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

3 Nasibeh Pourghasemian,¹ Parviz Ehsanzadeh¹, Maria Greger²

4 ¹Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of
5 Technology, Isfahan, 84156-83111, Iran.

6 ²Faculty of Applied Ecology and Agricultural Sciences, Hedmark University College,
7 Blæstad, 2418 Elverum, Norway.

8 Abstract

9 Soil pollution is a world-wide problem, with heavy metals being a major part of the concern.
10 To investigate the effect of temperature on cadmium (Cd) uptake and translocation, as well as
11 Cd tolerance in wild and cultivated species of safflower, a hydroponic experiment was
12 conducted under controlled conditions. The responses of four wild genotypes (Isfahan, Arak,
13 Azari, and Shiraz) and four cultivated genotypes (AC-Sterling, 2811, Saffire, and C111) of
14 safflower to nine levels of CdCl₂ (0, 0.5, 1, 5, 10, 20, 50, 100, and 500 μM) in solution were
15 examined under two temperatures (18 and 23 °C). Cadmium sensitivity was determined using
16 the Weibull model on the total dry weight of the plants. Cadmium uptake and translocation
17 were analyzed on 1 μM Cd treated plants. Results revealed that safflower genotypes differed
18 in terms of uptake, translocation, and tolerance to Cd, with AC-Sterling and Arak indicating
19 the most and the least tolerance to Cd, respectively. Relative Cd uptake and Cd concentration
20 in roots and shoots increased with an increase in temperature in all genotypes, with the
21 exception of AC-Sterling. Net accumulation of Cd via root increased with an increase in
22 temperature for the wild Azari and the cultivated 2811, Saffire, and C111, though it
23 decreased for the rest of genotypes. Cadmium translocation to shoots significantly increased
24 with increased temperature in all genotypes. Cadmium translocation from roots to shoots in
25 cultivated genotypes was significantly greater than in wild genotypes. Root Cd concentration

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

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

31 1. Introduction

32 Pollution of the biosphere with toxic levels of metals has accelerated dramatically since
33 the beginning of the industrial revolution (Nriagu, 1979). Soil pollution by heavy metals
34 including cadmium (Cd) is a global problem, which can cause agricultural lands to become
35 hazardous for wildlife and human populations. Environmental pollution with Cd is mainly
36 caused by mining and smelting, dispersal of sewage sludge, and the use of Cd-rich phosphate
37 fertilizers (Chaney, 1998). A study on roadside soils in Isfahan, Iran (Samani Majd et al.,
38 2006) indicated that the Cd level of these soils could reach 2.25 to 2.57 mg kg⁻¹. Cadmium
39 entry into the human body via the food chain is a major concern, because Cd accumulates
40 with a half-life exceeding 10 years, and it has been linked with renal tube dysfunction and
41 pulmonary emphysema (Gairola et al., 1992). Plants, which take up and accumulate Cd in
42 their roots and shoots, may also be negatively affected in their photosynthesis, growth, and
43 reproduction (Xiong and Peng, 2001).

44 Cadmium uptake and its effects on plants may be influenced by a variety of factors, e.g.
45 the plant species, cultivar, soil characteristics, and temperature. Genetic differences in
46 mineral uptake among plant species were observed decades ago (Saric, 1983), and even
47 cultivars of the same species often show large variation in tolerance to Cd toxicity (Koleli,
48 2004). In a series of studies Landberg and Greger (1996 and 2002b) and Greger and
49 Landberg (1999) showed variation in tolerance, uptake, and translocation of Cd among 200
50 wild and cultivated willow clones. Genotypic variation in Cd uptake and accumulation was

51 also found in birch, pine, and spruce (Österås et al., 2000), rice (Liu et al., 2007), wheat
52 (Greger and Löfstedt, 2004), and wild and modern wheat (Cakmak et al., 2000).

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

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

76

77 2. Materials and Methods

78 2.1 Plant material and growth conditions

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

85 The seedlings were treated for 14 days with Cd in the following initial concentrations:
86 0, 0.5, 1, 5, 10, 20, 50, 100, and 500 μM CdCl_2 . These levels of Cd were chosen mainly
87 because some studies have suggested that Cd levels of some urban soils in Iran are in the
88 order of 2.57 mg/kg (Samani et al., 2006). In this paper, initial levels of 0.5 and 1 μM are
89 referred to as moderate, 5, 10, and 20 μM as high, and 50, 100, and 500 as very high levels of
90 pollution. Each pot contained 6 plants mounted on styrofoam plates floating on the solution
91 surface. Plants were grown in a climate-controlled chamber equipped with metal halogen
92 lamps (Osram Powestar HOI-R, Hans, Oldenburg, Germany) under two different temperature
93 regimes: (1) 23 °C during the day and 20 °C at night and (2) 18 °C during the day and 16 °C
94 at night, both with photoperiods of 16 h light (with a photon flux density of $600 \pm 20 \mu\text{molm}^{-2}\text{s}^{-1}$)
95 and 8 h dark. The relative humidity of the chamber was 50%.

