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Genotypic variation in safflower (*Carthamus spp*) cadmium accumulation and tolerance affected by temperature and cadmium levels

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Abstract

Soil pollution is a world-wide problem, with heavy metals being a major part of the concern. To investigate the effect of temperature on cadmium (Cd) uptake and translocation, as well as Cd tolerance in wild and cultivated species of safflower, a hydroponic experiment was conducted under controlled conditions. The responses of four wild genotypes (Isfahan, Arak, Azari, and Shiraz) and four cultivated genotypes (AC-Sterling, 2811, Saffire, and C111) of safflower to nine levels of $CdCl_2$ (0, 0.5, 1, 5, 10, 20, 50, 100, and 500 µM) in solution were examined under two temperatures (18 and 23 °C). Cadmium sensitivity was determined using the Weibull model on the total dry weight of the plants. Cadmium uptake and translocation were analyzed on 1 µM Cd treated plants. Results revealed that safflower genotypes differed in terms of uptake, translocation, and tolerance to Cd, with AC-Sterling and Arak indicating the most and the least tolerance to Cd, respectively. Relative Cd uptake and Cd concentration in roots and shoots increased with an increase in temperature in all genotypes, with the exception of AC-Sterling. Net accumulation of Cd via root increased with an increase in temperature for the wild Azari and the cultivated 2811, Saffire, and C111, though it decreased for the rest of genotypes. Cadmium translocation to shoots significantly increased with increased temperature in all genotypes. Cadmium translocation from roots to shoots in cultivated genotypes was significantly greater than in wild genotypes. Root Cd concentration

in wild genotypes was significantly greater than in cultivated genotypes. It seems that wild and cultivated species of safflower differ in their response to Cd. Furthermore, temperature may affect the plant's tolerance to Cd, probably through accompanying changes in Cd uptake and translocation from root to shoot.

Key words: Safflower; Cadmium; Uptake; Translocation; Tolerance.

1. Introduction

Pollution of the biosphere with toxic levels of metals has accelerated dramatically since the beginning of the industrial revolution (Nriagu, 1979). Soil pollution by heavy metals including cadmium (Cd) is a global problem, which can cause agricultural lands to become hazardous for wildlife and human populations. Environmental pollution with Cd is mainly caused by mining and smelting, 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., 2006) indicated that the Cd level of these soils could reach 2.25 to 2.57 mg kg^{-1}. Cadmium entry into the human body via the food chain is a major concern, because Cd accumulates with a half-life exceeding 10 years, and it has been linked with renal tube dysfunction and pulmonary emphysema (Gairola et al., 1992). Plants, which take up and accumulate Cd in their roots and shoots, may also be negatively affected in their photosynthesis, growth, and reproduction (Xiong and Peng, 2001).

Cadmium uptake and its effects on plants may be influenced by a variety of factors, e.g. the plant species, cultivar, soil characteristics, and temperature. Genetic differences in mineral uptake among plant species were observed decades ago (Saric, 1983), and even cultivars of the same species often show large variation in tolerance to Cd toxicity (Koleli, 2004). In a series of studies Landberg and Greger (1996 and 2002b) and Greger and Landberg (1999) showed variation in tolerance, uptake, and translocation of Cd among 200 wild and cultivated willow clones. Genotypic variation in Cd uptake and accumulation was

also 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).

The effects of temperature on metal toxicity, uptake, and accumulation have been the subject of only a few studies. Elevated temperature increased concentration of Cd in *Elodea canadensis* (Fritioff et al., 2005) and *Solanum nigrum* (Macek et al., 1994). However, Ekvall and Greger (2003) found that two ecotypes of *Pinus sylvestris* reacted differently to temperature in their Cd uptake and translocation. Lu et al. (2009) showed that low temperature treatment (4 °C) significantly inhibited Cd uptake and reduced upward translocation of Cd to shoots by up to 90% in one ecotype of *Sedum alfredii*, whereas no such effect was observed in the other ecotypes investigated.

Safflower (*Carthamus tinctorius* L.) is gaining importance as an oil seed crop in many countries worldwide. The crop has been traditionally grown for its flower, used in food coloring and flavoring, dyes, and medicinal applications for centuries. In recent decades, however, it has been grown as a source of vegetable oil for human consumption and industrial purposes (Dajue and Mündel, 1996). Safflower is known to tolerate at least two major environmental stresses, i.e. salinity and drought (Sabzalian et al., 2008), particularly in cropping systems in dry regions and marginal areas. *Carthamus oxyacanthus* L., a wild relative, may have the genetic potential to further improve the stress tolerance of the cultivated safflower, *C. tinctorius*. The two species are crossable with viable progenies (Sabzalian et al., 2008). Little scientific data exist on the response of this oilseed crop to Cd stress, though there are some reports that it may be used as a hyper-accumulator crop for Cd-polluted soils (Sayyad et al., 2010; Shi et al., 2010). The objective of this work was, therefore, to investigate differences in Cd uptake, translocation, and tolerance among eight genotypes of safflower and to assess whether (1) these parameters were influenced by temperature and (2) the effects depended upon species and genotype.

2. Materials and Methods

2.1 Plant material and growth conditions

Four genotypes of cultivated safflower, *Carthamus tinctorius* (AC-Sterling, 2811, C111, and Saffire), and four genotypes of wild safflower, *C. oxyacanthus* (Arak, Azari, Isfahan, and Shiraz), were used in the experiments. After surface sterilization with 1% (w/v) calcium hypochlorite for 10 min, seeds were sown in paper moistened with distilled water and kept for six days for germination in a growth chamber. At the two-leaf stage the seedlings were transferred to plastic pots filled with 300 mL of Hoagland nutrient solution.

The seedlings were treated for 14 days with Cd in the following initial concentrations: 0, 0.5, 1, 5, 10, 20, 50, 100, and 500 µM $CdCl_2$. These levels of Cd were chosen mainly because some studies have suggested that Cd levels of some urban soils in Iran are in the order of 2.57 mg/kg (Samani et al., 2006). In this paper, initial levels of 0.5 and 1 µM are referred to as moderate, 5, 10, and 20 µM as high, and 50, 100, and 500 as very high levels of pollution. Each pot contained 6 plants mounted on styrofoam plates floating on the solution surface. Plants were grown in a climate-controlled chamber equipped with metal halogen lamps (Osram Powestar HOI-R, Hans, Oldenburg, Germany) under two different temperature regimes: (1) 23 °C during the day and 20 °C at night and (2) 18 °C during the day and 16 °C at night, both with photoperiods of 16 h light (with a photon flux density of 600 ± 20 $\mu molm^{-2}s^{-1}$) and 8 h dark. The relative humidity of the chamber was 50%.

When the volume of the nutrient solution in the pots had decreased by 10%, water was added to maintain the initial volume. The nutrient solution pH was 6.3 and did not change during the experiment.

2.2 Harvest of plants and analysis of Cd content

At the end of Cd treatment, plants were harvested and the roots washed in distilled water for 2 × 2 min. The roots were then separated from the shoots, and fresh weights of

roots and shoots were registered. The plant materials were dried at 105 °C for 24 h and the dry weight of roots and shoots determined. Thereafter, the plant materials were wet-digested in HNO_3 : $HClO_4$ (7:3, v/v) according to the method described by Frank (1975). The Cd content in roots and shoots was analyzed by atomic absorption spectrophotometry (SpectraA 55B, Varian, Agelant, USA) using a flame atomizer. A graphite oven (GTA 100) was used when necessary (i.e. at low concentration ranges). Standards were added to the samples to eliminate the interaction of the sample matrix.

