll biosynthesis and affects amino-levulinic acid (ALA) to decrease chlorophyll level (Vajpayee et al., 2001). Bertrand & Poirier (2005) reported chlorophyll degradation and enhanced membrane permeability damaged at high chromium concentration. Chlorophyll–a & b decreased up to 47&43% while carotenoids were affected 50% in Cr stressed rice plants (Ahmad & Wahid et al., 2011). They also investigated plants for NPK required for different metabolic activities and observed remarkable reduction line up to 82, 37 & 42%, respectively. Cr accumulation showed plants having no transport systems for Cr, and imbalanced chlorophyll and nutrient contents in affected parts (Diwan et al., 2012). The same findings were in this experiment and low Cr translocation from root to leaves was significantly observed, but Cr contents in plant organs increased along its application in soil. In roots we observed more Cr uptake 189.6 ppm than in leaves i.e. 6.96 ppm (Table-2). Due to Cr toxicity in leaves, chlorophyll contents reduced up to 31% predicted chloroplast structural abnormalities with the rift in N, P, K & Mg ions to destroy porphyrin ring (Table-1). Affected rate of photosynthesis was due to unusual photoactivity of chlorophyll under Cr stress.

5 Conclusion

From the present study, it can be concluded that use of hexavalent chromium is harmful for both sunflower cultivars. Different Cr doses (mg/kg) have affected physiological attributes of leaves. Same way, leaf chlorophyll contents also found to be affected by Cr toxicity. The stressed leaf physiological activity proposed to be associated with affected plant growth and yield due to possible less accumulation of metabolites. However, some hopeful findings also obtained at lower Cr doses where injurious effects were consistent and affordable than at higher Cr doses. Some emerging effects of Cr obtained at 50 mg/kg were much hopeful. The plant responses were positive and better even than at control. Hence, this dose can be recommended as a beneficial micronutrient for test hybrids. However, comparing physiological performance of two sunflower hybrids, SF-5009found to be more Cr tolerant than Hysun-33.

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