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BRIEF COMMUNICATION

Changes in biomass and photosynthetic parameters of tomato plants exposed to trivalent and hexavalent chromium

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Abstract

Tomato plants were treated for two weeks with different concentrations of Cr(III) or Cr(VI) compounds to compare their toxic effects. The concentration of total Cr in plant tissues increased linearly with its concentration in the growth medium and Cr accumulated largely in the roots, regardless of the form in which it was supplied to the plant. All measured plant growth parameters were negatively affected by Cr, but Cr(VI) showed much more pronounced toxic effects. Leaf net photosynthetic rate (P_N) was decreased by both Cr forms, and the decrease was also greater for Cr(VI). Cr(III) caused no significant effect on leaf stomatal conductance, whereas Cr(VI) reduced it. Cr(VI) also markedly reduced the variable to maximum chlorophyll *a* fluorescence ratio, measured in dark-adapted leaves.

Additional key words: chlorophyll fluorescence, Cr toxicity, *Lycopersicum esculentum*, net photosynthetic rate, stomatal conductance.

It has been unequivocally shown that Cr at levels above a threshold concentration negatively affects plant growth (see review by Shanker *et al*. 2005, Scocciant *et al*. 2006, Liu *et al*. 2008). Both the trivalent and hexavalent forms of chromium can cause serious damage to plants, but chromate is known to be more hazardous, possibly due to its higher oxidizing potential and mobility within the plant. It has been proposed that roots are able to convert Cr(VI) into Cr(III) (Liu *et al*. 1995, Lytle *et al*. 1998, Zayed *et al*. 1998, Aldrich *et al*. 2003), which would then be stored in vacuoles or retained in cation-exchange sites of the root cell wall (Skeffington *et al*. 1976, Mangabeira *et al*. 2006). Other authors (Zayed *et al*. 1998, Appenroth *et al*. 2000, 2001, Aldrich *et al*. 2003) claim that all plant parts have the ability to reduce Cr(VI) to Cr(III), supposedly as part of a generalized cellular detoxification strategy. However, one should be cautious with such an assumption because Cr(VI) reduction in the cell generates reactive Cr(V) and Cr(IV) intermediates, as well as free radicals (Shi and Dalal 1990, Liu *et al*. 1995) that may cause cellular damage and thus enhance, rather than ameliorate, chromium toxicity.

Chromium, particularly Cr(VI), causes severe disturbances in multiple aspects of plant metabolism and physiology, particularly in the plant water status (Barceló *et al*. 1986, Pandey and Sharma 2003), mineral nutrient balance (Barceló *et al*. 1998, Sharma and Pant 1994, Chatterjee and Chatterjee 2000, Dube *et al*. 2003, Pandey and Sharma 2003, Gardea-Torresdey *et al*. 2004, Liu *et al*. 2008), and respiration and photosynthesis (Bazynski *et al*. 1981, Losi *et al*. 1994, Appenroth *et al*. 2001, Zeid 2001, Dixit *et al*. 2002, Fernandes *et al*. 2002, Liu *et al*. 2008, Gupta *et al*. 2009). Most of these effects were recently reviewed (Shanker *et al*. 2005). Both the mitochondrial and photosynthetic electron transport chains have been shown to be disrupted by Cr (Losi *et al*. 1994, Dixit *et al*. 2002, Fernandes *et al*. 2002, Liu *et al*. 2008) leading to the formation of reactive oxygen species that can account for the severe oxidative stress that has been reported to be associated with Cr toxicity (Dixit *et al*. 2002, Shanker and Pathmanabhan 2004, Shanker *et al*. 2005, Liu *et al*. 2008).

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Abbreviations: Chl - chlorophyll; c_i - intercellular CO_2 concentration; F_v/F_m - variable to maximum Chl *a* fluorescence ratio; g_s - stomatal conductance; LMR - leaf/plant mass ratio; P_N - net photosynthetic rate; PS - photosystem; SLA - specific leaf area; WOC - water oxidizing centre.