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

99 2.2 Harvest of plants and analysis of Cd content

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

102 roots and shoots were registered. The plant materials were dried at 105 °C for 24 h and the
 103 dry weight of roots and shoots determined. Thereafter, the plant materials were wet-digested
 104 in HNO₃ : HClO₄ (7:3, v/v) according to the method described by Frank (1975). The Cd
 105 content in roots and shoots was analyzed by atomic absorption spectrophotometry (SpectraA
 106 55B, Varian, Agelant, USA) using a flame atomizer. A graphite oven (GTA 100) was used
 107 when necessary (i.e. at low concentration ranges). Standards were added to the samples to
 108 eliminate the interaction of the sample matrix.

109 2.3 Experimental design, calculations and statistical treatments

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

$$117 \text{ Relative Cd uptake (\%)} = \frac{\text{total Cd content in whole plants (\mu g)}}{\text{total amount of Cd in solution (\mu g)}} \times 100 \quad 1)$$

$$121 \text{ Net accumulation of Cd via root (\mu g Cd/gDW)} = \frac{\text{total amount of Cd in whole plants (\mu g)}}{\text{root dry weight (g)}} \quad 2)$$

$$127 \text{ Translocation of Cd to shoot (\%)} = \frac{\text{total content of Cd in shoot (\mu g)}}{\text{total content of Cd in whole plants (\mu g)}} \times 100 \quad 3)$$

$$133 \text{ Relative biomass production (\%)} = \frac{\text{gFW treated}_{14 \text{ days}} - \text{gFW treated}_{\text{start}}}{\text{gFW treated}_{\text{start}}} \times 100 \quad 4)$$

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; gFW untreated_{14

141 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
$$y = a + b \cdot e^{-(x/c)^d} \quad 5)$$

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
$$\text{TT}_{95b} = c (-\ln 0.95)^{1/d} \quad 6)$$

153
$$\text{EC}_{50} = c (-\ln 0.50)^{1/d} \quad 7)$$

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

160 3. Results

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

164 3.1 Dry matter production

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

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

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

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

188 3.2. Cd uptake

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

198 Net accumulation of Cd via root was significantly affected by genotype, wild genotype,
199 cultivated genotype, and interaction effects of temperature \times cultivated genotypes and
200 temperature \times wild genotypes (Table 3). Safflower genotypes contrasted in their net
201 accumulation of Cd via root in response to temperature (Fig. 5). Genotypes 2811, C111,
202 Saffire, and Azari accumulated more Cd when grown under 23 °C than when grown under 18
203 °C. Among wild genotypes, Arak and Isfahan had the most and least net accumulation of Cd ,
204 respectively, averaged over temperatures. Among cultivated genotypes, Saffire and AC-
205 Sterling showed the highest and lowest net Cd accumulation via root, respectively, averaged
206 over temperatures. Net accumulation of Cd via root increased with temperature in all
207 cultivated genotypes, except for AC-Sterling. However, increased temperature led to a
208 decrease in net Cd accumulation in all wild genotypes, with the exception of Azari (Fig. 5).

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

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

224 3.3. Cd translocation rate

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

229 3.4. Cd tolerance

230 Relative biomass production was significantly affected by all factors except species and
231 species \times temperature (Table 1). Biomass production of the safflower genotypes in response
232 to three groups of Cd levels (moderate: 0.05 and 1 μ M CdCl₂; high: 5, 10, and 20 μ M CdCl₂;
233 and very high: 50, 100 and 500 μ M CdCl₂) was measured under the two temperatures. Arak,

234 C111, and Saffire were classified as sensitive to moderate concentrations of Cd (0.5 and 1 μ M
235 CdCl₂) when grown under 23 °C for 14 days (Table 5); AC-Sterling and 2811 were more
236 resistant to moderate levels of Cd at 23 °C than any of the other genotypes. At high levels of
237 Cd (5, 10, and 20 μ M CdCl₂) at 23 °C, Arak, C111, and Saffire remained the most sensitive
238 and AC-Sterling and 2811 the most resistant. The only difference from the rankings at the
239 moderate level was the genotype Shiraz, which was sensitive to high Cd levels. For all
240 genotypes, growth drastically diminished with very high concentrations of Cd (50, 100, and
241 500 μ M CdCl₂), under 23 °C. The genotypes 2811 and AC-Sterling seemed more resistant
242 than the others because their growth under exposure to Cd pollution did not decrease as much
243 as that of the other genotypes.

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

249 Cd sensitivity was determined by the decrease in dry weight following the Cd
250 treatment. Interrelations between Cd, temperature, and genotype with regard to Cd sensitivity
251 were analyzed using the Weibull model (Table 6). According to this model, the lower are the
252 toxicity threshold (i.e. TT_{95b}) and the effective concentration (i.e. EC₅₀) that produce a
253 negative effect, the more sensitive is the genotype. It is apparent from EC₅₀ that safflower
254 plants grown at 18 °C have better resistance to Cd than those grown at 23 °C. The same result
255 was shown by the TT_{95b} for all genotypes but AC-Sterling and C111, which had better
256 resistance to Cd at 23 °C than at 18 °C. Grown at 18 °C, Arak and Azari were the most
257 sensitive, and 2811, Shiraz, and AC-Sterling the most resistant to Cd pollution based on both
258 TT_{95b} and EC₅₀. At 23 °C, Arak, Saffire, and Shiraz appeared most sensitive according to

