CHROMIUM TOXICITY INDUCED ALTERATIONS IN GROWTH, PHOTOSYNTHESIS, GAS EXCHANGE ATTRIBUTES AND YIELD FORMATION IN MAIZE

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Accumulation and soil enrichment with non-essential heavy metals like chromium (Cr) above threshold levels results in Cr toxicity in plants. In order to study the effect of varying Cr stress levels on maize growth, photosynthesis and gas exchange capacities and yield responses, two maize hybrids i.e., Wan Dan 13 and Run Nong 35 were grown in soil contained pots contaminated with different concentrations of Cr (0 μ M, 30 μ M, 60 μ M, 90 μ M, 120 μ M and 150 μ M). Cr toxicity substantially reduced total chlorophyll contents, net photosynthesis, transpiration rate, inter-cellular CO₂, stomatal conductance, water use efficiency and intrinsic water use efficiency. It further haltered growth (plant height, number of leaves/plant, leaf area, stem diameter, shoot fresh and dry weight) and yield and related attributes (ears/plant, number of kernels/row, 100-kernels weight, grain yield/plant, biological yield/plant) as well as harvest index both maize hybrids but the effects were more conspicuous in Run Nong 35 than Wan Dan 13. The overall toxicity of Cr to both maize hybrids were recorded as: 150μ M > 120μ M > 90μ M > 60μ M > 30μ M > 0μ M. In sum, Cr contamination negatively influenced on maize, however, Wan Dan 13 was better able to resist Cr-stress than Run Nong 35.

Keywords: Chlorophyll, gas exchange, chromium toxicity, soil contamination, water use efficiency.

INTRODUCTION

After industrial revolution, the environment started to contaminate swiftly with heavy metals and the case has become more severe as industries proliferate worldwide (Govil et al., 2008). Heavy metal accumulation has further aggravated by anthropogenic activities and postured a serious threat to the biosphere by entering in to the food chain (Chary et al., 2008; Batool et al., 2014; Riaz et al., 2014; Anjum et al., 2015; Al-Busaidi et al., 2015). Buildup of heavy metals pools in the ecosystem cause deleterious effects on plants, animals, human beings and all other micro flora and fauna being one of the main reasons of environmental destabilization (Nodelkoska et al., 2000; Ashraf et al., 2015; Anjum et al., 2016). Among heavy metals, chromium (Cr) is considered to have severe toxic effects in biotic systems. Concerns about Cr accumulation in agricultural lands are highly important due to its severe effects on crop productivity and food quality (Gill et al., 2014). Metal origin and speciation (responsible for its uptake, movement and subsequent toxicity with plant systems) determine Cr impact on plant physiological and biochemical

processes, from simple to multiple stages i.e., from reduced root and shoot growth to structural and functional changes of enzymes and mutagenesis (Shanker *et al.*, 2005).

Although trace amounts of Cr are essential for proper functioning of plants but increased levels of Cr in plants diminishes root and shoot growth, biomass accumulation and reduce grain yield (Hayat et al., 2012). Excessive Cr accumulation inhibits seed germination and plant growth and developmental processes (Rout et al., 2000). Furthermore, photosynthetic ability and chlorophyll biosynthesis are directly affected by Cr ions, resulting reduced carbon assimilation and respiratory activities (Prasad, 2004). Cr restricts various metabolic processes and resulted in growth inhibition, chlorosis, abridged photosynthesis, stunted root growth (Gill et al., 2014). Ali et al. (2013) declared Cr as the most toxic heavy metals that inhibit photosynthesis in plants in term of CO₂ fixation, photophosphorylation, electron transport mechanism and enzymatic processes. A remarkable inhibition of photosynthesis and hill reaction by Cr toxicity was also observed by Zeid (2001).

Furthermore, Bishnoi *et al.* (1993) reported photosystem-II (PS-II) is more vulnerable to Cr than photosystem I (PS I) in

chloroplasts of peas. Studies have documented a decrease in chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll contents and carotenoids under Cr stress (Rodriguez *et al.*, 2012). Moreover, a consistent exposure of plants to elevated levels of heavy metals including Cr normally results in photosynthetic pigments' degradation considerably (Hayat *et al.*, 2012) that may lead to growth and yield reduction (Sharma and Sharma, 2003). Cr-caused oxidative stress may harm physio-biochemical functions in plants and may induce alterations in morphological features and plant architecture (Shanker *et al.*, 2005; Ali *et al.*, 2015).

