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Effects of Cd on photosynthesis and growth of safflower (*Carthamus tinctorius* L.) genotypes

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Abstract

Heavy metals such as cadmium (Cd) may affect different physiological functions in plants. We carried out a hydroponic experiment under greenhouse conditions in order to evaluate the effect of Cd on photosynthetic and physiological parameters of safflower. The responses of six safflower genotypes (Nebraska-10, 2811, Kouseh, S149, C111, and K12) to four concentrations of $CdCl_2$ (0, 1.5, 3, and 4.5 mg L^{-1}) were examined. Mean shoot and root dry masses of safflower plants were reduced by nearly 57% after the treatment by 4.5 mg($CdCl_2$) L^{-1}. Contrary to the mean proline content, which increased by 121%, the mean total leaf area per plant, net photosynthetic rate, stomatal conductance to the CO_2, leaf chlorophyll *a*, *b*, and (*a*+*b*), carotenoid content, and quantum efficiency of PSII decreased by 84.4, 50.5, 50.0, 31.6, 32.2, 31.8, 32.9, and 11.2%, respectively, at the presence of 4.5 mg($CdCl_2$) L^{-1}. The mean Cd concentration in shoots and roots of safflower genotypes exhibited 52- and 157-fold increase, respectively, due to the addition of 4.5 mg($CdCl_2$) L^{-1} to the growing media. The mean malondialdehyde content was enhanced by 110% with the increasing $CdCl_2$ concentration, indicating the occurrence of a considerable lipid peroxidation in the plant tissues. Even though the membrane stability index was adversely affected by the application of 1.5 mg($CdCl_2$) L^{-1}, the decrease ranged from 45 to 62% when plants were treated with 4.5 mg($CdCl_2$) L^{-1}. Genotype Nebraska-10 seemed to be different from the remaining genotypes in response to the 4.5 mg($CdCl_2$) L^{-1}; its net photosynthetic rate tended to be the greatest and the Cd concentration in shoots and roots was the lowest among genotypes studied. This study proved Cd-induced decline in growth, photosynthesis, and physiological functions of safflower.

Additional key words: cadmium; chlorophyll; gas exchange; lipid peroxidation; proline.

Introduction

Pollution of the biosphere with toxic amounts of metals released from both industrial and agricultural sources has accelerated dramatically since the beginning of the industrial revolution (Nriagu 1979). Soil pollution by heavy metals including Cd can cause lands and water resources to become hazardous for plants, wildlife, and human populations. Heavy metal accumulation in soils is of concern in agricultural production due to adverse effects on food safety and marketability, crop growth due to phytotoxicity, and environmental health of soil organisms (Nagajyoti 2010). Cadmium is one of the most persistent elements in natural environments (Benavides *et al*. 2005). Pollution with Cd is mainly caused by mining and smelting, power stations, urban traffic, dispersal of sewage sludge, and the use of Cd-rich phosphate fertilizers (Chaney 1998). A study on roadside soils in Isfahan, Iran (Samani Majd *et al*. 2007) indicated that the Cd concenration in these soils could reach 2.25 to 2.57 mg kg^{-1}. Cadmium is mobile in the soil-plant system and is readily taken up by plants (Krevešan *et al*. 2003); Cd entry into the human body *via* the food chain is a major concern. It interferes with uptake, transport, and use of several elements and water by plants (Nagajyoti 2010). Plants, which take up and accumulate Cd in their organs,

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Abbreviations: C_i – substomatal CO_2 concentration; Cd – cadmium; Cd-S – Cd concentration in shoots; Cd-R – Cd concentration in roots; Chl – chlorophyll; CK – control; DMR – dry mass of roots; DMS – dry mass of shoots; F_0 – minimum fluorescence; F_m – maximum fluorescence; F_v/F_m – maximal quantum efficiency of PSII; g_s – stomatal conductance to the CO_2; LP – lipid peroxidation; MDA – malondialdehyde; MSI – membrane stability index; P_N – net photosynthetic rate; ROS – reactive oxygen species; TBA – thiobarbituric acid; TBARS – thiobarbituric acid reactive substances; TCA – trichloroacetic acid; TLA – total leaf area per plant.
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may be also negatively affected in their photosynthesis, growth, and reproduction (Xiong and Peng 2001).

Cadmium uptake and its effects on plants may be influenced by factors, such as plant species, cultivar, soil characteristics, and temperature. Genetic differences in mineral uptake among plant species were observed decades ago (Sarić 1983) and even cultivars of the same species often show large variation in tolerance to Cd toxicity (Köleli 2004). Genotypic variation in Cd uptake and accumulation was found in birch, pine, and spruce (Österås *et al*. 2000), rice (Liu *et al*. 2007), wheat (Greger and Löfstedt 2004), and wild and modern wheat (Cakmak *et al*. 2000). Cadmium may induce different changes in plant growth (Clemens *et al*. 2002, Liao *et al*. 2005), physiology (Baryla *et al*. 2001, Singh *et al*. 2006), and nutritional status (Ghnaya *et al*. 2005). Direct and indirect interactions of Cd with different components of the photosynthetic apparatus and, therefore, impairing electron transport, chlorophyll (Chl) biosynthesis, and carbon assimilation of different species, have been documented (Laspina *et al*. 2005, Cherif *et al*. 2012).

Safflower (*Carthamus tinctorius* L.) is gaining importance as a source of vegetable oil for human consumption and industrial purposes (Pourghasemian *et al.* 2013). Safflower is known to tolerate at least two major environmental stresses, *i.e*., salinity and drought (Sabzalian *et al*. 2008), particularly in cropping systems of dry regions and marginal areas. Little scientific data exists on the response of this oilseed crop to Cd stress, though there are some reports that it may be used as a hyperaccumulator crop for Cd-polluted soils (Sayyad *et al*. 2010, Shi *et al*. 2010). A recent study (Pourghasemian *et al*. 2013) has shown that there are genotypic differences in Cd translocation, uptake, and sensitivity in safflower. No further physiological details including photosynthetic performance and cell membrane stability in response to Cd has been provided in the abovementioned study. The objective of the present work was, therefore, to investigate differences in some physiological and photosynthetic attributes among six genotypes of safflower in response to Cd.

Materials and methods

Plant material and growth conditions: Experiment was carried out in a greenhouse equipped with a partial shade canopy at the Isfahan University of Technology, Isfahan, Iran, in 2012. Mean weekly temperatures in the greenhouse were 30.0, 30.3, 29.8, 29.4, 28.5, 28.1, 29.1, and 27.8°C for weeks one to eight, respectively. Seeds of six *C. tinctorius* genotypes, namely, Kouseh, Nebraska-10, K12, S149, 2811, and C111 were sown into 20 × 30 × 5 cm plastic trays filled with washed sand and grown under controlled conditions (light/dark regime of 12/12 h at 25/20ºC, relative humidity of 60–70%). From one week after emergence, seedlings were watered every day with a full-strength Johnson's solution (Johnson *et al.* 1957) (Table 1). Four healthy 2-week old seedlings (two-leaf stage) from each genotype were selected and transferred to the containers filled with four liters of aerated Johnson's nutrient solution. A total number of 72 containers were used in the experiment. Cd treatment started when plants were 20-day old, *i.e.*, one week after transplantation. In order to avoid any shock, seven days after transplantation, $CdCl_2$ was added in two and three equal installments of 1.5 mg L^{-1} on alternate days until the target concentrations of 3 and 4.5 mg L^{-1} were achieved, respectively. Albeit, only nutrient solution was used in control treatment (CK). Nutrient solutions were collected from each container on a daily basis and electrical conductivity and pH were measured with conductivity meter (*Cond7110, InoLab*, Germany) and pH meter (*PHM3030, Jenway*, UK), respectively. The pH of the solutions was maintained at 6.0–6.5 throughout the experiment. The nutrient solution was renewed weekly and the amount of evaporated water was replenished with distilled water every day.