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We carried out a comparative study of the toxicity of trivalent and hexavalent chromium forms on tomato plants in solution culture. We first measured the extent of the decrease in several plant biomass parameters caused by the two Cr forms and next measured the extent of photosynthesis inhibition in order to evaluate the photosynthesis-related contribution to the observed decrease in plant dry matter yield.

Tomato (*Lycopersicon esculentum* Mill. cv. Juncal) seeds were germinated in *Vermiculite* wetted with distilled water at room temperature under an irradiance of 150 µmol m^{-2} s^{-1}. Seeds were watered daily with distilled water and after germination the seedlings were watered with distilled water or nutrient solution, on alternate days. After one month, seedlings were selected for their uniformity and transferred into containers with aerated half strength Hoagland nutrient solution. They were grown at 16-h photoperiod with irradiance of 1200 µmol m^{-2} s^{-1}. After a 15-d period of plant adaptation to the new growth conditions, chromium treatments were initiated by adding the appropriate amounts of $CrCl_3 . 6\,H_2O$ or $K_2Cr_2O_7$ to give final Cr(III) or Cr(VI) concentrations of 0, 10, 20, 30, 40 and 50 µM and the plants were grown for another 15-d period.

After 15 d of exposure to chromium treatments, 15 plants per treatment were harvested and biomass determinations carried out. The plants dry mass was determined after 72-h drying at 80 ºC in a ventilated oven. Leaf area was measured with a *MUSTEK 1200UB Plus* scanner using *IMAGE J 1.33u* software.

Leaf net photosynthetic rate (P_N) and intercellular CO_2 concentrations (c_i) were determined between 10:00 and 11:00 with a portable infrared gas analyser (*LCi*, *ADC*, Hoddesdon, UK) on one leaf of each of 6 plants per treatment. Leaf stomatal conductance (g_s) was determined with a porometer (*AP4*, *DELTA-T Devices*, Cambridge, UK) at 10:00 on one leaf of each of 6 plants per treatment. Leaf fluorescence parameters were measured at the same time, after a 20-min dark adaptation period, on 6 leaves per treatment, using a plant efficiency analyzer (*PEA*, *Hansatech*, Norfolk, UK).

Root and leaf chromium concentrations were determined by atomic absorption spectrophotometry. Each sample was a mixture of 3 roots or leaves from the same treatment and there were 3 samples per treatment.

Data presented are the mean ± standard deviation (SD) of at least 3 independent measurements per treatment; comparison between means was carried out by unifactorial analysis of variance (*ANOVA*) and Student's *t*-test.

The tomato dry mass was significantly reduced by both the trivalent and hexavalent chromium at all the concentrations tested (Table 1). Cr(VI) caused a reduction of about two thirds in the plant dry mass at 20 µM concentration, whereas Cr(III) reduced the plant dry mass by only one third and at the highest concentration used of 50 µM. Leaf area was also significantly reduced by both Cr forms, but again Cr(VI) caused a more drastic reduction from the 20 µM concentration upwards. Leaf area was about a quarter of the control at the 50 µM Cr(VI) treatment, but about three quarters at the same Cr(III) concentration. Specific leaf area (SLA) displayed firstly a slight increase with increasing Cr concentrations, reflecting a mass decrease faster than a leaf area decrease, but gradually was reduced for both Cr forms at 30 µM concentrations and above. Leaf/plant mass ratio (LMR) showed a significant decrease for Cr(VI) treatments and reached its maximum

Table 1. Effects of Cr(III) and Cr(VI) on several plant growth and photosynthetic parameters of tomato leaf. Data are the mean ± SD and different letters indicate statistically significant differences at $P \leq 0.05$.