Maize (*Zea mays* L.) is an important cereal crop, grown around the world under a wide range of soil types. Being a C4 plant it is capable to thrive with metal-induced oxidative stresses; however, hyper accumulation of Cr may cause alteration in plant morpho-physiological and biochemical attributes that ultimately led to yield penalty (Shanker *et al.*, 2005). This study was therefore planned to study Cr induced changes in agronomic traits and yield responses, photosynthesis, chlorophyll contents and gas exchange attributes in two maize hybrids i.e., Ran Nong 35 and Wan Dan 13.

MATERIALS AND METHODS

Physiography and experimental details: The experiment was carried out during summer 2012 in net house at College of Agronomy and Biotechnology, Southwest University, Chongqing, China (latitudes 29° 49' 32" N, longitudes 106° 26' 02" E and 220 m above from sea level.). Healthy, homogenous, and uniform seeds of both maize hybrids purchased from two different companies. Wan Dan 13 was purchased from Chongqing Guo Ben Seed Company (Ltd.) Chongqing, China, while Run Nong 35 was provided by Sichuan Dragon Seed Company (Ltd.) Sichuan, China. Sodium hypochlorite solution (0.1%) was used to surface sterilize the seeds of both hybrids maize hybrids. After treating with the solution for 10 min, seeds were rinsed with double distilled water. Special PVC nursery trays were used to sow the seeds (two seeds/hill) then after two weeks, two seedlings were transferred to plastic pots (60 cm \times 34 cm \times 24 cm) filled with sandy loam soil containing 16.81 g kg⁻¹, organic matter, total NPK 2.14 g kg⁻¹, 3.76 g kg⁻¹, 11.02 g kg⁻¹, and available NPK 91.24 mg kg⁻¹, 31.62 mg kg⁻¹ 51.54 mg kg⁻¹, and pH 6.47. After being air-dried, the soil was passed through a 2-mm sieve to fill the pots. The plastic pots were kept at 29±1°C, 80% RH and 13 h photoperiod with irradiance of 40-50 μ mol⁻² s⁻¹ for seedling establishment. Hygrometers and thermocouples were used to record the data on RH and temperature (Vaissala HMP35, Helsinky, Finland). The experimental material comprised of two maize hybrids (Wan Dan 13 and Run Nong 35). Four different concentrations of chromium (30, 60, 90, 120 and 150µM) were prepared by diluting 1 mM stock solution of $K_2Cr_2O_7$ •7 H_2O using 5% Hoagland solution (Arnon and Hoagland, 1940) and mixed with soil of respective pots during pot filling whereas a control (0 μ M) was also maintained for comparison. Pots were arranged in a completely randomized design (CRD) with factorial arrangement with 12 pots per treatment in triplicate.

Growth and yield: Leaf area of plants was recorded at tasseling stage with LI-3100 leaf area meter (Li-Cor, Lincoln, NE) CI-203 (CID, Inc., USA) while a meter scale and an electronic weighing balance were used to measure plant height and fresh root and shoot mass, respectively. To record the plants dry weight, harvested plants were kept in an oven for 72 h at 80°C. After sampling, plant organs were separated and measured. To determine growth and yield related attributes, 30 plants (10 plants from each replicate) were sampled randomly and harvested at maturity. Harvest index (HI) was calculated as (grain yield/biological yield) \times 100.

Photosynthesis, photosynthetic pigments and gas exchange attributes: Fully expanded healthy leaves of maize were sampled to assess chlorophyll contents by using alcohol acetone hybrid method as ascribed by Arnon (1949). After grinding leaf sample (0.1 g), was placed in 15 ml centrifuge tube along with miscible liquids (10 ml) by 95.5% acetone and absolute ethyl alcohol in 1:1ratio and then after covering with black plastic bag it was kept at dark place until the sample transformed into white. Then with the help of UV-visible spectrophotometer, Chl a, Chl b and Chl a+b contents were measured at 645, 652 and 663 nm.