Table 1. Johnson's nutrient solution composition.

Element	Compound	[μmol L^{-1}]
Cl	KCl	50
B	H_3BO_3	25
Mn	$MnSO_4.H_2O$	2
Zn	$ZnSO_4.7H_2O$	2
Cu	$CuSO_4.5H_2O$	0.5
Mo	H_2MoO_4(85%MoO_3)	0.5
Fe	Fe-EDTA	50
N	KNO_3	16,000
K	$Ca(NO_3)_2 \cdot 4H_2O$	6,000
Ca	$NH_4H_2PO_4$	4,000
P	$MgSO_4 \cdot 7H_2O$	2,000
S		1,000
Mg		1,000

Leaf gas exchange, Chl fluorescence and Chl concentration parameters: Net photosynthetic rate (P_N), stomatal conductance to the CO_2 (g_s), and substomatal CO_2 concentration (C_i) were measured five weeks after application of $CdCl_2$ on 3 healthy leaves in each container with a calibrated portable gas exchange system (*LCi, ADC Bioscientific Ltd.,* UK). Conditions, under which measurements were made, were: leaf temperature of 26–33°C, CO_2 concentration of 370–390 mg kg^{-1}, and PPFD of 350–500 μmol m^{-2} s^{-1}.

Chl fluorescence parameters including maximum (F_m), minimum (F_0) fluorescence, and the maximum efficiency of PSII ($F_m - F_0/F_m$, *i.e.*, F_v/F_m) were measured on three young, fully expanded (dark-adapted for 20 min) attached leaves (Bazrafshan and Ehsanzadeh 2014) per container after two weeks of $CdCl_2$ application,

using a portable chlorophyll fluorometer (*Opti-Sciences, Inc.,* Hudson, NH, USA). Each data set for all attributes in each container represents the mean of three measurements.

Leaf Chl concentration of the safflower plants was determined after three weeks of Cd treatment using fully expanded young leaves. A fresh leaf sample of 0.5 g was ground and extracted with 15 mL of 80% (v/v) acetone. The slurry was filtered and absorbance was determined at 645 and 663 nm for Chl *a* and Chl *b*, respectively, using a UV-visible spectrophotometer (*HITACHI U 1800*, Japan). Concentrations of Chl *a*, Chl *b*, and total Chl (*a*+*b*) were estimated according to Arnon (1949). Leaf carotenoid content was determined according to Lichtenthaler and Wellburn (1983).

Growth parameters: All four plants from each experimental unit were subjected to the following measurements, and then harvested after six weeks of Cd treatment. Mean total leaf area per plant (TLA) was calculated after measuring TLA of two plants in each container, using a portable area meter (*Li 3000A, LI-COR, Inc.,* Lincoln, Nebraska, USA). Plant heights were measured and shoot (DMS) and root dry masses (DMR) were determined. For dry mass determination, samples were oven-dried at 70°C for 72 h and then weighed.

Lipid peroxidation, membrane stability index, and leaf proline content: Since malondialdehyde (MDA) is a product of lipid peroxidation (LP) by a thiobarbituric acid (TBA) reaction, the level of LP was measured in terms of a thiobarbituric acid reactive substances (TBARS) content of the leaf samples. Leaf samples of 0.1 g after five weeks of Cd treatment were homogenized in 2.5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged (3,000 × *g*) for 5 min. To 1 mL of aliquot of the supernatant 2.25 mL of 0.5% TBA in 20% TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 × *g* for 10 min, absorbance of the supernatant was recorded spectrophotometrically at 532 nm (*HITACHI U 1800*, Japan). The value for nonspecific absorption at 600 nm was subtracted. The TBARS content was calculated using its absorption coefficient of 150 mM^{-1} cm^{-1} and the results were expressed as nmol(MDA) g^{-1}(FM).

In order to measure membrane stability index (MSI), after six weeks of Cd treatment leaf discs of at least 80 mg were cut with scissors and placed in glass vials. The samples were washed twice with double-distilled water for 5 min. After draining the water off, 10 mL of double-distilled water was added to each vial. All vials were then placed on a shaker (150 rpm, 25ºC, 30 min). The conductivity of the medium was measured using a conductivity electrode (*Cond7110, InoLab*, Germany). Then all vials were transferred to a hot water bath for 10 min and the conductivity was measured. The MSI was calculated as described by Bajji *et al.* (2002).

Free proline content in the leaves was measured using the method of Bates *et al.* (1973). Fresh mature leaves (500 mg) were grinded in 10 mL of 3% aqueous sulphosalycylic acid and the extract was filtered. Two mL of the extract was added into the test tube with 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid. The reaction mixture was boiled in a water bath at 100°C for 1 h. After cooling the mixture on ice, 4 mL of toluene was added and thoroughly mixed, the toluene phase was separated and its absorbance measured at 520 nm using a *HITACHI U1800* spectrophotometer against toluene blank and the results were expressed as μmol mol^{-1}.

Harvest of plants and analysis of Cd content: At the end of Cd treatment, plants were harvested and roots washed in distilled water for 2 × 2 min. The roots were then separated from the shoots. Thereafter, the plant materials were wet-digested in HNO_3:$HClO_4$ (7:3, v/v) according to the method described by Frank (1976). The Cd concentration in shoots (Cd-S) and roots (Cd-R) were measured by atomic absorption spectrophotometry (*Perkin Elmer 3030*, USA) using a flame atomizer. Standards were added to the samples to eliminate the interaction of the sample matrix.

The amount of metal taken up by roots (Eq. 1) and the translocation of metal to the shoots (Eq. 2) were calculated.

$$\text{Translocation of Cd to shoots [\%]} = \frac{\text{total content of Cd in shoots [g]}}{\text{total content of Cd in whole plant [μg]}} \times 100 \quad (1)$$

$$\text{Net accumulation of Cd } \textit{via} \text{ roots [μg } g^{-1}\text{(DM)]} = \frac{\text{total amount of Cd in whole plant [μg]}}{\text{root dry matter [g]}} \quad (2)$$

Experimental design and statistical analysis: A factorial experiment (Cd at four concentrations and six genotypes) was conducted using a randomized complete block design with three replications. All data were subjected to analysis of variance using a *Statistical Analysis Software, version 9.1* (*SAS Institute Inc.,* Cary, North Carolina, USA). The means were separated using *Fisher*'s protected least significant differences. Differences were considered significant at $p \leq 0.05$.

Results

Plant growth: DMR was significantly affected by Cd and genotype (Table 2). Mean DMR of safflower genotypes indicated a 57.3% decrease at 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 2). Averaged over three

concentrations of $CdCl_2$, the DMR of C111 genotype was significantly smaller than that of Nebraska-10, but the rest of the genotypes did not significantly differ in this regard.

DMS was significantly affected by Cd (Table 2). Mean DMS of safflower genotypes indicated a 56.8% decrease at 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 2).

Plant TLA was significantly affected by Cd, genotype, and interaction effects of Cd × genotype (Table 2). Plant TLA of all safflower genotypes decreased severaly with an increase in the Cd concentration (Fig. 1). The extent of the decrease appeared to be more severe in 2811 and S149 genotypes, leading to the significant Cd × genotype interaction.

Plant height was significantly affected by Cd (Table 2). Mean plant height of safflower genotypes indicated a 39.0% decrease at 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 2).

Cadmium uptake attributes: Cd-R was significantly affected by the genotype, Cd^{2+} concentration and interaction effects of Cd × genotype (Table 2). All safflower genotypes showed the drastic increase in Cd-R when grown at 4.5 mg($CdCl_2$) L^{-1} in the solution, relative to CK (Fig. 2*A*).

Cd-R of the genotypes did not appear to differ under CK conditions. Though, the genotypes appeared different in terms of Cd-R, when grown at the presence of 4.5 mg($CdCl_2$) L^{-1}. Genotypes 'Nebraska-10' (92 mg kg^{-1}) and 'S149' (111 mg kg^{-1}) indicated the smallest and greatest Cd-R, respectively.