2.3 Experimental design, calculations and statistical treatments

To study the effect of temperature, genotype, and Cd level, a three replicates factorial randomized complete block design was used, in which a combined analysis over two temperatures was carried out. Plants harvested from the same pot (n = 6) were pooled into one replicate. The relative Cd uptake (equation 1), the amount of metal that had been taken up by root (equation 2), and the translocation of metal to the shoot (equation 3), the percent growth increase over the 14 days of treatment (given as relative biomass production, equation 4) were calculated after subtracting the control content of Cd.

$$\text{Relative Cd uptake (\%)} = \frac{\text{total Cd content in whole plants (µg)}}{\text{total amount of Cd in solution (µg)}} \times 100 \quad 1)$$

$$\text{Net accumulation of Cd via root (µg Cd/gDW)} = \frac{\text{total amount of Cd in whole plants (µg)}}{\text{root dry weight (g)}} \quad 2)$$

$$\text{Translocation of Cd to shoot (\%)} = \frac{\text{total content of Cd in shoot (µg)}}{\text{total content of Cd in whole plants (µg)}} \times 100 \quad 3)$$

$$\text{Relative biomass production (\%)} = \frac{\text{gFW treated}_{14\ \text{days}} - \text{gFW treated}_{\text{start}}}{} \times 100 \quad 4)$$

137 $$\text{gFW untreated}_{14\ \text{days}} - \text{gFW untreated}_{\text{start}}$$

138

139 In these equations $\text{gFW treated}_{14\ \text{days}}$ = fresh weight (g) of plants 14 days after Cd

140 treatment; $\text{gFW treated}_{\text{start}}$ = fresh weight (g) of plants before Cd treatment; $\text{gFW untreated}_{14}$

141 $_{\text{days}}$ = fresh weight (g) of control plants after 14 days; $\text{gFW untreated}_{\text{start}}$ = fresh weight (g) of

142 control plants on transfer to pots.

143 A modified Weibull model (Taylor et al., 1992) was used to compare dose-response

144 curves. Dry weight data were analyzed using the iterative nonlinear fitting procedure of JMP

145 version 2.0.2 software (SAS Institute, Cary, NC, USA) and the modified formula (equation

146 5).

147 $$y = a+b.e^{-(x/c)d} \qquad 5)$$

148 in which *y* is the plant response (dry weight) to the concentration of Cd in the growth medium

149 (*x*), *a* is the absolute minimum growth, *b* is the unaffected growth, and *c* and *d* are parameters

150 showing the shape of the curve. The parameter TT_{95b} and EC_{50} values were calculated by

151 equations 6 and 7, respectively.

152 $$TT_{95b} = c\ (-\ln 0.95)^{1/d} \qquad 6)$$

153 $$EC_{50} = c\ (-\ln 0.50)^{1/d} \qquad 7)$$

154 TT_{95b} and EC_{50} are toxicity threshold values (µM) indicating the initial metal concentrations

155 in which growth is reduced by 5% and 50%, respectively.

156 Data were subjected to analysis of variance (ANOVA) using the SAS statistical

157 program (SAS Institute Inc., 1999); where the F-value was significant, mean comparisons

158 were performed using the least significant difference (LSD) test at a 0.05 level of probability.

159

3. Results

Relative biomass production was evaluated at 8 levels (0.5, 1, 5, 10, 20, 50, 100, and 500µM) of Cd and has been presented here, accordingly. However, dry matter production attributes were evaluated and presented at two levels (0 and 1µM) of Cd.

3.1 Dry matter production

Root dry weight was significantly affected by temperature, Cd, genotype, wild genotype, cultivated genotype, species, and interaction effects of genotype × temperature, wild genotype × temperature, and cultivated genotype × temperature (Table 1). Root dry weight of C111, Saffire, Azari and Arak genotypes significantly decreased with an increase in temperature (averaged over 0 and 1µM levels of Cd), but there were no significant changes in dry weight for 2811 and AC-Sterling genotypes with increased temperature (Fig. 1). Cultivated genotypes outperformed wild genotypes in root dry weight (Table 2).

Shoot dry weight was significantly affected by temperature, Cd, genotype, wild genotype, cultivated genotype, species, and interaction effects of genotype × temperature, wild genotype × Cd, wild genotype × temperature, and cultivated genotype × temperature (Table 1). Arak and AC-Sterling genotypes indicated the greatest and smallest decrease, respectively, in shoot dry weight with increasing Cd level from 0 to 1µM (Fig. 2). Shoot dry weight for C111, Saffire, Azari, and Arak decreased significantly with increased temperature (averaged over 0 and 1µM levels of Cd) (Fig. 1); however, for AC-Sterling, shoot dry weight increased with an increase in temperature. Shoot dry weight for the remaining genotypes showed no significant changes with temperature. Cultivated genotypes outperformed wild genotypes in shoot dry weight (Table 2)

The root : shoot (dry weight) ratio was significantly affected by genotype and interaction effects of wild genotypes versus cultivated genotypes, genotype × temperature, and cultivated genotypes × temperature (Table 1). In contrast to the remaining genotypes,

which showed no significant changes with temperature, high temperature led to a significant decrease in root: shoot ratio for AC-Sterling, leading to a significant interaction of genotype × temperature (Fig 3).

3.2. Cd uptake

The ANOVA showed that relative Cd uptake was significantly affected by temperature, genotype, wild genotype, cultivated genotype, interaction effects of temperature × genotype and temperature × cultivated genotype (Table 3). Relative Cd uptake increased with temperature in all genotypes, except for AC-Sterling, which showed no significant changes with temperature. Azari and 2811showed the greatest (61.8%) and smallest (44.9%) increases in relative Cd uptake with temperature, respectively (Fig. 4). Among wild genotypes, Azari and Arak indicated the most and least relative Cd uptake, respectively, and among cultivated genotypes, Saffire and AC-Sterling showed the most and least relative Cd uptake, respectively.

Net accumulation of Cd via root was significantly affected by genotype, wild genotype, cultivated genotype, and interaction effects of temperature × cultivated genotypes and temperature × wild genotypes (Table 3). Safflower genotypes contrasted in their net accumulation of Cd via root in response to temperature (Fig. 5). Genotypes 2811, C111, Saffire, and Azari accumulated more Cd when grown under 23 °C than when grown under 18 °C. Among wild genotypes, Arak and Isfahan had the most and least net accumulation of Cd , respectively, averaged over temperatures. Among cultivated genotypes, Saffire and AC-Sterling showed the highest and lowest net Cd accumulation via root, respectively, averaged over temperatures. Net accumulation of Cd via root increased with temperature in all cultivated genotypes, except for AC-Sterling. However, increased temperature led to a decrease in net Cd accumulation in all wild genotypes, with the exception of Azari (Fig. 5).

Root Cd concentration was significantly affected by temperature, genotype, wild genotype, cultivated genotype, species, and interaction effects of temperature × genotype, temperature × cultivated genotype, temperature × wild genotype, and temperature × species (Table 1). Wild safflower genotypes outperformed cultivated genotypes in mean root Cd concentration at both temperatures (Table 4). All wild and cultivated genotypes showed increased root Cd concentration with increased temperature, except for AC-Sterling, in which a decrease in root Cd concentration was observed at 23 °C (Fig. 6).