Moreover, a portable infrared gas exchange analyzer (Li-6400, Li-Cor, Lincoln, Nebraska, USA) was utilized to measure the gas exchange attributes like net photosynthetic (A) and transpiration rate (E), inter-cellular CO_2 and stomatal conductivity (g_s) in the 3rd leaf from the top during 9:00 to 10:30 a.m. Fifteen leaves were chosen from each treatment with the following modifications: molar flow of air per unit leaf area was 407.60 mmol lol-1 1m-2 s-1, water vapour pressure into leaf chamber was 3.4 mbar, photosynthetically active radiation (PAR) at leaf surface was up to 1201 mol m⁻²s⁻¹ temperature of leaf ranged from 37.05 to 41.63°C, ambient temperature was 36.33-40.96°C, ambient CO₂ concentration was 399.4 mol mol⁻¹ and relative humidity (RH) was recorded up to 52.39%. Water use efficiency (WUE) as well as intrinsic water use efficiency (WUEi) was estimated as ratio between net photosynthesis to transpiration rate (A/E) and net photosynthesis to stomatal conductance (A/g_s) , respectively.

Data analysis: Date presented as the means of three replicates \pm SE for each treatment. SPSS 16.0 (Inc., Chicago, USA) was used to perform analysis of variance (ANOVA) for variables under study while Newman–Keuls test (p<0.05) was used to compare treatment means.

RESULTS

Maize morphology: Cr-induced significant reductions in agronomic attributes of maize in terms of plant height, number of leaves/plant, leaf area, stem diameter, ear length, and shoot fresh and dry weight. Plant height was reduced up to 5.22% in Wan Dan 30 and 6.39% in Run Nong 35 as Cr stress level increased from 0 to 150µM. Number of leaves per plant also reduced as Cr toxicity exacerbated but the difference was not significant in both hybrids at different Cr concentrations. Cr stress also reduced the leaf area of both hybrids significantly. Up to 3% and 10% leaf area reduction was observed in both maize hybrids (Wan Dan 30 and Run Nong 35, respectively) exhibited to 150 µM of Cr than control. Interestingly, Wan Dan 30 responded positively from 0 to 60 µM (increase in leaf area) then reduced gradually while gradual increase in Cr concentration reduced leaf area of Run Nong 35 progressively. Cr contaminated

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soil notably affected the maize stem diameter. 4.42% and 10.73% reductions were recorded in both maize hybrids at maximum Cr level compared with control. Additionally, Cr reduced fresh and dry biomass accumulation in both maize hybrids, but the effects were more prominent in Run Nong 35 than Wan Dan 30. No significant effects of Cr toxicity were observed in ear length in both maize hybrids (Table 1). Yield and related attributes: Cr toxicity severely affected m yield and its related attributes in both Run Nong 35 and Wan Dan 30 negatively. Ears/plant, number of kernels/ear, kernel rows/ear, number of kernels/row, 100-kernels weight, grain vield/plant, biological vield/plant, and harvest index affected differently at different Cr concentrations in both maize hybrids. Reductions in yield and related attributes for were recorded up to 21.05, 6.12, 4.55, 13.44, 7.35, 19.06, 13.21, and 5.49% in Wan Dan 13 while 31.58, 9.22, 7.05, 24.87, 26.67, 28.66, 20.97, and 10.28% in Run Nong 35,