Cd-S was significantly affected by the genotype, Cd, and interaction effects of Cd × genotype (Table 2). All safflower genotypes showed severe increases in Cd-S under 4.5 mg($CdCl_2$) L^{-1}, relative to CK (Fig. 2*B*). Cd-S of the genotypes did not differ under CK conditions. Though, Cd-S of the genotypes differed, when they were subjected to 4.5 mg($CdCl_2$) L^{-1}. Genotypes 'Nebraska-10' (9.3 mg kg^{-1}) and 'K12' (10.2 mg kg^{-1}) tended to indicate the smallest and greatest Cd-S, respectively.

Net accumulation of Cd *via* roots was significantly affected by Cd (Table 2). Treatment of safflower plants with Cd increased the Cd content of the plants; 4.5 mg($CdCl_2$) L^{-1} in the solution caused a more than 90-fold increase in the net Cd accumulation, compared to

Table 2. Analysis of variance (mean squares) and mean comparisons for plant height (Height), total leaf area (TLA), root dry mass (DMR), shoot dry mass (DMS), Cd concentration in shoots (Cd-S), Cd concentration in roots (Cd-R), translocation of Cd to shoots (Cd-T), and net accumulation of Cd *via* roots (Cd-A) of safflower genotypes subjected to different concentrations of Cd. Data in the brackets indicate df for Cd-S and Cd-R. Mean square – between group variance; df – degrees of freedom; ns – not significant; Error – within group variance. * – $P \leq 0.05$;** – $P \leq 0.01$. Data are the mean ± SE [n = 18 for $CdCl_2$; n = 12 (6 for Cd attributes) for genotype]. Values within a group in a column bearing *different superscript* are significantly different at $P \leq 0.05$.

Source of variation	df	Mean squares							
		DMR	DMS	TLA	Height	Cd-R	Cd-S	Cd-A	Cd-T
Replicate	2	0.0013^{ns}	0.02^{ns}	452.90^{ns}	9.30^{ns}	1.57^{ns}	0.006^{ns}	667.02^{ns}	82.96^{ns}
Cd	3(1)	0.0228^{**}	0.76^{**}	291025.58^{**}	2764.96^{**}	84393.15^{**}	840.420^{**}	200355.30^{**}	6353.84^{**}
Genotype	5	0.0008^{**}	0.02^{ns}	2025.92^{**}	11.6^{ns}	125.55^{**}	0.377^{**}	170.14^{ns}	54.73^{ns}
Cd × Genotype	15(5)	0.0001^{ns}	0.01^{ns}	1022.78^{**}	8.48^{ns}	120.89^{**}	0.266^{**}	163.93^{ns}	57.98^{ns}
Error	46(22)	0.0002	0.01	263.77	6.67	1.62	0.012	213.84	54.25
Mean comparisons									
		DMR [g]	DMS [g]	TLA [cm^2 per plant]	Height [cm]	Cd-R [μg(Cd) g^{-1}(DM)]	Cd-S	Cd-A	Cd-T [%]
$CdCl_2$ [mg L^{-1}]									
0		$0.75^{a} \pm 0.04$	$0.146^{a} \pm 0.005$	$338.23^{a} \pm 8.19$	$72.16^{a} \pm 0.90$	$0.62^{b} \pm 0.02$	$0.19^{b} \pm 0.01$	$1.65^{b} \pm 0.07$	$61.12^{a} \pm 1.53$
1.5		$0.38^{b} \pm 0.03$	$0.106^{b} \pm 0.003$	$149.59^{b} \pm 6.34$	$60.21^{b} \pm 0.51$	-	-		-
3		$0.33^{b} \pm 0.02$	$0.081^{c} \pm 0.003$	$88.48^{c} \pm 3.55$	$49.68^{c} \pm 0.51$	-	-	-	-
4.5		$0.32^{b} \pm 0.002$	$0.063^{d} \pm .02$	$52.55^{d} \pm 2.41$	$44.03^{d} \pm 0.57$	$97.46^{a} \pm 2.03$	$9.86^{a} \pm 0.10$	$150.86^{a} \pm 5.01$	$34.55^{b} \pm 1.98$
LSD (5%)		0.11	0.014	10.89	1.73	0.88	0.07	10.10	5.09
Genotype									
Nebraska-10		$0.54^{a} \pm 00.08$	$0.112^{a} \pm 0.01$	$162.45^{b} \pm 32.73$	$57.72^{a} \pm 3.42$	$44.05^{d} \pm 19.46$	$4.81^{c} \pm 2.07$	$72.36^{a} \pm 31.79$	$51.48^{a} \pm 6.19$
2811		$0.43^{a} \pm 0.07$	$0.105^{ab} \pm 0.01$	$181.2^{a} \pm 41.76$	$57.07^{ab} \pm 3.12$	$45.75^{c} \pm 20.20$	$4.87^{c} \pm 2.10$	$69.02^{a} \pm 30.53$	$47.2^{a} \pm 7.13$
Kouseh		$0.46^{a} \pm 0.04$	$0.102^{ab} \pm 0.01$	$154.39^{bc} \pm 33.43$	$55.69^{ab} \pm 2.98$	$45.47^{cd} \pm 20.09$	$4.75^{c} \pm 2.05$	$76.80^{a} \pm 33.92$	$48.44^{a} \pm 3.94$
S_{149}		$0.44^{a} \pm 0.05$	$0.093^{b} \pm 0.01$	$148.92^{c} \pm 32.98$	$56.45^{ab} \pm 3.59$	$53.80^{a} \pm 23.73$	$5.12^{b} \pm 2.19$	$81.23^{a} \pm 36.06$	$45.16^{a} \pm 6.44$
K_{12}		$0.42^{a} \pm 0.05$	$0.090^{b} \pm 0.008$	$148.77^{c} \pm 30.10$	$57.10^{ab} \pm 3.09$	$50.54^{b} \pm 22.31$	$5.24^{b} \pm 2.24$	$83.16^{a} \pm 37.90$	$50.84^{a} \pm 7.22$
C_{111}		$0.41^{b} \pm 0.05$	$0.092^{b} \pm 0.01$	$147.54^{c} \pm 30.01$	$55.09^{b} \pm 3.65$	$54.64^{a} \pm 24.12$	$5.36^{a} \pm 2.28$	$74.97^{a} \pm 32.81$	$43.91^{a} \pm 8.28$
LSD (5%)		0.13	0.017	13.46	2.12	1.5	0.13	17.51	8.81

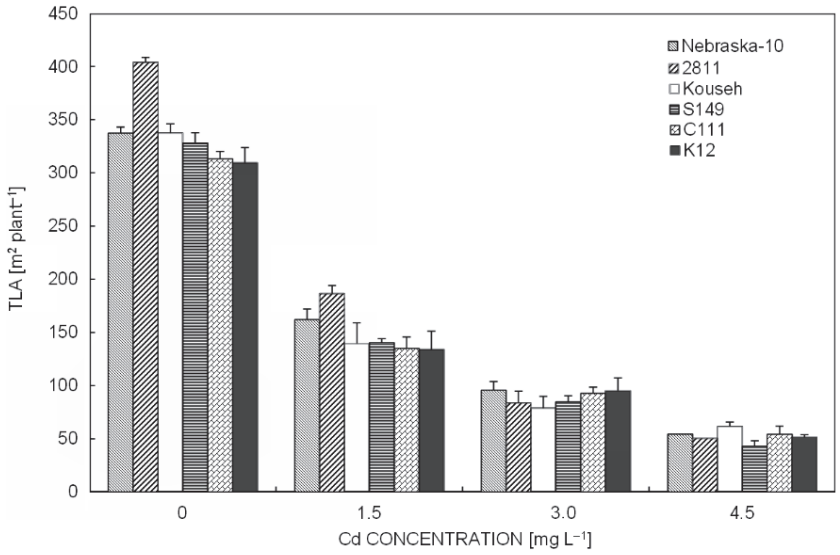

Fig. 1. Plant total leaf area in six genotypes of safflower when grown for six weeks under two concentrations of Cd. Data are the mean ± SE (n = 3). Columns designated by *the same letter* are not significantly different at $P \leq 0.05$ level as determined by LSD test.