Parameters	Control		10 µM Cr	20 µM Cr	30 µM Cr	40 µM Cr	50 µM Cr
Dry mass [g $plant^{-1}$]	6.38 ± 0.78^{a}	Cr(III)	5.05 ± 0.53^{b}	4.62 ± 0.49^{b}	4.51 ± 0.51^{b}	4.23 ± 0.43^{bc}	4.07 ± 0.43^{c}
		Cr(VI)	4.29 ± 0.57^{bc}	2.31 ± 0.21^{d}	2.19 ± 0.33^{d}	1.98 ± 0.23^{d}	2.00 ± 0.21^{d}
Leaf area [cm^2]	101.40 ± 19.8^{a}	Cr(III)	84.50 ± 13.2^{bc}	81.60 ± 16.5^{bc}	82.40 ± 18.7^{bc}	74.80 ± 13.3^{cd}	70.40 ± 15.3^{d}
		Cr(VI)	77.00 ± 9.9^{cd}	28.60 ± 4.4^{e}	26.90 ± 5.5^{e}	23.10 ± 5.4^{f}	24.30 ± 5.4^{f}
SLA [cm^2 g^{-1}(f.m.)]	27.51 ± 4.3^{bcd}	Cr(III)	28.24 ± 4.5^{bc}	30.34 ± 4.0^{a}	25.72 ± 27^{cde}	26.35 ± 3.3^{cde}	26.78 ± 3.7^{cde}
		Cr(VI)	28.97 ± 4.0^{ab}	27.98 ± 4.0^{bc}	26.46 ± 33^{cde}	24.15 ± 3.6^{de}	22.68 ± 2.2^{e}
LMR	0.67 ± 0.02^{bc}	Cr(III)	0.66 ± 0.02^{bc}	0.63 ± 0.03^{d}	0.70 ± 0.03^{a}	0.67 ± 0.04^{bc}	0.63 ± 0.04^{d}
		Cr(VI)	0.65 ± 0.02^{cd}	0.55 ± 0.03^{f}	0.56 ± 0.03^{f}	0.56 ± 0.03^{f}	0.58 ± 0.02^{f}
R/S	0.20 ± 0.04^{bc}	Cr(III)	0.17 ± 0.04^{c}	0.21 ± 0.06^{b}	0.13 ± 0.04^{d}	0.14 ± 0.04^{d}	0.13 ± 0.05^{d}
		Cr(VI)	0.18 ± 0.04^{c}	0.23 ± 0.04^{a}	0.20 ± 0.04^{b}	0.17 ± 0.02^{c}	0.14 ± 0.02^{d}
P_N [µmol m^{-2} s^{-1}]	$16.70\pm1.3\ ^{a}$	Cr(III)	14.70 ± 1.2^{b}	14.00 ± 1.5^{b}	13.60 ± 1.2^{bc}	13.10 ± 1.1^{bc}	12.80 ± 1.3^{c}
		Cr(VI)	13.80 ± 1.3^{bc}	8.20 ± 1.0^{d}	7.10 ± 1.0^{d}	6.30 ± 1.1^{e}	5.70 ± 0.8^{f}
g_s [mol m^{-2} s^{-1}]	0.46 ± 0.06^{a}	Cr(III)	0.44 ± 0.07^{a}	0.44 ± 0.07^{a}	0.43 ± 0.07^{a}	0.44 ± 0.06^{a}	0.43 ± 0.06^{a}
		Cr(VI)	0.41 ± 0.07^{b}	0.32 ± 0.06^{c}	0.19 ± 0.05^{d}	0.17 ± 0.05^{d}	0.14 ± 0.05^{f}
c_i [µmol mol^{-1}]	$237.0\ \pm16^{c}$	Cr(III)	$241.0\ \pm13.0^{bc}$	$245.0\ \pm14.0^{b}$	$247.0\ \pm13.0^{b}$	$254.0\ \pm13.0^{a}$	$257.0\ \pm15^{a}$
		Cr(VI)	$243.0\ \pm17.0^{b}$	$231.0\ \pm15.0^{c}$	$215.0\ \pm15.0^{d}$	$189.0\ \pm13.0^{e}$	$176.0\ \pm12^{f}$
F_v/F_m	0.80 ± 0.02^{a}	Cr(III)	0.79 ± 0.02^{ab}	0.81 ± 0.03	0.80 ± 0.02^{a}	0.75 ± 0.03^{bc}	0.71 ± 0.03^{c}
		Cr(VI)	0.78 ± 0.03^{b}	0.68 ± 0.02	0.61 ± 0.03^{e}	0.60 ± 0.03^{e}	0.58 ± 0.03^{f}

at 30 µM Cr(III). The root/shoot ratio was highest at 20 µM Cr(VI), and remained always higher at Cr(VI) than at Cr(III) at the same concentrations.