Table 1. Growth and related attributes of two maize h	vbrids as affected by different levels of chromium stress.
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Cr	Plant height	Number of	Leaf area	Stem	Ear length	Shoot fresh	Shoot dry
level	(cm)	leaves/plant	(cm ²)	diameter	(cm)	weight/plant	weight/plant
(µM)				(mm)		(g)	(g)
0	201.38 ± 1.87^{a}	14.33 ± 0.44^{ns}	254.65±1.97 ^a	25.55 ± 0.76^{a}	17.98 ± 0.29^{ns}	241.00±1.33 ^a	66.36±0.29 ^a
30	199.76±1.44 ^a	14.21 ± 0.34^{ns}	256.87±1.33ª	$24.84{\pm}0.34^{ab}$	17.92 ± 0.34^{ns}	237.55±1.33 ^a	65.32 ± 0.34^{a}
60	196.00±3.22 ^b	14.12 ± 0.44^{ns}	258.76±2.09ab	$24.71{\pm}0.44^{ab}$	17.83 ± 0.18^{ns}	230.21±2.09ab	$62.54{\pm}0.18^{ab}$
90	193.65±2.44 ^{bc}	13.99±0.59 ^{ns}	253.33±1.28 ^{ab}	$24.60{\pm}0.59^{ab}$	17.77±0.59 ^{ns}	227.87 ± 1.28^{ab}	60.34 ± 0.59^{b}
120	191.88±2.21°	$13.93{\pm}0.78^{ns}$	251.54±2.66 ^{ab}	$24.69{\pm}0.78^{ab}$	17.73 ± 0.78^{ns}	219.54±2.66 ^b	55.54±0.78°
150	190.87±0.98°	13.78 ± 0.43^{ns}	247.02 ± 0.99^{b}	24.42 ± 0.49^{b}	17.62 ± 0.65^{ns}	209.65±0.22°	53.76±0.65°
0	200.95 ± 2.76^{a}	14.32±0.66 ^{ns}	253.74±2.33ª	24.88±0.44 ^a	17.97±0.64 ^{ns}	238.87±1.46 ^a	64.99±0.64 ^a
30	198.76±3.11 ^b	$14.19{\pm}0.78^{ns}$	250.55±3.11 ^{ab}	$23.39{\pm}0.78^{ab}$	17.88 ± 0.95^{ns}	231.76±3.11 ^b	62.56 ± 0.95^{ab}
60	194.33±2.55°	14.08 ± 0.70^{ns}	247.56±2.57 ^{ab}	22.87 ± 0.70^{b}	17.75±0.70 ^{ns}	225.23±2.57b	59.76±0.70 ^b
90	191.04 ± 2.01^{d}	13.92±0.54 ^{ns}	244.99 ± 2.08^{b}	22.64±0.33 ^b	17.69±0.34 ^{ns}	214.87±2.08°	55.23±0.34°
120	189.65±0.88e	13.78 ± 0.38^{ns}	237.87±1.86°	22.52 ± 0.38^{b}	17.61 ± 0.38^{ns}	201.65 ± 4.00^{d}	51.76±0.38 ^d
150	188.11 ± 1.65^{f}	13.61 ± 0.32^{ns}	228.76 ± 2.11^{d}	22.21 ± 0.65^{b}	17.55 ± 0.20^{ns}	189.43±2.77 ^e	47.32 ± 0.20^{e}
	Cr level (μM) 0 30 60 90 120 150 0 30 60 90 120 150	CrPlant height (cm)level(cm) (μM) 0 0 201.38 ± 1.87^a 30 199.76 ± 1.44^a 60 196.00 ± 3.22^b 90 193.65 ± 2.44^{bc} 120 191.88 ± 2.21^c 150 190.87 ± 0.98^c 0 200.95 ± 2.76^a 30 198.76 ± 3.11^b 60 194.33 ± 2.55^c 90 191.04 ± 2.01^d 120 189.65 ± 0.88^c 150 188.11 ± 1.65^c	CrPlant height (cm)Number of leaves/plant μ (cm)leaves/plant 0 201.38 ± 1.87^a 14.33 ± 0.44^{ns} 30 199.76 ± 1.44^a 14.21 ± 0.34^{ns} 60 196.00 ± 3.22^b 14.12 ± 0.44^{ns} 90 193.65 ± 2.44^{bc} 13.99 ± 0.59^{ns} 120 191.88 ± 2.21^c 13.93 ± 0.78^{ns} 150 190.87 ± 0.98^c 13.78 ± 0.43^{ns} 0 200.95 ± 2.76^a 14.32 ± 0.66^{ns} 30 198.76 ± 3.11^b 14.19 ± 0.78^{ns} 60 194.33 ± 2.55^c 14.08 ± 0.70^{ns} 90 191.04 ± 2.01^d 13.92 ± 0.54^{ns} 120 189.65 ± 0.88^c 13.78 ± 0.38^{ns} 150 188.11 ± 1.65^c 13.61 ± 0.32^{ns}	CrPlant height (cm)Number of leaves/plantLeaf area (cm2) $ \mu M $ (cm) leaves/plant (cm^2) 