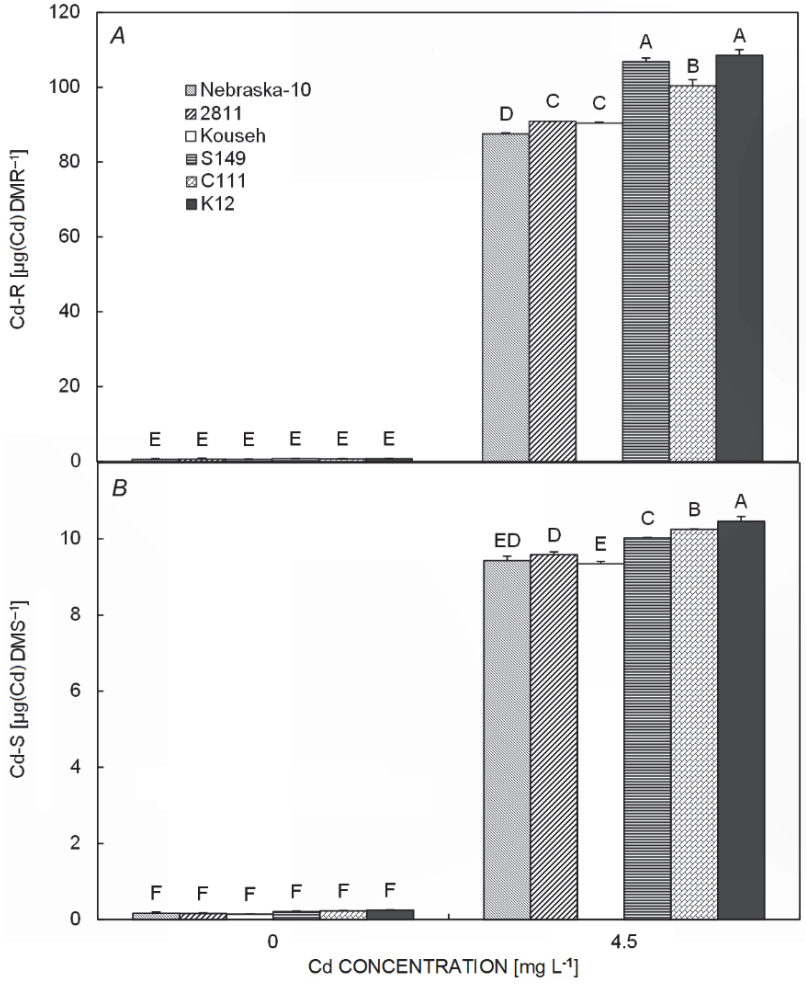

Fig. 2. Cd concentration of roots (*A*) and shoots (*B*) in six genotypes of safflower when grown for six weeks under two concentrations of Cd. Data are the mean ± SE (n = 3). Columns designated by *the same letter* are not significantly different at $P \leq 0.05$ level as determined by LSD test.

CK (Table 2). The net Cd accumulation in safflower genotypes ranged from 69 in 2811 genotype to 83 µg(Cd^{2+}) g^{-1}(DM) in K12, averaged over the two Cd concentrations (Table 2), which did not appear to be significantly different.

Cadmium translocation from roots to shoots was significantly affected by Cd (Table 2). The treatment of safflower plants with Cd reduced the Cd translocation (Table 2); 4.5 mg($CdCl_2$) L^{-1} in the solution indicated a 34.55% Cd translocation, compared to 61.12% in CK.

Table 3. Analysis of variance (mean squares) and mean comparisons for net photosynthetic rate (P_N), stomatal conductance to the CO_2 (g_s), substomatal CO_2 concentration (C_i), minimum (F_0) and maximum fluorescence (F_m), and quantum efficiency of PSII (F_v/F_m) of safflower genotypes subjected to different concentrations of Cd. Mean square – between group variance; df – degrees of freedom; ns – not significant; error – within group variance; * – $P \leq 0.05$; ** – $P \leq 0.01$. Data are the mean ± SE (n = 18 for $CdCl_2$; n = 12 for genotype). Values within a group in a column bearing *different superscript* are significantly different at $P \leq 0.05$.

Source of variation	df	Mean squares					
		P_N	g_s	C_i	F_0	F_m	F_v/F_m
Replicate	2	0.118^{ns}	0.0003^{ns}	10.79^{ns}	19.05^{ns}	3.09^{ns}	0.00016^{ns}
Cd	3	53.665^{**}	0.08073^{**}	25157.57^{**}	918.38^{**}	3228.56^{**}	0.02662^{**}
Genotype	5	1.555^{**}	0.00128^{**}	177.73^{**}	12.15^{ns}	14.78^{ns}	0.00024^{ns}
Cd × Genotype	15	0.0322^{**}	0.00054^{**}	15.34^{*}	5.15^{ns}	5.33^{ns}	0.00010^{ns}
Error	46	0.040	0.00011	7.70	7.07	6.31	0.00020
Mean comparisons		P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(CO_2) m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	F_0	F_m	F_v/F_m
$CdCl_2$ [mg L^{-1}]							
0		$8.00^{a} \pm 0.18$	$0.296^{a} \pm 0.006$	$193.77^{d} \pm 1.00$	$68.00^{a} \pm 1.09$	$286.27^{a} \pm 0.27$	$0.780^{a} \pm 0.005$
1.5		$6.00^{b} \pm 0.08$	$0.183^{b} \pm 0.003$	$212.72^{c} \pm 0.71$	$63.55^{b} \pm 0.35$	$271.05^{b} \pm 0.98$	$0.741^{b} \pm 0.001$
3.0		$5.02^{c} \pm 0.04$	$0.163^{c} \pm 0.002$	$235.27^{b} \pm 1.18$	$59.83^{c} \pm 0.45$	$261.88^{c} \pm 0.57$	$0.710^{c} \pm 0.001$
4.5		$3.97^{d} \pm 0.07$	$0.148^{d} \pm 0.002$	$280.55^{a} \pm 1.40$	$51.16^{d} \pm 0.31$	$255.38^{d} \pm 0.31$	$0.692^{d} \pm 0.002$
LSD (5%)		0.13	0.007	1.86	1.78	1.68	0.009
Genotype							
Nebraska-10		$6.17^{a} \pm 0.55$	$0.2150^{a} \pm 0.02$	$232.75^{b} \pm 10.24$	$59.66^{b} \pm 2.05$	$270.83^{a} \pm 3.88$	$0.734^{ab} \pm 0.01$
2811		$5.51^{c} \pm 0.43$	$0.1941b^{c} \pm 0.01$	$228.50^{c} \pm 9.86$	$61.16a^{b} \pm 2.15$	$268.08^{b} \pm 3.53$	$0.728^{ab} \pm 0.01$
Kouseh		$6.07a^{b} \pm 0.48$	$0.2016^{b} \pm 0.01$	$236.00^{a} \pm 10.22$	$62.16^{a} \pm 1.92$	$268.5^{b} \pm 3.48$	$0.730^{a} \pm 0.01$
S149		$5.95^{b} \pm 0.45$	$02016^{b} \pm 0.01$	$232.58^{b} \pm 9.84$	$60.75a^{b} \pm 1.95$	$268.58^{b} \pm 3.57$	$0.738^{a} \pm 0.01$
K12		$5.54^{c} \pm 0.43$	$0.1883^{c} \pm 0.01$	$228.16^{c} \pm 9.31$	$59.41^{b} \pm 1.97$	$268.16^{b} \pm 3.55$	$0.728^{ab} \pm 0.01$
C111		$5.26^{d} \pm 0.36$	$0.1875^{c} \pm 0.01$	$225.50^{c} \pm 9.27$	$60.66^{ab} \pm 1.97$	$267.75^{b} \pm 3.33$	$0.726^{b} \pm 0.01$
LSD (5%)		0.16	0.0087	2.28	2.18	2.06	0.011

Cadmium translocation in safflower genotypes ranged from 43.9% in C111 genotype to 51.48% in Nebraska-10, averaged over the two Cd concentrations (Table 2), which did not differ significantly.