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

262 4. Discussion

263 We observed an overall increase in relative Cd uptake with growth under high
264 temperature (23 °C) in this experiment, in both wild and cultivated species (Fig. 4). However,
265 wild and cultivated safflower plants showed somewhat contrasting responses to temperature
266 in net accumulation of Cd via root (Fig. 5). A higher temperature may affect Cd
267 concentration in the plant tissues indirectly, by increasing total dry matter, and in effect
268 diluting the Cd content (Fritioff et al., 2005). It could also have a direct impact on plant Cd
269 uptake through its effect on some internal factor(s). Earlier investigations (Gonzalez-Davila
270 et al., 1995) showed that higher temperatures lead to increased extracellular concentrations of
271 heavy metals. These authors reasoned that the equilibrium between the cell wall exchange
272 sites and the metal in solution changes with temperature. Plant cell walls consist of materials
273 (e.g. pectic polysaccharides and glycoprotein) that act like ion exchangers (Allan and Jarrell
274 1989; Wang et al 1992). Then, the cell wall exchange properties may leave impacts on ion
275 availability for uptake, ion diffusion rates in the apoplast and membrane transporters.
276 Intracellular ion accumulation may also increase with the increasing cation exchange capacity
277 (CEC) of cell walls due to the ion gradient established around of the plasma membrane
278 (Wang et al 1992). It has been speculated that high temperatures could alter the cell
279 membrane's lipid composition, and therefore decrease its fluidity, which in turn may
280 facilitate both passive and active metal fluxes through the membrane (Lynch and Steponkus,
281 1987). Reports on varietal and species differences in heavy metal uptake and accumulation
282 are contradictory. Chen et al. (2008) found that temperature did not affect Cd accumulation in
283 *Vigna radiata* plants; Fritioff et al. (2005), however, found that heavy metal accumulation

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

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

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

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

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

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

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

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

343 Our study further showed that CdCl₂ concentrations greater than 20 µM are detrimental
344 to both cultivated and wild safflowers (Table 5). A 50% decrease in dry mass per plant after a
345 6-day exposure of bean seedlings to 3 µM Cd was reported by Poschenrieder et al. (1989),
346 who argued that the decreased dry matter was likely associated with the plants' decreased
347 water potential and relative water content. .Cultivated AC-Sterling seemed more resistant to
348 low and moderate concentrations of CdCl₂ than the other cultivated safflower genotypes used
349 in this study. Whether AC-Sterling benefits from some kind of Cd-excluding mechanism or
350 not needs more investigation. Wild safflower genotypes were found to be, on average, more
351 sensitive to Cd pollution than cultivated safflowers. Among the wild safflower genotypes,
352 Arak appeared to be the most vulnerable to Cd pollution (Tables 2, 5, and 6)

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

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

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

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

374 5. Conclusions

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

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489 Xiong, Z.T., Peng, Y.H., 2001. Response of pollen germination and tube growth to cadmium with special
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491 Table. 1. Analysis of variance of relative biomass production and dry matter production (root dry weight, shoot
 492 dry weight, and root : shoot dry weight) of seedlings of eight safflower genotypes cultivated at two
 493 temperatures, in response to Cd pollution. Relative biomass production was studied at 8 levels (0.5, 1, 5, 10, 20,
 494 50, 100, and 500 μM) of Cd; dry matter production attributes were studied at two levels (0 and 1 μM) of Cd.
 495

Source of variation	Relative biomass production		Dry matter production			
	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 ^{ns}
Replication (Temperature)	4	0.001	4	0.0006	0.008	0.00056
Cadmium	7	6.5**	1	0.143**	2.114**	0.0013 ^{ns}
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 \times Genotype	49	0.015**	7	0.0007 ^{ns}	0.013**	0.000029 ^{ns}
Wild \times Cd	21	0.031**	3	0.000153 ^{ns}	0.020*	0.000015 ^{ns}
Cultivated \times Cd	21	0.006**	3	0.000158 ^{ns}	0.005 ^{ns}	0.000049 ^{ns}
Species \times Cd	7	0.014*	1	0.00034 ^{ns}	0.019*	0.000012 ^{ns}
Cd \times Temperature	7	0.0177**	1	0.00018 ^{ns}	0.007 ^{ns}	0.000004 ^{ns}
Genotype \times Temperature	7	0.026**	7	0.0064**	0.180**	0.0021**
Wild \times Temperature	3	0.024**	3	0.0050**	0.032**	0.00087 ^{ns}
Cultivated \times Temperature	3	0.069**	3	0.0098**	0.370**	0.0041*
Species \times Temperature	1	0.008 ^{ns}	1	0.0007 ^{ns}	0.005 ^{ns}	0.00019 ^{ns}
Cd \times Genotype \times Temperature	49	0.014**	7	0.0001 ^{ns}	0.003 ^{ns}	0.000048 ^{ns}
Error	252	0.002	60	0.00071	0.0046	0.0005

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

498 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
 499 averaged over 0 and 1 µM levels of Cd and 3 replicates.