0201.38±1.87a14.33±0.44ns254.65±1.97a30199.76±1.44a14.21±0.34ns256.87±1.33a60196.00±3.22b14.12±0.44ns258.76±2.09ab90193.65±2.44bc13.99±0.59ns253.33±1.28ab120191.88±2.21c13.93±0.78ns251.54±2.66ab150190.87±0.98c13.78±0.43ns247.02±0.99b0200.95±2.76a14.32±0.66ns253.74±2.33a30198.76±3.11b14.19±0.78ns250.55±3.11ab60194.33±2.55c14.08±0.70ns247.56±2.57ab90191.04±2.01d13.92±0.54ns244.99±2.08b120189.65±0.88c13.78±0.38ns237.87±1.86c150188.11±1.65f13.61±0.32ns228.76±2.11d	CrPlant heightNumber of leaves/plantLeaf area (cm²)Stem diameter (mm) 0 201.38 ± 1.87^a 14.33 ± 0.44^{ns} 254.65 ± 1.97^a 25.55 ± 0.76^a 30 199.76 ± 1.44^a 14.21 ± 0.34^{ns} 256.87 ± 1.33^a 24.84 ± 0.34^{ab} 60 196.00 ± 3.22^b 14.12 ± 0.44^{ns} 258.76 ± 2.09^{ab} 24.71 ± 0.44^{ab} 90 193.65 ± 2.44^{bc} 13.99 ± 0.59^{ns} 253.33 ± 1.28^{ab} 24.60 ± 0.59^{ab} 120 191.88 ± 2.21^c 13.93 ± 0.78^{ns} 251.54 ± 2.66^{ab} 24.69 ± 0.78^{ab} 150 190.87 ± 0.98^c 13.78 ± 0.43^{ns} 247.02 ± 0.99^b 24.42 ± 0.49^b 0 200.95 ± 2.76^a 14.32 ± 0.66^{ns} 253.74 ± 2.33^a 24.88 ± 0.44^a 30 198.76 ± 3.11^b 14.19 ± 0.78^{ns} 250.55 ± 3.11^{ab} 23.39 ± 0.78^{ab} 60 194.33 ± 2.55^c 14.08 ± 0.70^{ns} 247.56 ± 2.57^{ab} 22.87 ± 0.70^b 90 191.04 ± 2.01^d 13.92 ± 0.54^{ns} 237.87 ± 1.86^c 22.52 ± 0.38^b 120 189.65 ± 0.88^e 13.78 ± 0.38^{ns} 237.87 ± 1.86^c 22.52 ± 0.38^b 150 188.11 ± 1.65^f 13.61 ± 0.32^{ns} 228.76 ± 2.11^d 22.21 ± 0.65^b	Cr Plant height (cm) Number of leaves/plant Leaf area (cm ²) Stem Ear length (diameter (cm) level (cm) leaves/plant (cm ²) diameter (mm) (cm) 0 201.38±1.87 ^a 14.33±0.44 ^{ns} 254.65±1.97 ^a 25.55±0.76 ^a 17.98±0.29 ^{ns} 30 199.76±1.44 ^a 14.21±0.34 ^{ns} 256.87±1.33 ^a 24.84±0.34 ^{ab} 17.92±0.34 ^{ns} 60 196.00±3.22 ^b 14.12±0.44 ^{ns} 258.76±2.09 ^{ab} 24.71±0.44 ^{ab} 17.83±0.18 ^{ns} 90 193.65±2.44 ^{bc} 13.99±0.59 ^{ns} 253.33±1.28 ^{ab} 24.60±0.59 ^{ab} 17.77±0.59 ^{ns} 120 191.88±2.21 ^c 13.93±0.78 ^{ns} 247.02±0.99 ^b 24.42±0.49 ^b 17.62±0.65 ^{ns} 0 200.95±2.76 ^a 14.32±0.66 ^{ns} 253.74±2.33 ^a 24.88±0.44 ^a 17.97±0.64 ^{ns} 30 198.76±3.11 ^b 14.19±0.78 ^{ns} 250.55±3.11 ^{ab} 23.39±0.78 ^{ab} 17.88±0.95 ^{ns} 60 194.33±2.55 ^c 14.08±0.70 ^{ns} 247.56±2.57 ^{ab} 22.87±0.70 ^b 17.75±0.70 ^{ns} 90 191.04±2.01 ^d	Cr Plant height (cm) Number of leaves/plant Leaf area (cm ²) Stem Ear length (cm) Shoot fresh weight/plant (µM) (cm) (cm ²) diameter (mm) (cm) weight/plant 0 201.38±1.87 ^a 14.33±0.44 ^{ns} 254.65±1.97 ^a 25.55±0.76 ^a 17.98±0.29 ^{ns} 241.00±1.33 ^a 30 199.76±1.44 ^a 14.21±0.34 ^{ns} 256.87±1.33 ^a 24.84±0.34 ^{ab} 17.92±0.34 ^{ns} 237.55±1.33 ^a 60 196.00±3.22 ^b 14.12±0.44 ^{ns} 258.76±2.09 ^{ab} 24.71±0.44 ^{ab} 17.83±0.18 ^{ns} 230.21±2.09 ^{ab} 90 193.65±2.44 ^{bc} 13.99±0.59 ^{ns} 251.54±2.66 ^{ab} 24.69±0.78 ^{ab} 17.77±0.59 ^{ns} 227.87±1.28 ^{ab} 120 191.88±2.21 ^c 13.93±0.78 ^{ns} 251.54±2.66 ^{ab} 24.69±0.78 ^{ab} 17.73±0.78 ^{ns} 219.54±2.66 ^b 150 190.87±0.98 ^c 13.78±0.43 ^{ns} 247.02±0.99 ^b 24.42±0.49 ^b 17.62±0.65 ^{ns} 209.65±0.22 ^c 0 200.95±2.76 ^a 14.32±0.66 ^{ns} 253.74±2.33 ^a 24.88±0.44 ^a 17.97±0.64 ^{ns} 238.87±1.46 ^a <tr< td=""></tr<>