Photosynthetic attributes: P_N, g_s, and C_i were significantly affected by Cd, genotype, and interaction effects of Cd × genotype (Table 3). P_N for all genotypes significantly decreased, when exposed to either 1.5, 3, or 4.5 mg($CdCl_2$) L^{-1}, relative to CK (Fig. 3*A*). The extent of the decrease differed, however, with the genotype; Nebraska-10 indicated the greatest decrease among all genotypes. g_s for all genotypes indicated the significant decrease under 1.5, 3, and 4.5 mg($CdCl_2$) L^{-1} in the solution, relative to CK conditions (Fig. 3*B*). The decrease in g_s with increasing Cd^{2+} concentration in the solution was genotype-dependent, *i.e.*, the decrease in Nebraska-10 was greater, compared to other genotypes. Substomatal CO_2 concentration for all genotypes increased with increase in the Cd^{2+} concentration in the solution (Fig. 3*C*); the extent of the increase was greater for Nebraska-10, compared to the remaining genotypes. Despite greater changes in photosynthetic parameters of the genotype Nebraska-10, photosynthetic performance of this genotype under 4.5 mg($CdCl_2$) L^{-1} tended to be one of the greatest among the genotypes studied.

F_0, F_m, and F_v/F_m were significantly affected by Cd (Table 3). Mean F_0, F_m, and F_v/F_m of the safflower genotypes indicated 24.1, 10.8, and 11.5% decrease, respectively, at 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 3).

Leaf Chl *a*, *b*, (*a*+*b*), and carotenoid contents were significantly affected by Cd and genotype (Table 4) and the mean values showed 31.6, 32.2, 31.9, and 32.9% decline, respectively, under 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 4). The genotypes '2811' and 'C111' showed the greatest and smallest means of Chl *a*, respectively (Table 4).

Genotypes 2811 and Nebraska-10 showed the greatest and C111 the smallest Chl *b* contents. Genotype 2811 showed the greatest and C111 and S149 the smallest total Chl contents. Genotypes 2811 and Nebraska-10 showed the greatest and C111 the smallest carotenoid contents.

The leaf proline content was significantly affected by Cd, while lipid peroxidation and MSI were significantly affected by Cd, genotype, and interaction effects of Cd × genotype (Table 4). The mean leaf proline content of safflower genotypes exhibited 121.4% increase when exposed to 4.5 mg($CdCl_2$) L^{-1} compared with CK (Table 4).

The tissue MDA content (as a measure of lipid

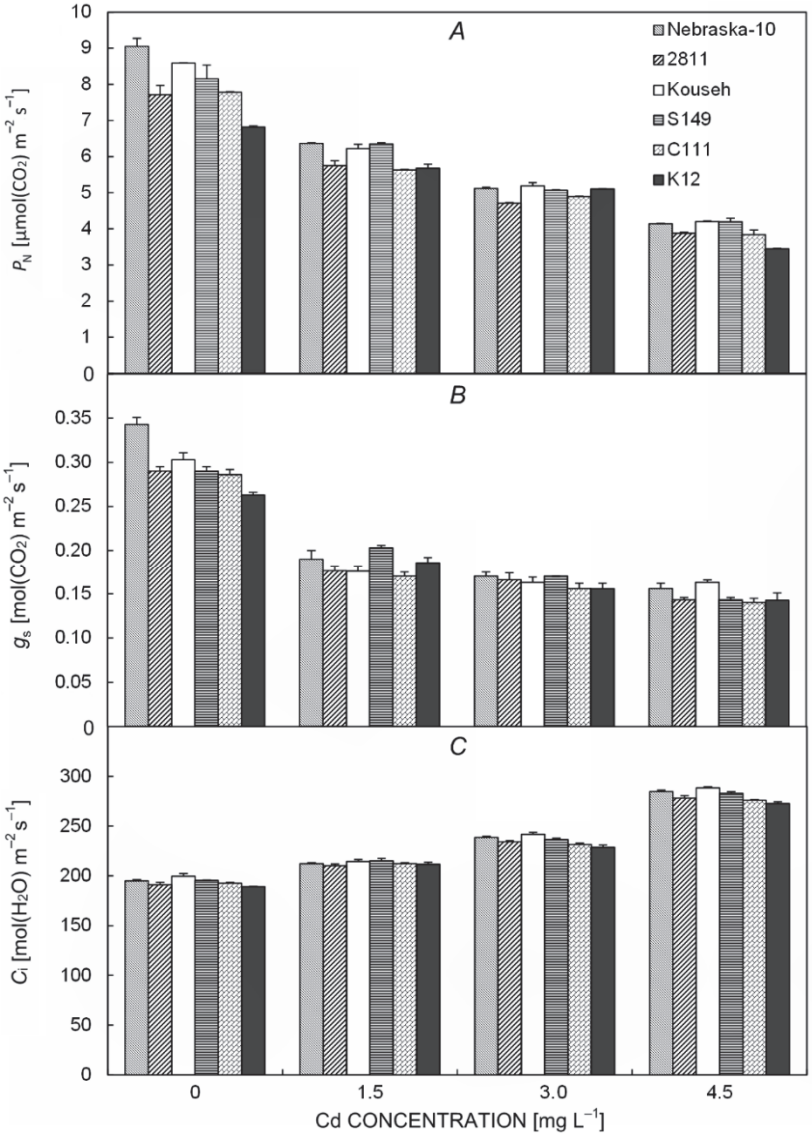

Fig. 3. Net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), and substomatal CO_2 concentration (C_i, *C*) in six genotypes of safflower when grown for six weeks under four concentrations of Cd. Data are the mean ± SE (n = 3). Columns designated by *the same letter* are not significantly different at $P \leq 0.05$ level as determined by LSD test.

peroxidation) of different safflower genotypes increased from nearly 2–2.4 in CK to nearly 5–5.5 nM g^{-1} at 4.5 mg($CdCl_2$) L^{-1} (Fig. 4*A*). In contrast to lipid peroxidation, MSI for all genotypes decreased with the Cd concentration, though, the extent of the decrease varied between genotypes (Fig. 4*B*).

Discussion

Cd toxicity impacted significantly all aspects of plant growth, *i.e.*, DMR, DMS, plant height, and TLA (Table 2), whereby leading to alterations in general growth in safflower genotypes. The Cd-induced stunted growth of safflower was likely related to the general stress-induced syndrome proposed by Potters *et al.* (2007, 2009). According to this notion, distinct sublethal stresses may induce a set of morphogenic responses, known as the stress-induced morphogenic response, as a part of a general acclimation strategy by which plant growth is redirected to diminish stress exposure. Increased ROS production, antioxidant activities, and alterations in transport and metabolism of plant growth regulators, such as auxin and ethylene, are presumably part of this strategy. Cd has been shown to interfere with plant growth in different plant species, including Scots pine (Ekvall and Greger 2003), sunflower (Laspina *et al.* 2005), barley (Guo *et al.* 2007), mustard (Iqbal *et al.* 2010), tomato (Cherif *et al.* 2012), *Medicago truncatula* (Saeidi *et al.* 2012), and the Cd-hyperaccumulator, *Atriplex* plants (Nedjimi and Daoud 2009). It seems, according to our findings, that Cd pollution led to the severe reduction in the TLA of safflower genotypes and at Cd concentrations as low as 1.5 mg($CdCl_2$) L^{-1}; it could be sufficient for nearly the 50% decrease in this important attribute of plant production capacity. Plant

Table 4. Analysis of variance (mean squares) and mean comparisons for leaf chlorophyll (Chl) *a*, Chl *b*, and total Chl (*a*+*b*), carotenoid contents (Car), leaf proline content (Pro), lipid peroxidation (LP), and membrane stability index (MSI) of safflower genotypes subjected to different concentrations of Cd. Mean square – between group variance; df – degrees of freedom; ns – not significant; Error – within group variance; * – $P \leq 0.05$; ** – $P \leq 0.01$. Data are the means ± SE (n = 18 for $CdCl_2$; n = 12 for genotype). Values within a group in a column bearing *different superscript* are significantly different at $P \leq 0.05$.