Treatment		Root dry weight	Shoot dry weight	
Temperature	23	0.23 ^b	1.026 ^b	501
(°C)	18	0.28 ^a	1.174 ^a	502
Species	Cultivated	0.29 ^a	1.240 ^a	503
	Wild	0.21 ^b	0.95 ^b	504
				505
				506
				507

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

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517 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
 518 genotypes cultivated at two temperatures and in the presence of 1 μ M 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 \times Temperature	7	251*	4421**	61 ^{ns}	421**	26.2**
Wild \times Temperature	3	161 ^{ns}	2785*	18.3 ^{ns}	194*	35.4*
Cultivated \times Temperature	3	318*	3847**	116 ^{ns}	690**	25.5 ^{ns}
Species \times Temperature	1	319 ^{ns}	11052*	24.5 ^{ns}	297*	1.3 ^{ns}
Error	28	75	582	58	42.4	10.5

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

521

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

Treatment		Root Cd concentration	Cd translocation	524
				525
				526
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				528
Temperature (°C)	23	26.7.35 ^a	56.8 ^a	529
	18	14.2 ^b	49.2 ^b	530
				531
Species	Cultivated	16.2 ^b	60.5 ^a	532
	Wild	21.9 ^a	45.3 ^b	533
				534
				535

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

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

Relative biomass production (%)																543	
23 °C								18 °C								544	
Cultivated				Wild				Cultivated				Wild				545	
2811	C111	Saffire	AC	Isfahan	Azari	Arak	Shiraz	2811	C111	Saffire	AC	Isfahan	Azari	Arak	Shiraz	546	
0.5	89.6 \pm 0.8	85.8 \pm 2.7	84.3 \pm 5.5	99.6 \pm 8.1	87.9 \pm 3.5	99.2 \pm 2.8	74.3 \pm 1.4	86.5 \pm 6.0	96.41 \pm 4.7	90.6 \pm 3.4	89.5 \pm 2.7	94.4 \pm 2.1	92.6 \pm 0.8	91.5 \pm 1.2	66.4 \pm 0.8	92.5 \pm 7.1	547
1	83.3 \pm 2.5	63.7 \pm 4.0	69.7 \pm 3.2	86.7 \pm 3.7	71.3 \pm 4.5	71.8 \pm 0.6	60.7 \pm 4.7	75.7 \pm 1.2	82.9 \pm 1.2	83.5 \pm 0.8	78.7 \pm 1.2	79.6 \pm 1.1	72.7 \pm 2.1	91.5 \pm 0.1	69.1 \pm 1.5	81 \pm 4.5	548
5	50.3 \pm 0.5	48.4 \pm 1.3	43.2 \pm 1.2	60.8 \pm 1.1	56.7 \pm 1.7	46.2 \pm 2.5	40.1 \pm 3.2	41.7 \pm 1.0	67.9 \pm 4.6	61.2 \pm 0.7	59.3 \pm 2.3	63.1 \pm 2.2	33.9 \pm 1.1	48.2 \pm 1.8	45.2 \pm 3.4	64.5 \pm 2.7	549
10	37.6 \pm 0.4	30.0 \pm 1.5	28.2 \pm 2.2	38.8 \pm 0.6	31.6 \pm 1.8	35.2 \pm 1.7	25.7 \pm 0.3	27.1 \pm 0.5	49.6 \pm 1.3	36.3 \pm 1.8	40.5 \pm 1.9	53.1 \pm 3.1	25.8 \pm 0.8	42.3 \pm 4.6	32.5 \pm 1.9	57.8 \pm 2.2	550
20	17.4 \pm 0.7	17.8 \pm 0.2	15.1 \pm 0.1	17.8 \pm 0.8	17.3 \pm 0.1	19.9 \pm 0.8	14.9 \pm 0.2	10.6 \pm 0.7	23.0 \pm 1.6	20.9 \pm 0.5	19.5 \pm 0.3	28.8 \pm 1.1	19.7 \pm 0.9	18.0 \pm 0.3	13.4 \pm 0.6	27.3 \pm 0.5	551
50	6.17 \pm 0.4	4.7 \pm 0.4	0.3 \pm 0.1	3.2 \pm 0.3	5.3 \pm 0.3	4.4 \pm 0.2	-0.2 \pm 0.4	1.8 \pm 0.3	5.0 \pm 0.4	4.4 \pm 0.5	5.7 \pm 0.1	7.37 \pm 0.9	6.97 \pm 0.3	7.7 \pm 0.3	6.8 \pm 0.2	7.1 \pm 0.7	552
100	-1.26 \pm 0.1	-2.6 \pm 0.2	3.1 \pm 0.0	-3.9 \pm 0.3	-1.2 \pm 0.1	0.8 \pm 0.1	-3.4 \pm 0.2	-2.7 \pm 0.1	-2.4 \pm 0.1	-0.6 \pm 0.4	-2.3 \pm 0.6	-4.2 \pm 0.4	2.3 \pm 0.7	3.5 \pm 0.1	0.8 \pm 0.4	-1.09 \pm 0.6	553
500	-2.57 \pm 0.3	-3.2 \pm 0.2	-5.4 \pm 0.3	-5.3 \pm 0.3	-5.1 \pm 0.1	-3.4 \pm 0.7	-5.1 \pm 0.2	-4.3 \pm 0.2	-7.1 \pm 0.1	-8.8 \pm 0.4	-8.7 \pm 0.3	-6.5 \pm 0.6	-1.4 \pm 0.5	-2.5 \pm 0.3	-4.0 \pm 0.3	-6.6 \pm 0.6	554

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

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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 ‡ Calculations are based on the dry weight of plants.

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

570 ‡ The Terms TT_{95b} and EC₅₀ indicate the Cd concentration (µM) where the plant dry weight is declined by 5 and 50%, respectively.

571

572 Legends for figures.

573 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
574 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.

575 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
576 °C and 18 °C) and three replicates ± SE. LSD at 0.05 = 0.110.