Shoot Cd concentration was significantly affected by temperature, genotype, cultivated genotype, and interaction effects of temperature × genotype and temperature × cultivated genotypes (Table 3). All cultivated safflower genotypes showed significant increases in shoot Cd concentration at 23 °C compared with 18 °C, with the exception of AC-Sterling, which showed a non-significant decrease in shoot Cd concentration at 23 °C (Fig. 6). The shoot Cd concentration for AC-Sterling was significantly smaller than for all other genotypes but 2811 (Fig. 6). All wild genotypes had increased shoot Cd concentration with temperature, but only Azari's increase was significant (Fig. 6).

3.3. Cd translocation rate

Cd translocation was significantly affected by temperature, genotype, and species (Table 3); it significantly increased with temperature (Table 4). AC-Sterling and Azari, respectively, showed the most and least translocation of Cd (Fig. 7). Cultivated genotypes showed significantly more Cd translocation than the wild genotypes (Table 4).

3.4. Cd tolerance

Relative biomass production was significantly affected by all factors except species and species × temperature (Table 1). Biomass production of the safflower genotypes in response to three groups of Cd levels (moderate: 0.05 and 1 µM $CdCl_2$; high: 5, 10, and 20 µM $CdCl_2$; and very high: 50, 100 and 500µM $CdCl_2$) was measured under the two temperatures. Arak,

C111, and Saffire were classified as sensitive to moderate concentrations of Cd (0.5 and 1µM $CdCl_2$) when grown under 23 °C for 14 days (Table 5); AC-Sterling and 2811 were more resistant to moderate levels of Cd at 23 °C than any of the other genotypes. At high levels of Cd (5, 10, and 20µM $CdCl_2$) at 23 °C, Arak, C111, and Saffire remained the most sensitive and AC-Sterling and 2811 the most resistant. The only difference from the rankings at the moderate level was the genotype Shiraz, which was sensitive to high Cd levels. For all genotypes, growth drastically diminished with very high concentrations of Cd (50, 100, and 500 µM $CdCl_{2)}$, under 23 °C. The genotypes 2811 and AC-Sterling seemed more resistant than the others because their growth under exposure to Cd pollution did not decrease as much as that of the other genotypes.

When plants were grown under 18 °C for 14 days, with moderate levels of Cd, growth in Azari declined the least, and growth in Arak and Isfahan declined the most (Table 5). Under high and very high levels of Cd, at 18 °C, AC-Sterling, 2811, and Shiraz appeared the most resistant, and Arak and Isfahan the most sensitive, respectively, since growth was least negatively affected in the first group, and most negatively affected in the latter.

Cd sensitivity was determined by the decrease in dry weight following the Cd treatment. Interrelations between Cd, temperature, and genotype with regard to Cd sensitivity were analyzed using the Weibull model (Table 6). According to this model, the lower are the toxicity threshold (i.e. TT_{95b}) and the effective concentration (i.e. EC_{50}) that produce a negative effect, the more sensitive is the genotype. It is apparent from EC_{50} that safflower plants grown at 18 °C have better resistance to Cd than those grown at 23 °C. The same result was shown by the TT_{95b} for all genotypes but AC-Sterling and C111, which had better resistance to Cd at 23 °C than at 18 °C. Grown at 18 °C, Arak and Azari were the most sensitive, and 2811, Shiraz, and AC-Sterling the most resistant to Cd pollution based on both TT_{95b} and EC_{50}. At 23 °C, Arak, Saffire, and Shiraz appeared most sensitive according to

both TT_{95b} and EC_{50}, and AC-Sterling and C111 most resistant according to TT_{95b} alone. According to EC_{50}, however, C111 ranked more sensitive and AC-Sterling more resistant than the other genotypes.

4. Discussion

We observed an overall increase in relative Cd uptake with growth under high temperature (23 °C) in this experiment, in both wild and cultivated species (Fig. 4). However, wild and cultivated safflower plants showed somewhat contrasting responses to temperature in net accumulation of Cd via root (Fig. 5). A higher temperature may affect Cd concentration in the plant tissues indirectly, by increasing total dry matter, and in effect diluting the Cd content (Fritioff et al., 2005). It could also have a direct impact on plant Cd uptake through its effect on some internal factor(s). Earlier investigations (Gonzalez-Davila et al., 1995) showed that higher temperatures lead to increased extracellular concentrations of heavy metals. These authors reasoned that the equilibrium between the cell wall exchange sites and the metal in solution changes with temperature. Plant cell walls consist of materials (e.g. pectic polysaccharides and glycoprotein) that act like ion exchangers (Allan and Jarrell 1989; Wang et al 1992). Then, the cell wall exchange properties may leave impacts on ion availability for uptake, ion diffusion rates in the appoplast and membrane transporters. Intracellular ion accumulation may also increase with the increasing cation exchange capacity (CEC) of cell walls due to the ion gradient established around of the plasma membrane (Wang et al 1992). It has been speculated that high temperatures could alter the cell membrane's lipid composition, and therefore decrease its fluidity, which in turn may facilitate both passive and active metal fluxes through the membrane (Lynch and Steponkus, 1987). Reports on varietal and species differences in heavy metal uptake and accumulation are contradictory. Chen et al. (2008) found that temperature did not affect Cd accumulation in *Vigna radiata* plants; Fritioff et al. (2005), however, found that heavy metal accumulation

increased in two submersed plant species (*Elodea canadensis* and *Potamogeton natans*) as the temperature increased from 5 °C to 20 °C.

This study showed that averaged over temperatures wild safflowers had higher levels of Cd in their roots than cultivated safflowers (Table 4). Furthermore, both wild and cultivated safflowers had more Cd in their roots when grown at 23 °C than when grown at 18 °C. In contrast to mean root Cd concentrations, which increased significantly under high temperature in both cultivated and wild safflowers, mean shoot Cd concentration increased significantly in cultivated safflowers under high temperature, but not in wild safflowers (Fig. 6). Species differences in root Cd concentration have been reported between *Eloda canadensis* and *Potamogeton natans* (Fritioff et al., 2005), as have differences in shoot Cd concentrations between the submerged *Elodea canadenisi* and the non-submerged *Carex rostrata* (Nyquist and Greger, 2009). Liu et al. (2010), comparing two rice cultivars for Cd concentrations in their roots and shoots under Cd pollution, found that the two cultivars differed by 91.9% in their root Cd concentrations and 106.2% in shoot concentrations. Positive effects of higher temperatures on root and shoot Cd concentrations have been reported for *Solanum tuberosum* (Baghour et al., 2001) and *Brassica pekinensis* (Moreno et al., 2002).

AC-Sterling differed from the rest of genotypes (wild and cultivated) in its relative Cd uptake in response to temperature. In contrast to the others, AC-Sterling relative Cd uptake, and consequent root and shoot Cd concentrations, showed no significant increase (Figs. 4, 5, and 6) with temperature. Fritioff et al. (2005) speculated that some plant ecotypes contain extracellular binding sites for heavy metals such as Pb. Apparently these extracellular binding sites are less affected by temperature than the intracellular binding sites of plant organs (Beckett and Brown, 1984). We speculate, therefore, that the lack of an effect of temperature

on Cd accumulation in AC-Sterling is probably due to its having a higher proportion of extracellular binding sites for Cd than the other safflower genotypes.

A dilution effect also seems to have played a role in some of the differences in Cd uptake, at least in the safflower genotypes Saffire, Azari, Arak, and C111. These genotypes had significant decreases in their root and shoot dry weights under 0 and 1 µM $CdCl_2$ levels when grown at a high temperature (Fig 1). Decreased tissue concentrations of Cd attributable to enhanced growth, and hence a dilution effect, has been shown in Scots pine (Ekvall and Greger, 2003).