Source of variation	df	Mean squares Chl *a*	Chl *b*	Chl (*a*+*b*)	Car	Pro	LP	MSI
Replicate	2	0.0034^{*}	0.00018^{ns}	0.0046^{ns}	0.39^{ns}	0.00023^{ns}	0.0043^{ns}	2.63^{ns}
Cd	3	0.0549^{**}	0.01080^{**}	0.1144^{**}	10.25^{**}	0.2746^{**}	33.73^{**}	4564.45^{**}
Genotype	5	0.0079^{**}	0.00203^{**}	0.0177^{**}	1.34^{**}	0.00005^{ns}	0.225^{*}	169.96^{**}
Cd × Genotype	15	0.0011^{ns}	0.00044^{ns}	0.0025^{ns}	0.14^{ns}	0.00005^{ns}	0.0104^{**}	1.68^{*}
Error	46	0.0009	0.00029	0.0017	0.17	0.00023	0.0042	0.83

Mean comparisons	Chl *a*	Chl *b*	Chl (*a*+*b*) [mg g^{-1}(FM)]	Car	Pro [µmol mol^{-1}]	LP [nM(MDA) g^{-1}]	MSI [%]
$CdCl_2$ [mg L^{-1}]							
0	$0.411^{a} \pm 0.01$	$0.180^{a} \pm 0.005$	$0.590^{a} \pm 0.01$	$5.41^{a} \pm 0.15$	$0.243^{d} \pm 0.003$	$2.14^{d} \pm 0.99$	$87.33^{a} \pm 0.99$
1.5	$0.353^{b} \pm 0.01$	$0.149^{b} \pm 0.006$	$0.505^{b} \pm 0.01$	$4.70^{b} \pm 0.13$	$0.405^{c} \pm 0.003$	$3.52^{c} \pm 0.02$	$70.07^{b} \pm 0.78$
3.0	$0.318^{c} \pm 0.007$	$0.135^{c} \pm 0.004$	$0.459^{c} \pm 0.01$	$4.22^{c} \pm 0.11$	$0.451^{b} \pm 0.002$	$4.46^{b} \pm 0.03$	$59.08^{c} \pm 0.74$
4.5	$0.281^{d} \pm 0.006$	$0.122^{d} \pm 0.003$	$0.402^{d} \pm 0.008$	$3.63^{d} \pm 0.07$	$0.538^{a} \pm 0.003$	$5.34^{a} \pm 0.04$	$50.40^{d} \pm 0.91$
LSD (5%)	0.020	0.011	0.028	0.28	0.010	0.04	0.61
Genotype							
Nebraska-10	$0.355^{b} \pm 0.017$	$0.160^{a} \pm 0.009$	$0.517^{b} \pm 0.02$	$4.803^{a} \pm 0.024$	$0.415^{a} \pm 0.03$	$3.697^{d} \pm 0.35$	$71.26^{a} \pm 4.33$
2811	$0.385^{a} \pm 0.02$	$0.165^{a} \pm 0.009$	$0.552^{a} \pm 0.03$	$5.002^{a} \pm 0.27$	$0.402^{b} \pm 0.03$	$3.820^{c} \pm 0.34$	$67.39^{b} \pm 4.81$
Kouseh	$0.335^{cb} \pm 0.01$	$0.145^{b} \pm 0.008$	$0.478^{c} \pm 0.02$	$4.428^{b} \pm 0.23$	$0.416^{a} \pm 0.11$	$3.747^{d} \pm 0.34$	$71.00^{a} \pm 4.16$
S_{149}	$0.320^{c} \pm 0.01$	$0.136^{b} \pm 0.007$	$0.458^{c} \pm 0.01$	$4.242^{b} \pm 0.16$	$0.412^{ab} \pm 0.03$	$3.917^{b} \pm 0.36$	$64.43^{c} \pm 4.06$
K_{12}	$0.331^{bc} \pm 0.01$	$0.140^{b} \pm 0.008$	$0.473^{c} \pm 0.02$	$4.340^{b} \pm 0.24$	$0.401^{b} \pm 0.03$	$4.001^{a} \pm 0.36$	$63.50^{d} \pm 4.09$
C_{111}	$0.317^{c} \pm 0.01$	$0.134^{b} \pm 0.004$	$0.453^{c} \pm 0.01$	$4.155^{b} \pm 0.19$	$0.409^{ab} \pm 0.03$	$4.035^{a} \pm 0.37$	$62.74^{e} \pm 4.17$
LSD (5%)	0.025	0.014	0.034	0.347	0.012	0.053	0.75

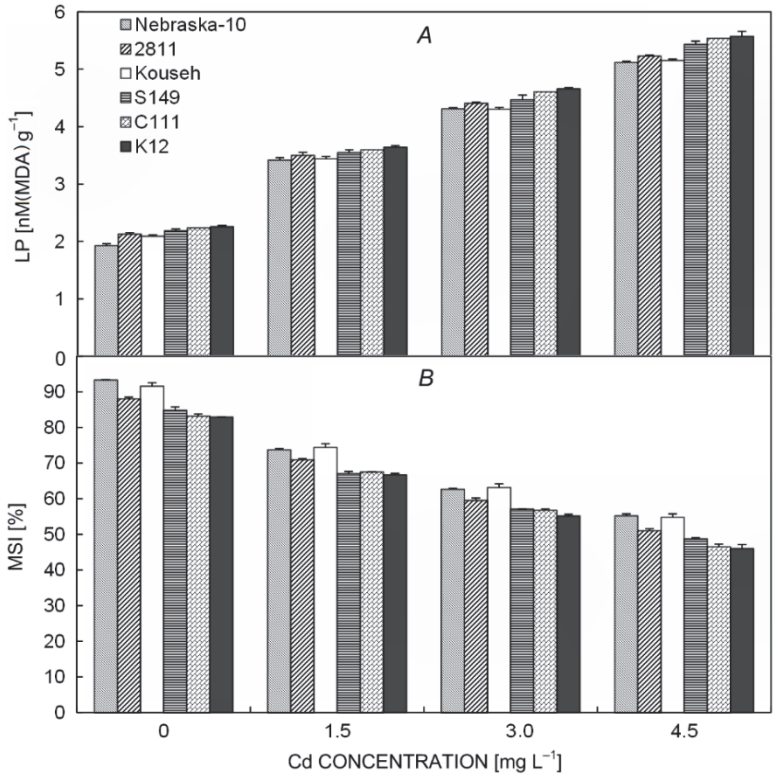

Fig. 4. Lipid peroxidation (LP), estimated *via* tissue malondialdehyde (MDA) content (*A*) and membrane stability index (MSI) (*B*) in six genotypes of safflower when grown for six weeks under four concentrations of Cd. Data are the mean ± SE (n = 3). Columns designated by *the same letter* are not significantly different at $P \leq 0.05$ level as determined by LSD test.

growth and total leaf area of Cd-exposed oilseed rape plants were lowered significantly (Larsson *et al.* 1998).