s in the table are the means of three replicates \pm SE. Values share a letter in common within columns do not differ icantly according to Newman–Keuls test (p<0.05).

Maize hybrids	Cr level (µM)	Ears/plant	Number of kernels/row	100-kernels weight	Grain yield/plant (g)	Biological yield/plant (g)	Harvest index (%)
Wan Dan 13	0	1.33±0.02 ^a	31.33±0.50 ^a	26.19±0.66ª	151.54±1.87 ^a	328.76±3.65ª	46.09±0.99 ^a
	30	1.27±0.01 ^{ab}	$30.44{\pm}0.77^{ab}$	25.90±0.76 ^{ab}	145.87 ± 1.76^{ab}	322.87±4.21 ^{ab}	45.18±0.77 ^{ab}
	60	1.22±0.03 ^{ab}	29.54±0.54 ^{bc}	25.61±0.44 ^{abc}	140.54 ± 1.98^{b}	315.87±2.54 ^{bc}	44.49 ± 0.76^{ab}
	90	1.18±0.04 ^{bc}	29.03±0.88°	24.87±0.88 ^{abc}	133.34±1.22°	309.65±1.99°	44.31 ± 0.88^{ab}
	120	1.09±0.03 ^{cd}	26.88 ± 0.34^{d}	24.51±0.34 ^{bc}	129.65±0.99°	294.43 ± 5.33^{d}	44.03 ± 0.34^{ab}
	150	1.05 ± 0.02^{d}	27.12±1.44 ^d	24.22±0.51°	122.65 ± 0.66^{d}	285.33±4.87 ^e	43.56±0.99b
Run Nong 35	0	1.33±0.04 ^a	30.44±1.33 ^a	24.67±0.76 ^a	150.76±2.09 ^a	328.00±4.55ª	45.93±1.04 ^a
-	30	1.25±0.03 ^{ab}	29.34±0.98ª	22.87±0.35 ^b	141.54±0.99 ^b	317.76±3.87 ^b	44.54 ± 0.98^{ab}
	60	1.18±0.03 ^b	27.02 ± 0.55^{b}	20.45±0.55 ^{cd}	136.76±1.54 ^b	309.87±2.99°	44.13±0.87 ^{ab}
	90	1.09±0.04°	26.11±0.34 ^{bc}	19.02±0.34 ^{cd}	126.98±1.22°	294.65±4.43 ^d	43.01±0.34bc
	120	1.02±0.05°	24.60±1.43 ^{cd}	18.65 ± 0.86^{d}	119.65±0.65 ^d	283.11±5.43 ^e	42.48±1.43 ^{bc}
	150	0.91 ± 0.03^{d}	22.87±0.65 ^d	18.09 ± 0.44^{d}	107.55±1.65e	259.22 ± 2.99^{f}	41.21±1.05°

Les in the table are the means of three replicates \pm SE. Values share a letter in common within columns do not differ ificantly according to Newman–Keuls test (p< 0.05).

respectively at 150 μ M Cr application compared with control. We further found that with a linear increase in Cr toxicity from 0 to 150 μ M, yield and related components of both maize hybrids were also decreased linearly; however, severity was more in Run Nong 35 than Wan Dan 13 (Table 2).