Both total biomass production and the ratio of shoot to root mass have been reported to be correlated with ion uptake. Cheeseman and Wickenes (1986) observed a highly significant correlation between the shoot : root ratio and nutrient uptake, and the same correlation could possibly be found for Cd. In the present study, however, no interrelations were found between changes in root : shoot ratio with temperature and changes in Cd uptake with temperature. Since Cd did not have a significant impact on the root : shoot ratio in safflower genotypes, it could be speculated that Cd does not affect the allocation of photoassimilates between roots and shoots.

Because we observed a general trend of more root-to-shoot Cd translocation in the cultivated safflowers than in the wild safflowers (Table 4), one might expect that the cultivated safflowers would have smaller root concentrations of Cd than the wild safflowers. Our results agree with those of Österås et al. (2000), who found that Norway spruce, Scots pine, and European white birch differed in their Cd translocation from root to shoot. Our results also showed that growing at a high temperature enhanced Cd translocation (Table 4). The positive effects of high temperature on Cd translocation from roots to shoots have been shown in species such as *Pinus sylvestris* (Ekvall and Greger, 2003) and *Sedum alfredii* (Lu et al., 2009). Ekvall and Greger (2003) reasoned that when plants are grown at higher

temperatures, their Cd translocation increases as a consequence of an enhanced transpiration stream. Both symplastic and appoplastic Cd translocation pathways have been suggested for different ecotypes of *Sedum alfredii* (Lu et al., 2009). It has also been reported that low temperature may decrease Cd translocation through the symplastic pathway. Therefore, root-to-shoot Cd translocation in ecotypes, genotypes, and/or species whose dominant pathways for metal translocation are known to be symplastic might increase with temperature more markedly than in other species. Whether Cd translocation in safflowers is dominantly symplastic or appoplastic needs more investigation, however, the greater translocation that we observed at 23 °C than at 18 °C may indicate the dominance of a symplastic pathway in this oilseed crop.

Our study further showed that $CdCl_2$ concentrations greater than 20 µM are detrimental to both cultivated and wild safflowers (Table 5). A 50% decrease in dry mass per plant after a 6-day exposure of bean seedlings to 3 µM Cd was reported by Poschenrieder et al. (1989), who argued that the decreased dry matter was likely associated with the plants' decreased water potential and relative water content. .Cultivated AC-Sterling seemed more resistant to low and moderate concentrations of $CdCl_2$ than the other cultivated safflower genotypes used in this study. Whether AC-Sterling benefits from some kind of Cd-excluding mechanism or not needs more investigation. Wild safflower genotypes were found to be, on average, more sensitive to Cd pollution than cultivated safflowers. Among the wild safflower genotypes, Arak appeared to be the most vulnerable to Cd pollution (Tables 2, 5, and 6)

In a study conducted by Shi et al. (2010), the response of two safflower cultivars to Cd pollution was found to be both cultivar- and dose-dependent. They found the shoot biomass of the plants decreased by 42.3% for the NS-4 cultivar, but increased by 3% for the YM cultivar under 25 mg/kg Cd pollution, in comparison to the control. It was previously reported (Wu et al., 2003) that a Cd concentration of 5 µM could drastically alter biomass production

in barley genotypes, though the Cd damage was clearly genotype-dependent. Landberg and Greger (2002a) showed that the tolerance index for sensitive clones of *Salix viminalis* was nearly 20% for roots and 25% for shoots, while in resistant clones both roots and shoots had a tolerance index of nearly 80%.

In the present study, the Weibull model showed no resistance to Cd in the wild safflowers (Table 6), although some cultivated safflowers seemed, at least to some extent, able to resist Cd pollution. At high temperatures, however, even cultivated safflowers may be vulnerable to damage from Cd pollution.

Our results also suggest that as Cd translocation increases with increasing temperature, Cd sensitivity also rises. Landberg and Greger (1996) have suggested that a greater translocation of metals can damage the photosynthetic apparatus, rendering willow plants sensitive to the heavy metals pollution. Our results confirmed those of Oncel et al. (2000), who indicated that Cd toxicity to plants increased with temperature. The only exception observed in our study was the genotype AC-Sterling, in which plant biomass increased at high temperature. Future studies will, we hope, shed light on the internal mechanisms by which this cultivated genotype resists the effects of Cd added to the nutrient solution.

5. Conclusions

In conclusion, this study showed that there were genotypic differences in Cd translocation, uptake, and sensitivity in safflowers. Wild and cultivated safflowers behaved differently in both Cd translocation and root concentration. Safflower genotypes had somewhat contradictory responses to temperature in both uptake and tolerance of Cd. More research is necessary to clarify the mechanism(s) of the between- and within-species differences observed in safflower, particularly with regard to its response to Cd under different temperatures.

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Table. 1. Analysis of variance of relative biomass production and dry matter production (root dry weight, shoot dry weight, and root : shoot dry weight) of seedlings of eight safflower genotypes cultivated at two temperatures, in response to Cd pollution. Relative biomass production was studied at 8 levels (0.5, 1, 5, 10, 20, 50, 100, and 500 µM) of Cd; dry matter production attributes were studied at two levels (0 and 1 µM) of Cd.

	Relative biomass production		Dry matter production			
Source of variation	df	Mean Squares		Mean Squares		
			df	Root D.W.	Shoot D.W.	Root:Shoot D.W.
Temperature	1	0.201**	1	0.0544**	0.526**	$0.0033^{n.s}$
Replication (Temperature)	4	0.001	4	0.0006	0.008	0.00056
Cadmium	7	6.5**	1	0.143**	2.114**	$0.0013^{n.s}$
Genotype	7	0.206**	7	0.0286**	0.182**	0.0049**
Wild	3	0.069**	3	0.0302**	0.221**	0.0040**
Cultivated	3	0.024**	3	0.0310**	0.167**	0.0062**
Species	1	0.00084^{ns}	1	0.0175**	0.114**	0.00036^{ns}
Cd × Genotype	49	0.015**	7	$0.0007^{n.s}$	0.013**	$0.000029^{n.s}$
Wild × Cd	21	0.031**	3	$0.000153^{n.s}$	0.020*	$0.000015^{n.s}$
Cultivated × Cd	21	0.006**	3	$0.000158^{n.s}$	$0.005^{n.s}$	$0.000049^{n.s}$
Species × Cd	7	0.014*	1	$0.00034^{n.s}$	0.019*	$0.000012^{n.s}$
Cd × Temperature	7	0.0177**	1	$0.00018^{n.s}$	$0.007^{n.s}$	$0.000004^{n.s}$
Genotype × Temperature	7	0.026**	7	0.0064**	0.180**	0.0021**
Wild × Temperature	3	0.024**	3	0.0050**	0.032**	$0.00087^{n.s}$
Cultivated ×Temperature	3	0.069**	3	0.0098**	0.370**	0.0041*
Species ×Temperature	1	0.008^{ns}	1	0.0007^{ns}	0.005^{ns}	0.00019^{ns}
Cd × Genotype × Temperature	49	0.014**	7	$0.0001^{n.s}$	$0.003^{n.s}$	$0.000048^{n.s}$
Error	252	0.002	60	0.00071	0.0046	0.0005

df: degrees of freedom; Mean Squares: between group variance; ns: non-significant; Error: within group variance; *P ≤ 0.05; **P ≤ 0.01.