In the present study, the greatest DMR and DMS were detected in genotype Nebraska-10 (Table 2) and the smallest values were observed in genotype K12. On the other hand, the latter genotypes indicated the smallest and greatest Cd-R and Cd-S, respectively. A decrease in a tissue Cd concentration due to an enhanced growth, *i.e.,* a dilution effect, has been proposed by Ekvall and Greger (2003). It could be concluded that the observed differences between the safflower genotypes in terms of Cd-R and Cd-S were, at least partly, due to the dilution effect. Our results are consistent with earlier reports; Ekvall and Greger (2003) have reported that genotypic and/or ecotypic differences exist for Cd uptake and translocation to shoots. Our results were also in agreement with several earlier reports which have indicated that much of Cd taken up by plants is retained in roots and only a portion is translocated to shoots (Benavides *et al.* 2005, Guo *et al.* 2007, Tran and Popova 2013). Both the amounts accumulated and translocated vary with species and genotypes (Tran and Popova 2013). As Blum (1997) has pointed out, Cd accumulation in the plant tissues is often in the order: roots > stems > leaves > fruits > seeds. Larsson *et al.* (1998) indicated that Cd concentration in roots of Cd-exposed oilseed rape plants could reach 5 to 7-fold of that in shoots. Nedjimi and Daoud (2009) observed that approximately 75% of the Cd taken up was retained in the *Atriplex halimus* roots, which is in concert with our findings in safflower plants.

Dramatic increases in Cd-S (52-fold) and Cd-R (157-fold) under 4.5 mg($CdCl_2$) L^{-1}, relative to CK (Table 2), together with 43.9% (genotype 'C111') and 51.5 % (genotype 'Nebraska-10') translocation of Cd *via* roots, indicated that Cd was easily transferred in soil-plant system. The nearly 21% (Fig. 2*A*) and 11% (Fig. 2*B*) differences in the root and shoot Cd accumulation, respectively, between the genotypes at the 4.5 mg($CdCl_2$) L^{-1} suggest existence of some differences in Cd absorption and, hence, physiological consequences of exposure to Cd in the examined genotypes. Increases of Cd concentrations in roots and shoots with an enhancement of external Cd concentrations have been emphasized by other researchers (Ekvall and Greger 2003).

It seemed that the extent of differences between g_s of the safflower genotypes decreased when plants were grown under Cd-polluted conditions (Fig. 3*B*). It is clear that Cd inhibits stomatal opening but the exact mechanism is not agreed upon. Stomatal movements could be altered through Cd-induced reduction in cell wall elasticity (Barceló *et al.* 1986). Furthermore, interference of Cd with phytohormone biosynthesis and/or ionic movements in stomatal guard cells have been speculated as the probable cause of Cd impact on plant water relations and stomatal movements (Poschenrieder *et al.* 1989). Interference of Cd with nutrition and movements of key ions, *i.e.,* K^+ and Ca^{2+} (Nedjimi and Daoud 2009) and/or phytohormones, such as abscisic acid, is perhaps involved. Alterations in status of plant growth regulators, such ethylene and auxin, have already been attributed to a stress-induced morphogenic response that is evoked by distinct sublethal stresses (Potters *et al.* 2007). Changes in abscisic acid (*i.e.*, due to Cd at the present study) and, therefore, stomatal movements as a part of a general acclimation strategy cannot be ruled out. The increase in mean C_i under 1.5, 3, and 4.5 mg($CdCl_2$) L^{-1} (Table 3) indicated serious influence on photosynthetic activity in safflower even under 1.5 mg($CdCl_2$) L^{-1}. It seemed that the photosynthetic activity of all genotypes was severely disrupted by concentrations greater than 1.5 mg($CdCl_2$) L^{-1} (Fig. 3*A*). Photosynthesis of the genotype 'Nebraska-10' seemed to suffer more from Cd-induced stress, compared to the remaining safflower genotypes, as indicated by its P_N (Fig. 3*A*) and g_s (Fig. 3*B*). The reduction in P_N in the Cd-treated safflower plants was, at least partly, attributable to a certain limitation of CO_2 supply due to the decreased g_s (Table 3) that was, presumably, caused by a partial closure of stomata. Various factors at different structural-functional levels may impair the photosynthetic activity of Cd-treated plants. Therefore, plant response to Cd is a complex phenomenon, *i.e.*, Cd may evoke an array of events at molecular, physiological, and morphological levels (Singh *et al.* 2006, Cherif *et al.* 2012). Alterations in chloroplast ultrastructure, lowering Chl and Car contents, and lowering the activity of key CO_2 assimilation enzymes, *i.e.*, Rubisco and phospho*enol*pyruvate carboxylase, whereby leading to adverse impacts on photosynthetic rate, have been proposed in literature (Tran and Popova 2013). In our experiment, Chl and Car contents of safflower were adversely affected by Cd. Furthermore, Cd effects on photosynthetic performance of safflower may be categorized into two types: (*1*) the response of stomata to stress (*i.e.* stomatal limitation), and (*2*) the diffusion-independent effects (*i.e.*, nonstomatal limitation) of the stressor factor. Stomatal closure in response to environmental constrains is commonly seen in different species. The data presented here for safflower indicated that stomatal opening and, hence g_s, decreased due to the presence of Cd in the nutrient solution. However, the extent to which stomatal closure affects photosynthetic capacity can be speculated by the magnitude of the alteration in C_i (Seemann and Critchley 1985). Our results indicated that the reduction in g_s (Fig. 3*B*) did not lead to a photoassimilation-dependent depletion of the substomatal CO_2 in safflower genotypes (Fig. 3*C*). A decline of P_N associated with an increase in C_i has, frequently, been interpreted as a direct effect of the stress factor on the photosynthetic capacity. Such changes in the photosynthetic capacity that are not proportional to diffusional limitations could be attributed to either a change in a size of photosynthetic apparatus and/or an alteration in the efficiency of operation of this machinery (Seemann and Critchley 1985). Our results,

therefore, indicate that the declined P_N in safflower genotypes in response to Cd could be only partly explained by a decreased g_s; *i.e.*, nondiffusional limitations were involved in the decline of photosynthesis. It seems that a decreased mesophyll conductance, which suggests inhibition of photosynthetic activity at the biochemical level, contributed to the lowered photosynthetic performance of the safflower genotypes. Inhibitory effect of Cd on photosynthetic activity of different plant species including beans (Vassilev *et al.* 2005) and maize (Krantev *et al.* 2008) has been documented. The 50% decrease in mean P_N of the Cd-treated safflower genotypes (Table 3) agreed well with the 60% decrease reported by an earlier work (Vassilev *et al.* 2005) on Cd-exposed bean plants.