577 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.
578 Each value is a mean of two Cd levels (0 and 1 µM) and three replicates ± SE. LSD at 0.05 = 0.033.

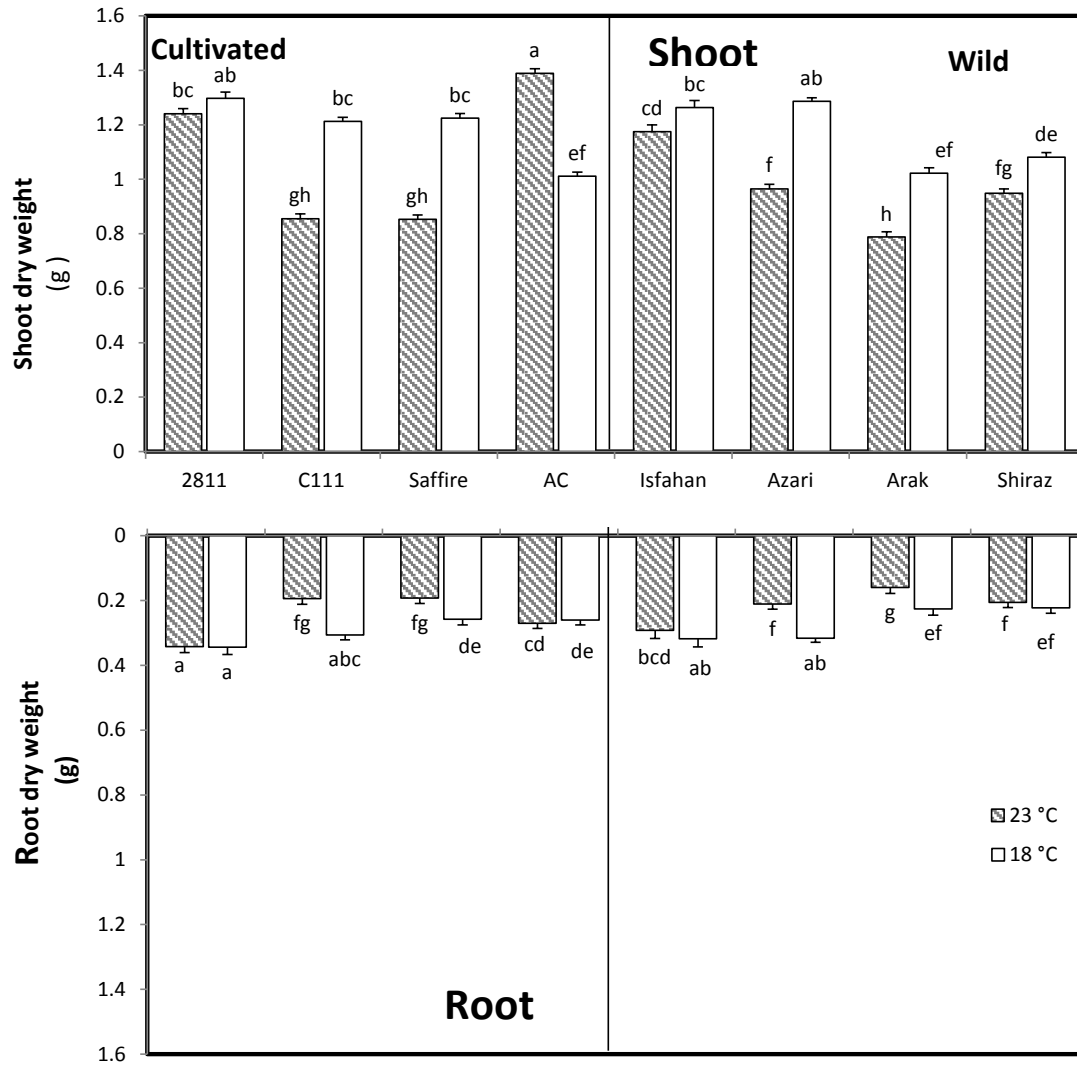
579 Figure 4. Relative Cd uptake calculated as amount of Cd taken up in whole plants in relation to total Cd added in the medium. Plants of eight genotypes of safflower were
580 grown for 14 days in two temperatures (23 °C and 18 °C). Each value is a mean of three replicates ± SE. LSD at 0.05 = 14.8.

581 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
582 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.