Table 2. Root and shoot dry weight (g per pot) of two species (cultivated and wild) of safflower grown for 14 days in two temperatures (23°C and 18°C). Data are averaged over 0 and 1 µM levels of Cd and 3 replicates.

Treatment		Root dry weight	Shoot dry weight
Temperature (°C)	23	0.23 [b]	1.026 [b]
	18	0.28 [a]	1.174 [a]
Species	Cultivated	0.29 [a]	1.240 [a]
	Wild	0.21 [b]	0.95 [b]

a, b: different letters within a column represent significant differences ($P < 0.05$) between means.

Table 3. Analysis of variance of relative Cd uptake, net Cd accumulation via root, Cd translocation, and root and shoot Cd concentration of seedlings of eight safflower genotypes cultivated at two temperatures and in the presence of 1 µM of CdCl2.

Source of variation	df	Mean Square				
		Relative Cd uptake	Net Cd accumulation via root	Cd translocation	Root Cd concentration	Shoot Cd concentration
Temperature	1	7510**	367^{ns}	661*	643**	312**
Replication (temperature)	4	159	446	71.9	50.1	6.62
Genotype	7	302**	5349**	459*	552**	35.1**
Wild	3	296*	3228*	119^{ns}	236*	15.2^{ns}
Cultivated	3	333*	8653**	35^{ns}	922**	56.7**
Species	1	227^{ns}	1800^{ns}	2746**	389*	29.8^{ns}
Genotype × Temperature	7	251*	4421**	61^{ns}	421**	26.2**
Wild × Temperature	3	161^{ns}	2785*	18.3^{ns}	194*	35.4*
Cultivated × Temperature	3	318*	3847**	116^{ns}	690**	25.5^{ns}
Species × Temperature	1	319^{ns}	11052*	24.5^{ns}	297*	1.3^{ns}
Error	28	75	582	58	42.4	10.5

Mean Square: between group variance; df: degrees of freedom; ns: non-significant; Error: within group variance; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 4. Root Cd concentration (µg/g root DW) and translocation (%) of two species (cultivated and wild) of safflower when grown for 14 days in two temperatures (23°C and 18 °C) under 1 µM level of Cd. Each value is a mean of three replicates.

Treatment		Root Cd concentration	Cd translocation
Temperature (°C)	23	26.7.35 [a]	56.8 [a]
	18	14.2 [b]	49.2 [b]
Species	Cultivated	16.2 [b]	60.5 [a]
	Wild	21.9 [a]	45.3 [b]

a, b: different letters within a column represent significant differences ($P < 0.05$) between means.

Table5. Means (±SE) for relative biomass production of eight genotypes of safflower after 14 days cultivation at two temperatures and at eight levels of Cd. Biomass production is given as percent growth increase in relation to untreated plants (n = 3).

Relative biomass production (%)																
	23 °C								18 °C							
	Cultivated				Wild				Cultivated				Wild			
	2811	C111	Saffire	AC	Isfahan	Azari	Arak	Shiraz	2811	C111	Saffire	AC	Isfahan	Azari	Arak	Shiraz
0.5	89.6±0.8	85.8±2.7	84.3±5.5	99.6±8.1	87.9±3.5	99.2±2.8	74.3±1.4	86.5±6.0	96.41±4.7	90.6±3.4	89.5±2.7	94.4±2.1	92.6±0.8	91.5±1.2	66.4±0.8	92.5±7.1
1	83.3±2.5	63.7±4.0	69.7±3.2	86.7±3.7	71.3±4.5	71.8±0.6	60.7±4.7	75.7±1.2	82.9±1.2	83.5±0.8	78.7±1.2	79.6±1.1	72.7±2.1	91.5±0.1	69.1±1.5	81±4.6
5	50.3±0.5	48.4±1.3	43.2±1.2	60.8±1.1	56.7±1.7	46.2±2.5	40.1±3.2	41.7±1.0	67.9±4.6	61.2±0.7	59.3±2.3	63.1±2.2	33.9±1.1	48.2±1.8	45.2±3.4	64.5±3.7
10	37.6±0.4	30.0±1.5	28.2±2.2	38.8±0.6	31.6±1.8	35.2±1.7	25.7±0.3	27.1±0.5	49.6±1.3	36.3±1.8	40.5±1.9	53.1±3.1	25.8±0.8	42.3±4.6	32.5±1.9	57.8±2.2
20	17.4±0.7	17.8±0.2	15.1±0.1	17.8±0.8	17.3±0.1	19.9±0.8	14.9±0.2	10.6±0.7	23.0±1.6	20.9±0.5	19.5±0.3	28.8±1.1	19.7±0.9	18.0±0.3	13.4±0.6	27.3±3.1
50	6.17±0.4	4.7±0.4	0.3±0.1	3.2±0.3	5.3±0.3	4.4±0.2	-0.2±0.4	1.8±0.3	5.0±0.4	4.4±0.5	5.7±0.1	7.37±0.9	6.97±0.3	7.7±0.3	6.8±0.2	7.1±0.7
100	-1.26±0.1	-2.6±0.2	3.1±0.0	-3.9±0.3	-1.2±0.1	0.8±0.1	-3.4±0.2	-2.7±0.1	-2.4±0.1	-0.6±0.4	-2.3±0.6	-4.2±0.4	2.3±0.7	3.5±0.1	0.8±0.4	-1.09±0.6
500	-2.57±0.3	-3.2±0.2	-5.4±0.3	-5.3±0.3	-5.1±0.1	-3.4±0.7	-5.1±0.2	-4.3±0.2	-7.1±0.1	-8.8±0.4	-8.7±0.3	-6.5±0.6	-1.4±0.5	-2.5±0.3	-4.0±0.3	-6.6±0.6

Each value is a mean of three replicates. LSD at 0.05 = 6.121.

567 Table 6. Interpreting the differences in Cd toxicity among eight genotypes of safflower using the modified Weibull frequency distribution model (n = 3, ±SE).

Genotype	Temperature	Weibull Parameter					TT_{95b}≠	EC_{50}≠
		a	b	c	d	R2		
AC	23°C	-0.12	15.9±0.55	12.2±1.02	0.88±0.09	99.2	0.41±0.10	8.00±0.59
	18°C	-0.62	9.7±0.40	16.8±1.93	0.71±0.07	99.0	0.26±0.08	10.00±0.96
C111	23°C	-0.47	17.3±0.76	7.6±1.21	0.53±0.04	98.1	0.68±0.11	2.50±0.47
	18°C	-0.13	7.5±0.41	13.5±2.02	0.73±0.10	98.2	0.24±0.09	8.20±0.65
Saffire	23°C	-0.60	13.7±0.52	7.2±0.97	0.56±0.05	96.6	0.04±0.01	3.75±0.04
	18°C	-0.49	10.8±0.35	15.4±1.57	0.71±0.06	98.6	0.23±0.06	6.23±0.77
2811	23°C	-0.66	4.0±0.40	10.3±0.89	0.64±0.04	98.5	0.10±0.02	5.87±0.41
	18°C	-0.71	10.4±0.34	18.2±2.70	0.66±0.08	98.5	0.20±0.08	10.47±0.98
Isfahan	23°C	0.40	18.3±0.93	5.9±1.05	0.57±0.07	97.4	0.03±0.02	3.10±0.45
	18°C	-0.41	12.8±0.67	12.9±1.95	0.74±0.10	94.8	0.23±0.09	7.82±0.88
Azari	23°C	0.19	13.0±0.55	9.5±1.16	0.67±0.07	98.7	0.11±0.04	5.48±0.57
	18°C	-0.17	14.2±0.50	10.6±1.04	0.70±0.04	96.7	0.14±0.03	6.24±0.47
Arak	23°C	-1.40	12.8±1.09	15.0±4.30	0.31±0.04	95.7	0.009±0.0002	4.54±0.09
	18°C	-1.22	15.1±1.07	11.3±2.40	0.49±0.06	97.1	0.03±0.02	5.36±0.95
Shiraz	23°C	-0.66	18.3±0.49	6.7±0.67	0.54±0.04	97.1	0.03±0.02	3.41±0.32
	18°C	-0.54	10.8±0.48	15.2±1.62	0.72±0.28	97.2	0.25±0.10	9.14±0.96

568 ǂ Calculations are based on the dry weight of plants.