Figure 1. Influence of chromium (Cr) stress on (a) Chl a, (b) Chl b, (c) Chl a+b, and (d) Chl a/b ratio in the leaves of two maize hybrids . Capped bars above means represent S.E. of three replicates. FW: fresh weight.

Chlorophyll contents: Cr stress reduced the chlorophyll concentration in both maize hybrids however effects were more apparent in Run Nong 35 than Wan Dan 13 at all levels of Cr stress. Gradational increase in Cr stress reduced Chl a, Chl b, Chl a+b up to 27.71, 60.33 and 38.37% in Wan Dan 13 while 50.81, 83.19 and 61.37% in Run Nong 35 at 150 μ M, compared with control. Further, Chl b was affected more severely than Chl a and total a+b. Decreased Chl b contents raised the value of Chl a/Chl b ratio in both maize hybrids. Higher values for Chl a/Chl b ratio for Run Nong 35 depicted the physiological reduction in Chl a contents at high levels of chromium. In sum, chlorophyll biosynthesis decreased in maize fresh tissues as the chromium supply was increased but the percent decrease in Chl b was higher than Chl a. (Fig. 1).

Photosynthetic activity and gas exchange attributes: Photosynthetic activity and gas exchange attributes of both maize hybrids significantly affected when exposed to Cr stress. Data pertaining to net photosynthetic rate as influenced by different Cr toxicity levels revealed that plant photosynthetic activity strongly inhibited at high levels of Cr. In Wan Dan 13, gradual increase in Cr concentrations from 0 to 120 μ M, net photosynthetic rate was reduced significantly but not at 120 to 150 µM. About 29.70% and 42.27% reduction in photosynthetic rate was recorded in Wan Dan 13 and Run Nong 35, respectively than control. Furthermore, transpirational rates also exhibited a gradual decrease with increase in Cr concentration in both maize hybrids. As Cr increased from 0 to 150 µM, transpitrational rate decreased to 13.87% and 18.96% at highest Cr level in Wan Dan 13 and Run Nong 35, respectively than control. Cr also hindered stomatal conductance and inter-cellular CO₂ at all levels of Cr, however, no significant difference were found in intra-cellular CO₂ up to 120 µM in Wan Dan 13 then decreased abruptly as Cr level increased to 150 µM but Run Nong 35 showed a significant reduction in inter-cellular CO₂ at every level of Cr toxicity. Compared with control, 33.33% and 55.56% reduction in stomatal conductance while 9.41% and 16.47% reduced inter-cellular CO₂ was recorded at 150 µM in both maize hybrids (Wan Dan 13 and Run Nong 35), respectively. Further, water-use efficiency (WUE) as well as intrinsic water-use efficiency (WUEi) also deteriorated with Cr concentration that further reduced water economy with more intensively in Run Nong 35 (Fig. 2).

DISCUSSION

The current investigation aimed at understanding the Crinduced changes in agronomic variability, yield responses and photosynthesis and gas exchange attributes of maize under Cr stress. The extent of Cr phyto-toxicity depends on various factors like environmental conditions, soil and plant types as well as chromium concentration at the experimental site (Shanker *et al.*, 2005). Possibly, elevated Cr levels might

Cr induced-alterations in maize



Figure 2. Influence of chromium (Cr) stress on (a) net photosynthesis (b) transpiration rate (c) inter-cellular CO₂ (d) stomatal conductance (e) water use efficiency (WUE) and (f) intrinsic water use efficiency (WUEi) in two maize hybrids. Capped bars above means represent S.E. of three replicates.

cause nutrition imbalance and interrupted their role in anabolic pathways that ultimately halted normal plant developmental processes (Sharma *et al.*, 2003).