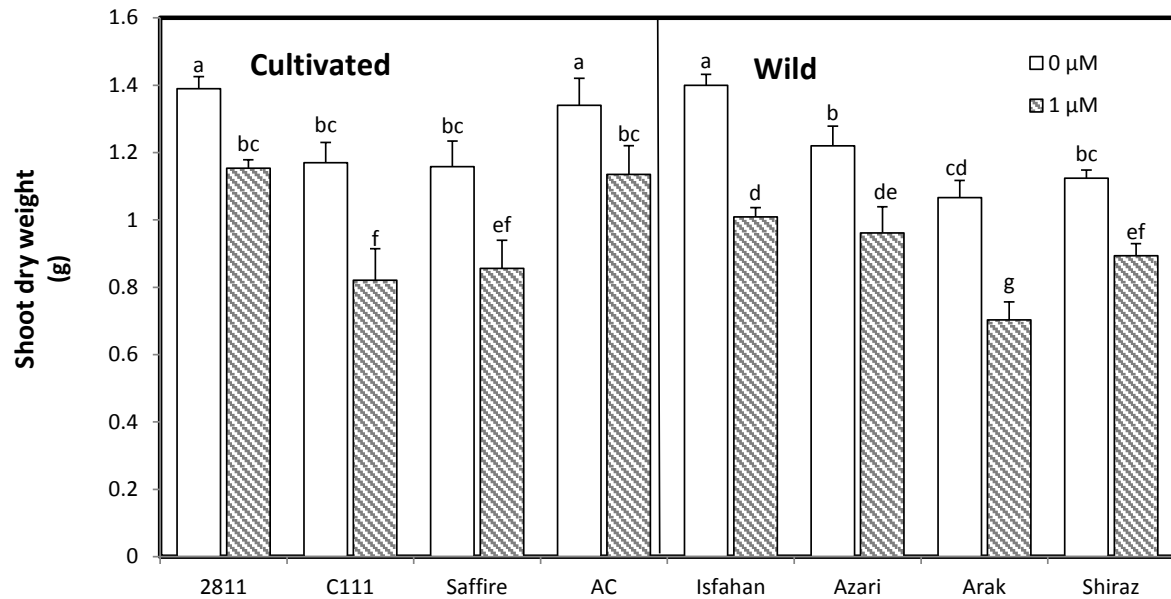
583 Figure 6. Cd concentration of shoot and root (µg Cd/g DW) of eight genotypes of safflower when grown for 14 days under 23 °C and 18 °C temperatures in 1 µM level of Cd.
584 Each value is a mean of three replicates ± SE. LSD (0.05) for Cd content of shoot and root is 5.2 and 10.9, respectively.

585 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-
586 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
587 | replicates ± SE. LSD at 0.05 = 9.02.

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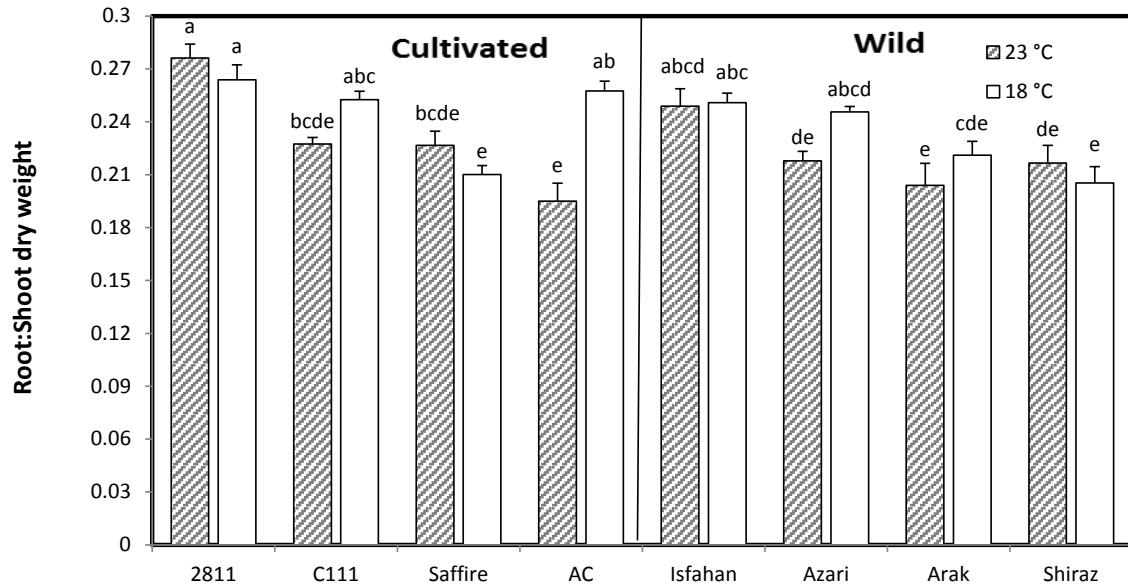


589
 590 Figure 1. Dry weight of shoot and root (g per pot) of eight genotypes of safflower when grown for 14 days
 591 under 23 °C and 18 °C temperatures. Each value is a mean of two Cd levels (0 and 1 μ M) and three replicates \pm
 592 SE. LSDs (0.05) for shoot and root dry weights are 0.110 and 0.039, respectively.
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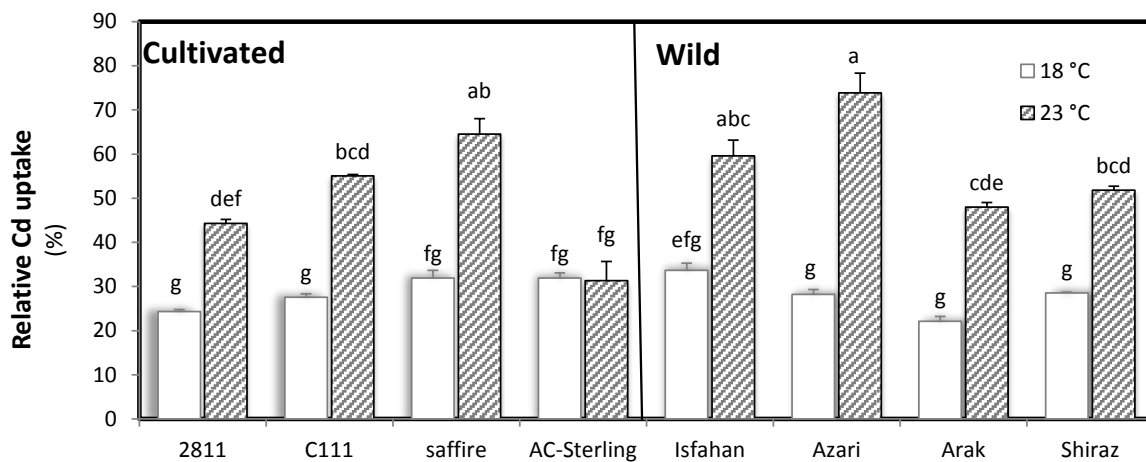
597
 598 Figure 2. Shoot dry weight (g per pot) of eight genotypes of safflower when grown for 14 days under 0 and 1
 599 μM levels of Cd. Each value is a mean of two temperatures (23 and 18 $^{\circ}\text{C}$) and three replicates \pm SE. LSD at
 600 0.05 = 0.110.