569 Ł Weibull parameters a and b are based on dry weight per pot (n=6).

570 ≠The Terms TT_{95b} and EC_{50} indicate the Cd concentration (µM) where the plant dry weight is declined by 5 and 50%, respectively.

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Legends for figures.

Figure 1. Dry weight of shoot and root (g per pot) of eight genotypes of safflower when grown for 14 days under 23 °C and 18 °C temperatures. Each value is a mean of two Cd levels (0 and 1 µM) and three replicates ± SE. LSDs (0.05) for shoot and root dry weight are 0.110 and 0.039, respectively.

Figure 2. Shoot dry weight (g per pot) of eight genotypes of safflower when grown for 14 days under 0 and 1 µM levels of Cd. Each value is a mean of two temperatures (23 °C and 18 °C) and three replicates ± SE. LSD at 0.05 = 0.110.

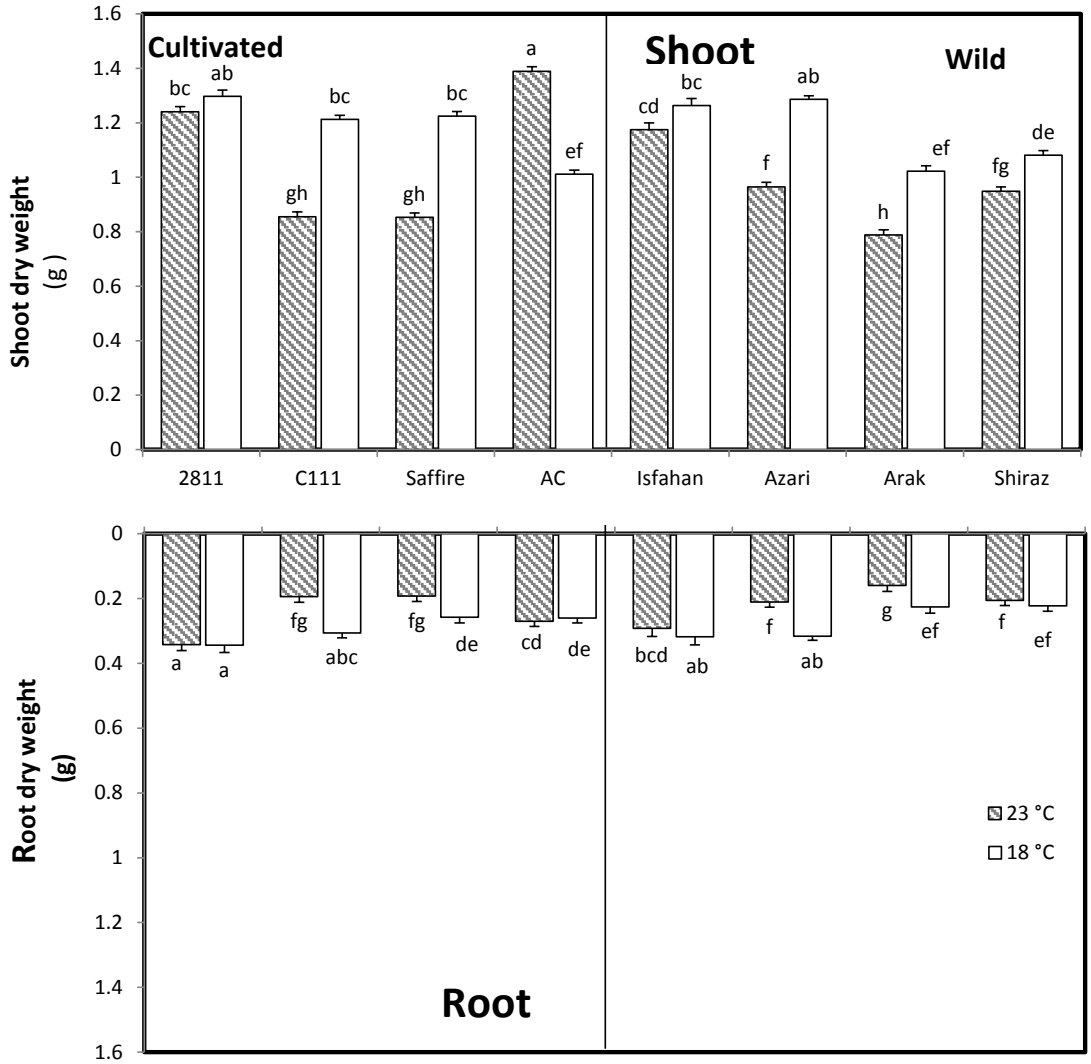
Figure 3. Root : hoot dry weight ratio (g root dry weight/g shoot dry weight) of eight genotypes of safflower when grown for 14 days under 23 °C and 18 °C temperatures. Each value is a mean of two Cd levels (0 and 1 µM) and three replicates ± SE. LSD at 0.05 = 0.033.

Figure 4. Relative Cd uptake calculated as amount of Cd taken up in whole plants in relation to total Cd added in the medium. Plants of eight genotypes of safflower were grown for 14 days in two temperatures (23 °C and 18 °C). Each value is a mean of three replicates ± SE. LSD at 0.05 = 14.8.

Figure 5. Net accumulation of Cd via root (uptake calculated as total amount of Cd taken up in whole plants in relation to dry weight of roots) of seedlings of eight genotypes of safflower when grown for 14 days under 23 °C or 18 °C temperatures in 1 µM level of Cd. Each value is a mean of three replicates ± SE. LSD at 0.05 = 39.9.

Figure 6. Cd concentration of shoot and root (µg Cd/g DW) of eight genotypes of safflower when grown for 14 days under 23 °C and 18 °C temperatures in 1 µM level of Cd. Each value is a mean of three replicates ± SE. LSD (0.05) for Cd content of shoot and root is 5.2 and 10.9, respectively.

Figure 7. Translocation of Cd to shoot calculated as amount of Cd in shoot in relation to total amount of Cd taken up. Seedlings of eight genotypes (2811, C111, Saffire, AC-Sterling, Isfahan, Azari, Arak, and Shiraz) of safflower were grown for 14 days in 1 µM level of Cd. Each value is a mean of two temperatures (23 °C and 18 °C) and 3 replicates ± SE. LSD at 0.05 = 9.02.