Maize growth and yield formation under Cr stress: When exposed, chromium altered plant growth, developmental pattern and yield formation. Our findings depicted distinguished decrease in different growth and yield related attributes in both maize hybrids while a pronounced toxic effect of Cr on maize was also observed when plants are exhibited to higher levels of chromium. Cr-induced reductions in agronomic characters i.e., plant height, leaf area, stem diameter, shoot fresh and dry weight as well as yield and related attributes i.e., ears/plant, number of kernels, kernels weight, grain and biological yield and harvest index of both maize hybrids (Table 1 & 2). Cr-induced disruption in growth might be ascribed to Cr transportation to the above ground part of the plant that may have a direct effect on

cellular metabolism, which finally contributes to the diminution in growth and development. Carbon, the most important element for dry matter production, constitute about 80-90% of total dry matter achieved by the plant were also reduced significantly under Cr stress in different plants as reported by Sharma and Sharma (1993). Numerous studies were undertaken in past to investigate the Cr nature in soil and its interaction with plant growth and resulted that Cr impedes plant growth and yield formation when go beyond the threshold levels (Joshi et al., 1999; Sharma et al., 2003; Arun et al., 2005). Our findings are also corroborated with Diwan et al. (2012) who also declared that Cr inhibits plant growth severely and toxic effects are concentration dependent and genotype specific. The ability of Wan Dan 13 to produce more yield than Run Nong 35 might be due to slow uptake and transportation of Cr from roots to above ground plant parts. However, the depression in grain and

biological yield in both maize hybrids might be due to differential chromium concentration in the rhizosphere.

Alterations in photosynthesis, photosynthetic pigments and gas exchange attributes under Cr stress: Plant exposed to Cr toxic soils affected physiological indices and chlorophyll biosynthesis of both Wan Dan 13 and Run Nong 35 (Fig. 1&2). Photosynthesis, being the most essential process of carbohydrate synthesis and regarded as the basic phenomena for building organic substances affected severely under heavy metal stress in general and Cr stress in particular (Bishnoi et al., 1993). Chloroplastic and ultra-structural damages, disruption of electron transport mechanisms and electron diversions from PSI due to Cr-induced changes might possible to reduced photosynthetic efficiency of both maize hybrids under study. Similar reasoning regarding photosynthetic inhibition due to Cr stress has been reported by Hayat et al. (2012). Furthermore, Ali et al. (2013) also stated Cr-induced changes may cause chloroplast damage, abnormal lamellar system, and ultra-structural changes which led to reduced photosynthesis and other assimilatory mechanisms. Probably, electron yielded from photochemical reactions might not be used for carbon fixation as evinced by reduced photosynthetic rates of Cr-stressed plants (Shanker et al., 2005). Reduced stomatal conductance at maximum Cr level (150 µM in present case) shows oxidative potential of Cr which may cause significant damage to the leaf mesophyll cells and guard cells of stomata (Shanker et al., 2005). Significant reductions in photosynthesis and gas exchange capacities due to Cr toxicity in Vigna mungo L. was also reported by Hussain et al. (2007).

Cr also inhibited chlorophyll contents significantly that possibly due to its interaction with the enzymes responsible for chlorophyll biosynthesis (Ouzounidou, 1995). Conversely, both reduced and enhanced chlorophyll contents in Cr stressed plants have been reported previously (Dixit et al., 2002; Samantary, 2002). In our experiment, Cr reduced Chl a, Chl b, Chl a+b in both maize hybrids but the reduction was concentration dependent and genotypespecific. Higher concentrations of Cr in growing medium resulted in lower the chlorophyll contents. Vajpayee et al. (2001) found in Nymphaea alba that Cr reduced α aminolaevulinic acid dehydratase activity that led to reductions in photosynthetic pigments.

In sum, Cr inhibited plant growth yield response, chlorophyll contents and photosynthetic and gas exchange capacities in both Wan Dan 13 and Run Nong 35 while the effects were more conspicuous in Run Nong 35. Further, phyto-toxic effects of Cr in maize were concentration dependent with more severe at higher concentrations. Sustained performance of Wan Dan 13 even under severe Cr stress exhibited its persistent capability to withstand against Cr stress.

Acknowledgments: The present research was supported by the National Natural Science Foundation Project (31271673) and Special Fund for Agro-scientific Research in the Public Interest (No. 201503127).

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