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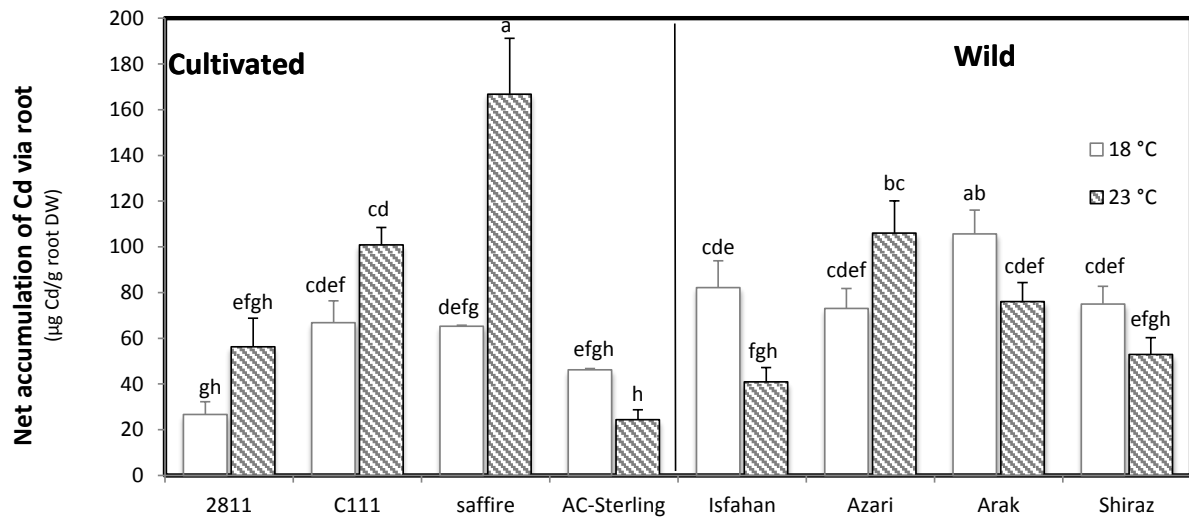
617
 618 Figure 3. Root: shoot dry weight ratio (g root dry weight/g shoot dry weight) of eight genotypes of safflower
 619 when grown for 14 days under 23 °C and 18 °C temperatures. Each value is a mean of two Cd levels (0 and 1
 620 μM) and three replicates ± SE. LSD at 0.05 = 0.033.

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 634 Figure 4. Relative Cd uptake calculated as amount of Cd taken up in whole plants in relation to total Cd added
 635 in the medium. Plants of eight genotypes of safflower were grown for 14 days in two temperatures (23 °C and
 636 18 °C). Each value is a mean of three replicates ± SE. LSD at 0.05 = 14.8.

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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.