589
590 Figure 1. Dry weight of shoot and root (g per pot) of eight genotypes of safflower when grown for 14 days
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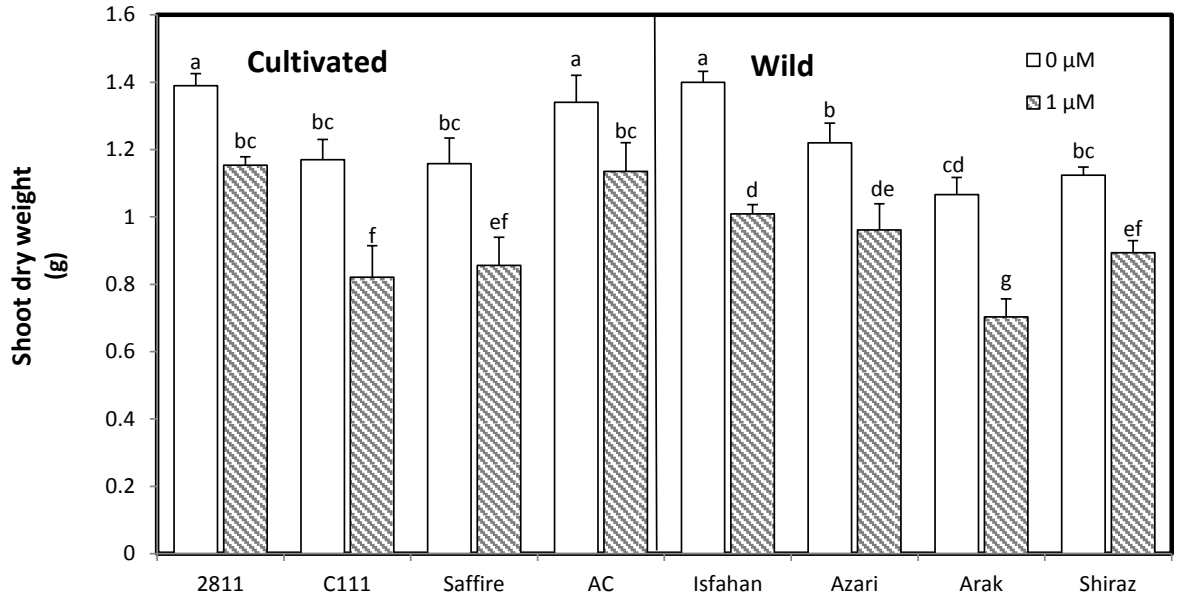

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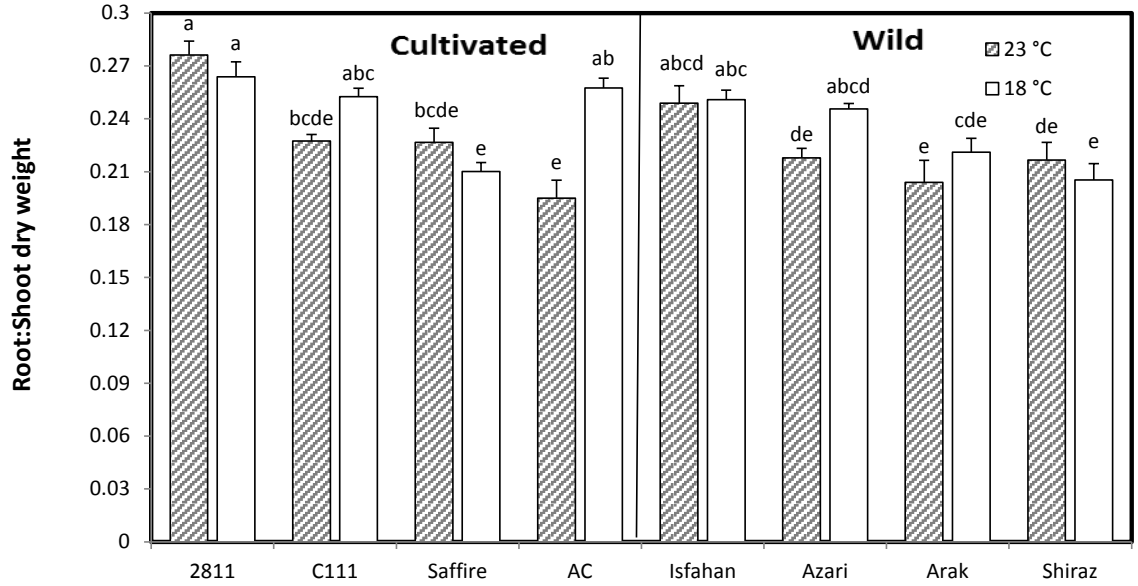

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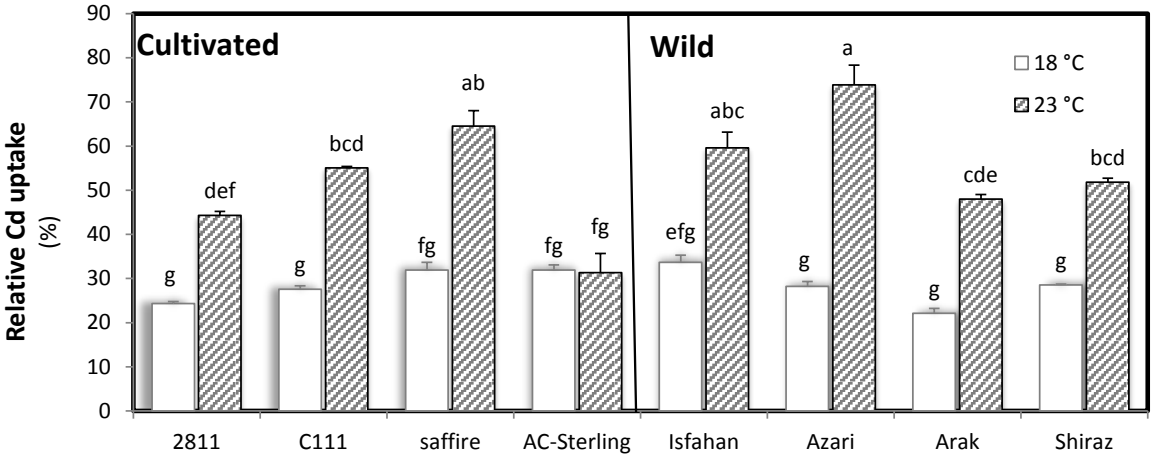


Figure 4. Relative Cd uptake calculated as amount of Cd taken up in whole plants in relation to total Cd added in the medium. Plants of eight genotypes of safflower were grown for 14 days in two temperatures (23 °C and 18 °C). Each value is a mean of three replicates ± SE. LSD at 0.05 = 14.8.