The photosynthetic apparatus has been frequently reported as one of the target sites of Cd action in plants (Sanità di Toppi and Gabbrielli 1999, Cherif *et al.* 2012). Different components of the photosynthetic apparatus including Chl, thylakoid membranes, and PSII could be the subject of harmful interactions with Cd. Such effects on ATP and NADPH utilization lead to a decrease in a rate of linear electron transport and quantum efficiency of PSII (Zribi *et al.* 2009). Stress-induced stomatal closure leads further to the depletion of substomatal CO_2 and accumulation of energy-containing products of electron transport that result in perturbation of photosynthetic light harvesting complexes (Sayed 2003 and references therein). Stress-induced inhibition of Chl synthesis, such as the one documented with Cd-stressed safflower in the present study, leads to preferential reduction of the light-harvesting pigment-protein associated with PSII through decreases in content of Chl *a/b* binding proteins. Increases or decreases in fluorescence under stress conditions suggest a disorder in the photosynthetic-related events in chloroplasts (Judy *et al.* 1990). In fact, under many environmental stresses, plants use less radiation energy for photosynthesis and may use some mechanisms to dissipate safely excessive light energy to avoid photoinhibition and photo-oxidation. Therefore, an inverse relationship is expected to exist between *in vivo* Chl fluorescence and photosynthetic activity. In the present study, lower Chl and Car contents and photosynthetic activity (CO_2 assimilation) in the Cd-treated safflower plants was associated with increased fluorescence intensity and lowered F_v/F_m of the plants (Table 3). It is, therefore, evident that the perturbed photosynthetic metabolism induced by Cd modified the fluorescence emission kinetics of the safflower genotypes. Cd-treated *Tradescantia* plants indicated 3–7% increases in F_0 when exposure time was lesser than 3 h, but they indicated 12% decrease in F_0, when plants were exposed to Cd for 5 h (Judy *et al.* 1990). Our results corresponded with those of Larsson *et al.* (1998), who found 33% and 73% decreases in F_v/F_m and F_m, respectively, when oilseed rape was subjected to 5 μM of $CdCl_2$. F_0 is usually expected to increase under stressful conditions, mainly because the efficiency of energy transfer from antenna Chl *a* to reaction centers decreases and/or PSII reaction centers become inactivated. As Larsson *et al.* (1998) have argued, however, if the latter nonfunctional PSII centers act as dissipating sinks, it may lead to the decrease in F_0.

Approximately one-third reduction in the mean concentration of different photosynthetic pigments in safflower plants grown under 4.5 mg($CdCl_2$) L^{-1} (Table 4) could be attributed to the reduction in Chl and Car biosynthesis and/or ROS-induced oxidation of these pigments which is characteristic to heavy metals stress (Cherif *et al.* 2012). A marked decline of Chl content in Cd-treated sunflower plants, similar to our finding, has been described by Laspina *et al.* (2005). Oilseed rape plants treated with Cd indicated drastic decreases, ranging from 60 to 80%, in the Chl and Car contents (Larsson *et al.* 1998). Increase in the external Cd concentration reduced the Chl concentration in the Cd-hyperaccumulator, *Atriplex* plants (Nedjimi and Daoud 2009). Genotype 2811 outperformed the remaining genotypes in terms of the mean Chl *a*, Chl *b*, Chl (*a*+*b*), and Car contents (Table 4). The decrease in leaf Chl and Car concentrations of the safflower genotypes could be one of the factors contributing to the previously discussed alterations in their P_N. Moreover, since the decreased Chl concentration was accompanied by the decrease in F_v/F_m (Table 3), this could be considered as a further evidence of the fact that the g_s was not the sole factor responsible for the decrease in P_N of the safflower genotypes studied.

The exact role of proline in response to environmental stresses is not agreed upon (Delauney and Verma 1993). Researchers have suggested several roles for the accumulation of nitrogen-containing compounds during a variety of stresses; this common adaptive response of plants to adverse environmental conditions is not purely osmotic in nature (Hare and Cress 1997). Such compounds may play diverse functions in response to stress, their function is dependent on the species, tissue, and the type of stress (Gilbert *et al.* 1998). Proline accumulation in plant cells is either through an increase in proteolysis or a decrease in protein synthesis (Ashraf and Harris 2004). This energetically expensive process of proline accumulation may ensure cellular homeostasis under stressful conditions (Hare and Cress 1997). The accumulation of proline under stress conditions can protect the cell by stabilizing subcellular structures (*e.g.*, proteins and enzymes) and buffering the cellular redox potential (Demiral and Türkan 2005). In addition to acting as an osmolyte, proline can also confer enzyme protection, increase membrane stability, and act as a part of the antioxidative defense system under stressful conditions (Nasir Khan *et al.* 2007). As it has been discussed by Hare and Cress (1997), an increased $NADP^+$/NADPH ratio due to high proline biosynthesis may enhance the activity of oxidative pentose phosphate pathway and, as a consequence, provide precursors to meet demands for increased secondary metabolites production during stress.

Furthermore, changes in proline metabolism may constitute a form of metabolic signaling within a plant. Proline has been proposed as a substrate for tricarboxylic acid cycle during plant recovery from stress (Hare and Cress 1997). Proline accumulation in plant tissues, therefore, may be part of a general adaptation in response to several stresses (Delauney and Verma 1993). In an earlier study on several plant species (Alia and Saradhi 1991), Cd was found to be the strongest proline-inducing heavy metal amongst four heavy metals examined. These researchers, therefore, proposed this osmolyte as a useful marker for heavy metal pollution in plants. Chen and Kao (1995) findings confirm our data; they indicated that declines in root growth of rice seedlings were associated with proline accumulation in roots. Our findings on safflower agreed with the conclusions made on Cd-stressed common ice plants (Shevyakova *et al.* 2003), where proline accumulation was attributed, at least in part, to development of water deficit in the Cd-stricken roots. Since proline accumulation in the safflower was associated with Cd accumulation in roots and shoots, it seems that this osmolyte played a protective role against Cd stress in safflower at least in the genotypes used in this experiment. It may be inferred that proline has contributed, at least partially, to Cd tolerance in safflower by providing an adaptive advantage (Delauney and Verma 1993) through counteracting the water deficiency that is brought about in heavy metal-stressed plants (Shevyakova *et al.* 2003).

Significant decreases in MSI were observed in all safflower genotypes after the plants were exposed to increasing concentrations of Cd for six weeks (Fig. 4*B*). The reduction of MSI from 82–92% under control to 46–56% under 4.5 mg($CdCl_2$) L^{-1} indicated severe damage to the membrane integrity in all safflower genotypes used in this study. The observed impairment in plant membrane integrity might be a further confirmation of a more general disturbing impact which is exerted by heavy metals on cellular functions. Plant cell membranes are among the primary sites of metal injury (Singh *et al.* 2006). Electron flow in PSII is often blocked by metal ions, leading to the production of ROS. Pollutant metals are involved in different types of ROS-generating mechanisms and, hence, oxidative stress (Benavides *et al.* 2005). LP has been associated with cell damages caused by different biotic and abiotic stresses and is often used as an indicator of stress-induced oxidative damage to the cellular membranes (Katsuhara *et al.* 2005). The contrasting trends observed for MSI (Fig. 4*B*) and LP (Fig. 4*A*), *i.e.*, tissue MDA content, in response to increasing concentrations of Cd were indicative of LP-induced damage to the leaf cells of the safflower genotypes. The MDA content in plant tissue is an indicator of oxidative stress-induced peroxidation of membrane lipids and it increases when plants are subjected to toxic concentrations of metals such as Cd (Guo *et al.* 2007, Son *et al.* 2012). Oxidative responses to Cd has been reported in several plant species (Benavides *et al.* 2005) and there are other reports that document LP under Cd treatment, *i.e.*, in sunflower (Laspina *et al.* 2005) and bean plants (Vassilev *et al.* 2005). It is reasonable, therefore, to hypothesize that the Cd-initiated stress was exacerbated by the oxidative stress, leading to the growth decline of safflower genotypes in the present study.

Conclusion: Cd was found to be a putative major factor constraining safflower performance, at least when grown at the presence of 4.5 mg($CdCl_2$) L^{-1}. The seriously adverse impact of Cd on the growth of the safflower genotypes was associated with the drastic reduction in MSI and increase in LP along with 52- and 156-fold increases in Cd-S and Cd-R, respectively. The stunted growth was further associated with significant alterations in gas exchange, photosynthetic pigments, and F_v/F_m. In safflower, proline appeared to play a role in adaptive response to Cd. We found clear indications of Cd-induced perturbations of photosynthesis that modified significantly the fluorescence emission kinetic characteristics of safflower plants. Minimal LP and Cd-S and Cd-R under 4.5 mg($CdCl_2$) L^{-1} in the genotype Nebraska-10 were associated to maximal g_s, P_N, and MSI, compared with the remaining safflower genotypes. Mechanisms distinguishing this genotype from the others remained unclear and should be further studied.

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