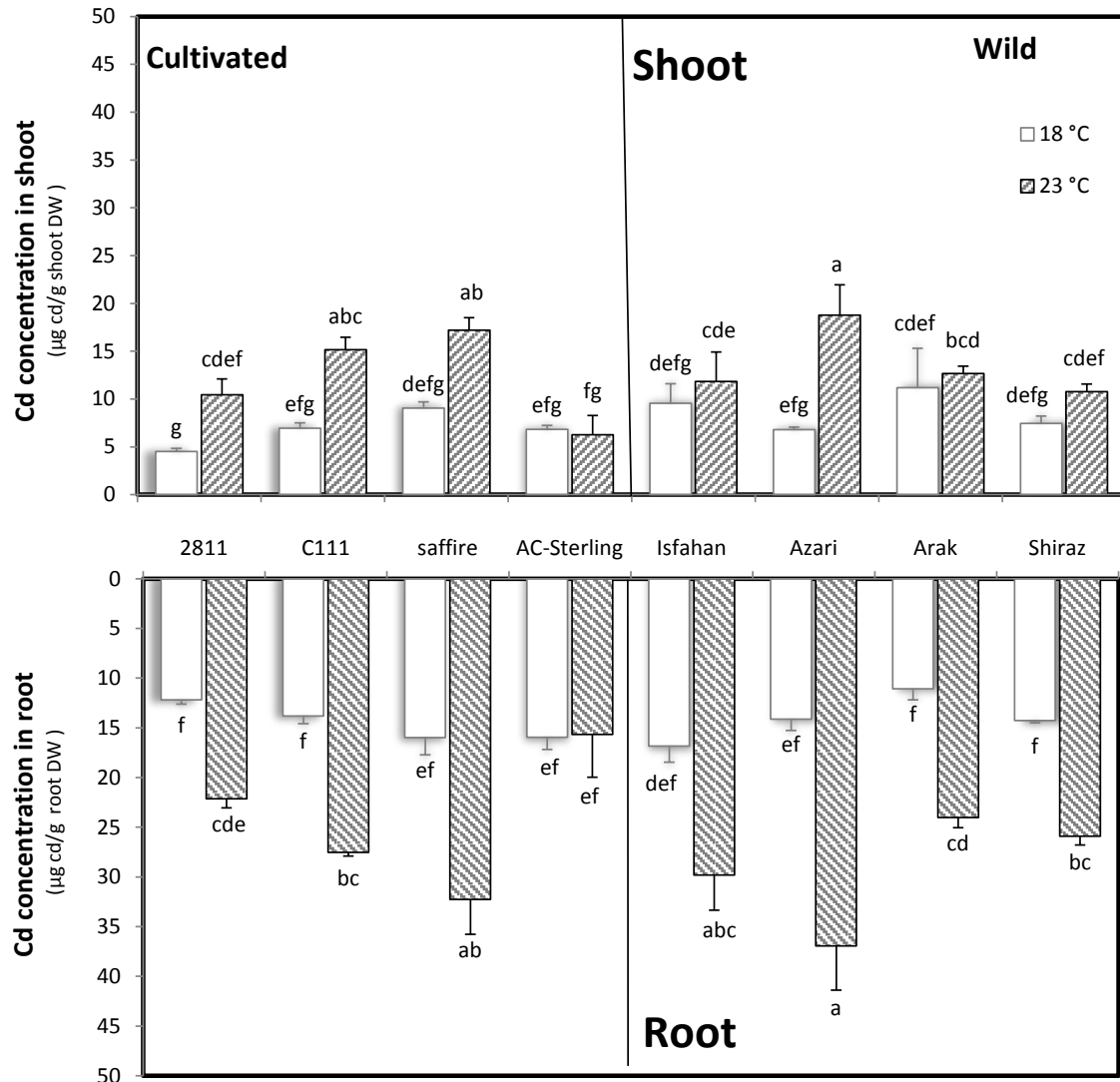


Figure 6. Cd concentration of shoot and root ($\mu\text{g Cd/g DW}$) of eight genotypes of safflower when grown for 14 days under 23 °C and 18 °C temperatures in 1 μM level of Cd. Each value is a mean of three replicates \pm SE. LSDs (0.05) for Cd content of shoot and root are 5.2 and 7.75, respectively.

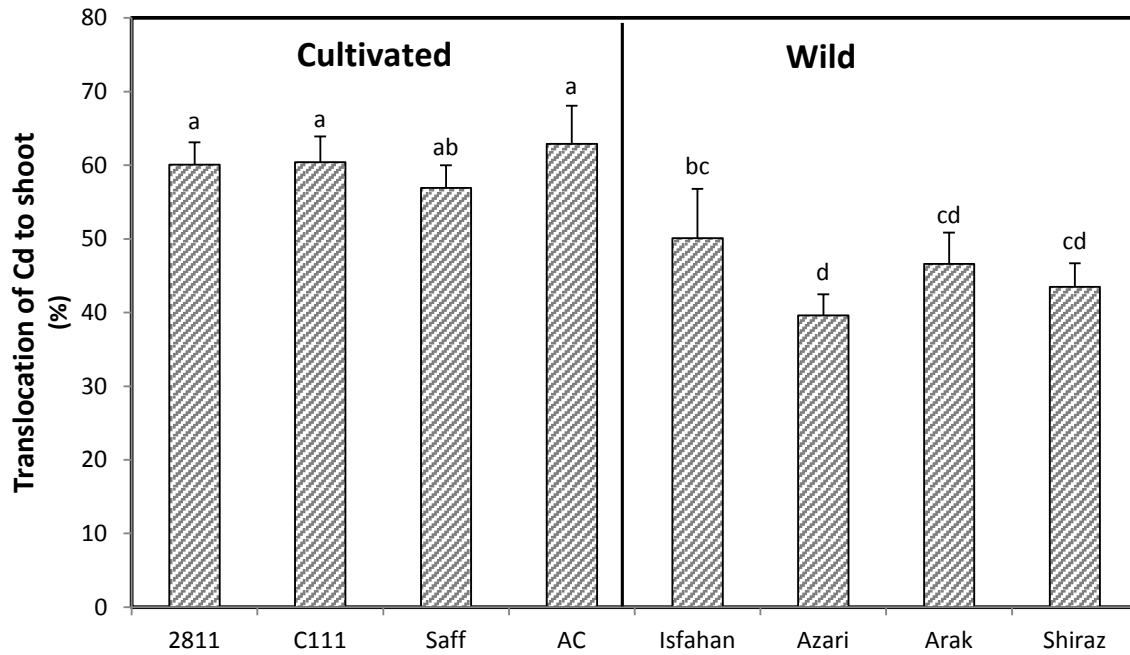


Figure 7. Translocation of Cd to shoot calculated as amount of Cd in shoot in relation to total amount of Cd taken up. Seedlings of eight genotypes (2811, C111, Saffire, AC-Sterling, Isfahan, Azari, Arak, and Shiraz) of safflower were grown for 14 days in 1 μ M level of Cd. Each value is a mean of two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