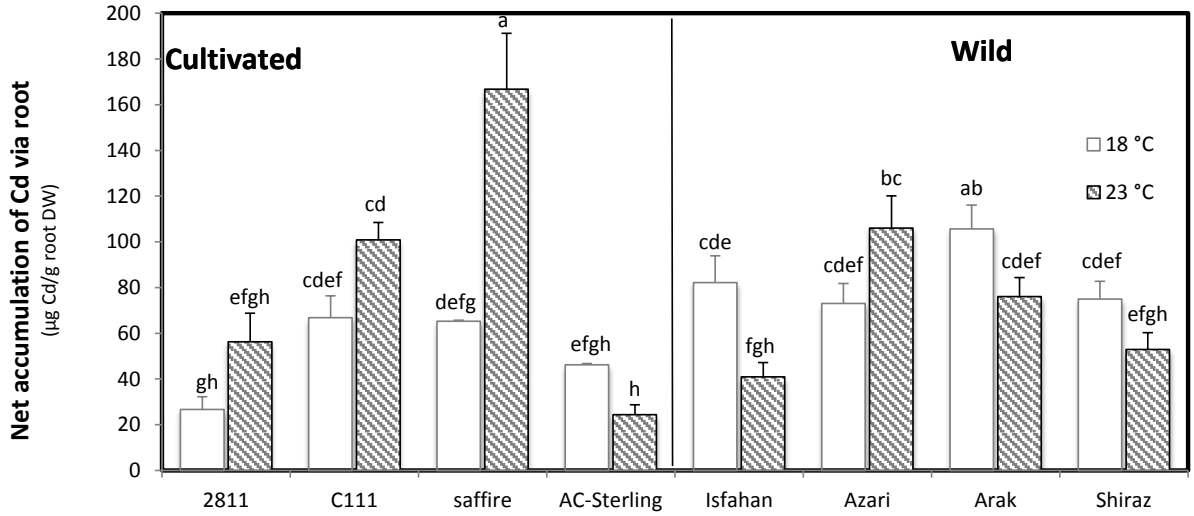
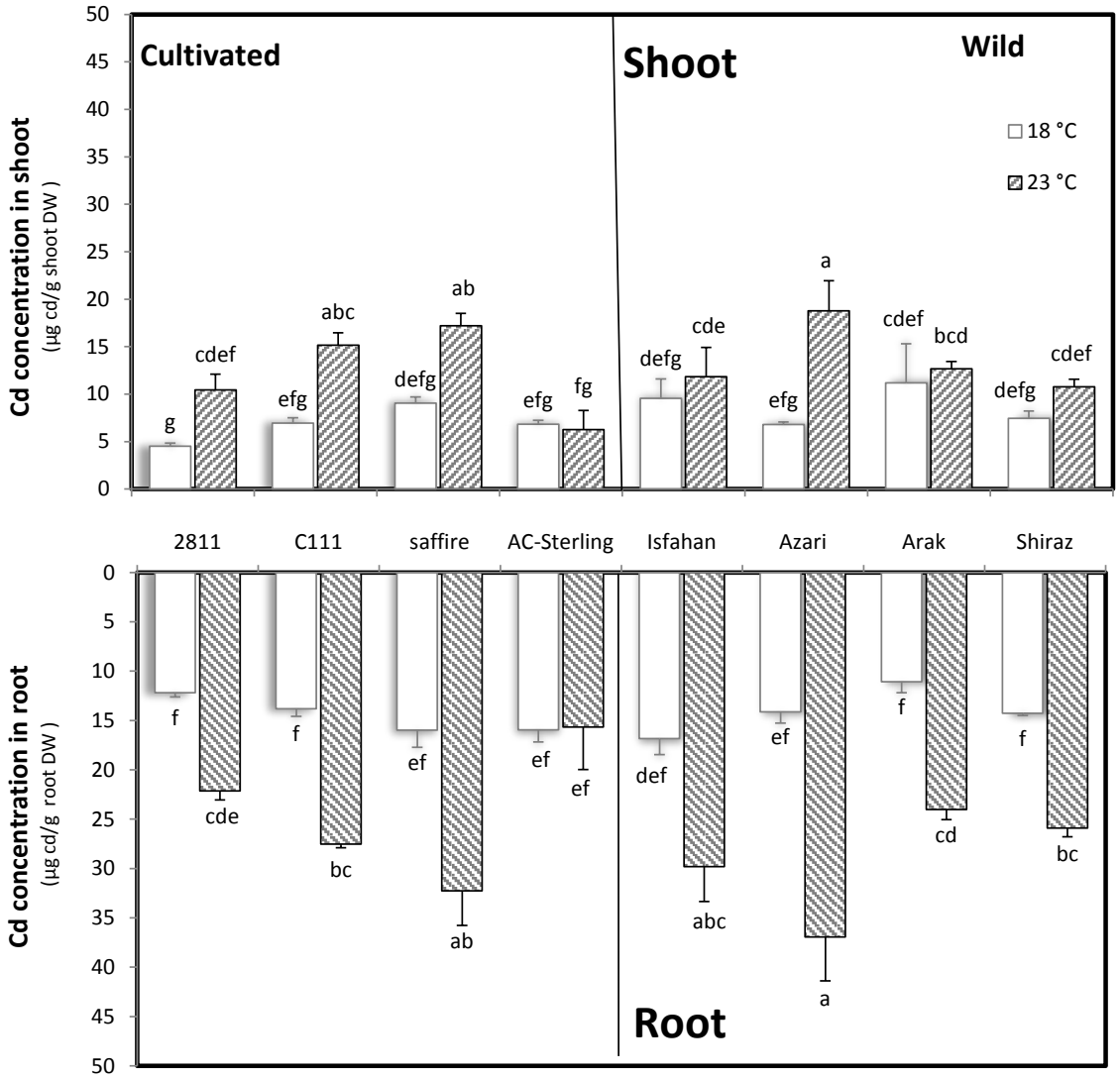


Figure 5. Net accumulation of Cd via root (uptake calculated as total amount of Cd taken up in whole plants in relation to dry weight of roots) of seedlings of eight genotypes of safflower when grown for 14 days under 23 °C or 18 °C temperatures in 1 µM level of Cd. Each value is a mean of three replicates ± SE. LSD at 0.05 = 39.9.



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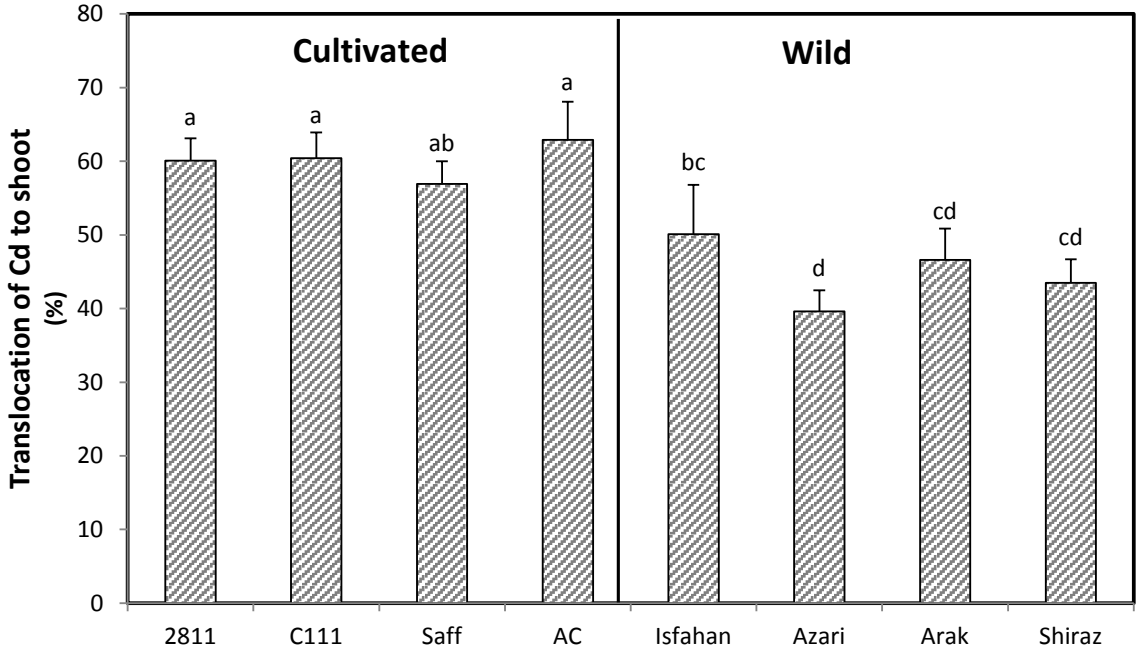

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