Two general conclusions can be drawn from these results. Firstly, Cr(VI) affects more physiological processes than Cr(III) and, secondly, at identical concentrations Cr(VI) exerts a much stronger toxic effect than Cr(III). Both Cr forms reduce plant growth. Analysis of the R/S ratio shows that Cr(VI) affects the plant shoot more severely than the root part, the R/S ratio always being higher for Cr(VI) than Cr(III) at the same concentration. The observation that higher levels of Cr accumulate in the plant shoot when the metal is absorbed as Cr(VI) may explain the stronger inhibitory effects on leaf development and physiological processes found for Cr applied in the chromate form.

Photosynthesis has been found to be a major target for Cr inhibition (Bazynski *et al.* 1981, Losi *et al.* 1994, Appenroth *et al.* 2001, Liu *et al.* 2008). P_N was reduced by both Cr forms from the 10 µM concentration upwards, but whereas the decrease caused by Cr(III) was gradual and slow, that caused by Cr(VI) was much more marked, particularly for concentrations of 20 µM and higher (Table 1). Cr(III) caused no significant effect on leaf stomatal conductance at all of the concentrations tested, but Cr(VI) caused a major reduction in g_s values at 20 µM and higher concentrations; at 50 µM concentration, Cr(VI) reduced leaf g_s to a third of controls. The manner in which Cr(VI) decreased guard cell pressure potential and induced stomatal closure is not clear, but stomatal closure was not caused by a reduction in leaf water availability, as the water potential of Cr(VI)-treated leaves remained at values close to the -0.4 MPa found in the controls. Shanker *et al.* (2005) propose that Cr(VI) inhibits the plasma membrane H^+-ATPase required for stomatal opening, but there is no clear evidence for this. For Cr(III) a slight, though significant, increase in c_i occurred, possibly caused by decrease in CO_2 consumption, but at Cr(VI) an initial increase in c_i was followed by a decrease at the 30, 40 and 50 µM concentrations, as the result of the reduction in g_s. The observed reductions in g_s, however, do not seem to be the primary cause for the measured decrease in P_N, as preliminary experiments using above-ambient CO_2 concentrations to compensate for eventual CO_2 restriction imposed by stomatal closure did not enhance the photosynthetic rates of Cr(VI)-treated leaves, indicating that Cr limits photosynthesis through nonstomatal effects. One such possible effect is on the photosynthetic light energy conversion itself. Indeed, our data show that Cr(VI) at moderate and high concentrations strongly reduces the maximum efficiency of photosystem (PS) 2 primary photochemistry, measured by the variable to maximum fluorescence ratio (F_v/F_m) in the dark-adapted leaves. The F_v/F_m showed a less than 10 % decline induced by Cr(III) and only at concentrations of 40 and 50 µM; Cr(VI), however, induced a decrease in F_v/F_m for all concentrations tested, showing a marked decrease for concentrations of 20 µM and higher (Table 1). This decrease reveals a progressive increase in the number of PS 2 units that are functionally disabled, directly or indirectly by Cr(VI) toxicity. Appenroth *et al.* (2001) also found that the number of inactivated PS 2 units increased markedly in the presence of Cr(VI), as a result of a decrease in the number of Q_B binding centers. Recently, Liu *et al.* (2008) similarly reported that the F_v/F_m ratio was decreased by treatments with 100 µM Cr in *Amaranthus viridis*, although to a much lesser extent than was found here. It is known that Cr, particularly Cr(VI), affects the photosynthesis in multiple manners and this makes particularly difficult to identify the primary site(s) of action. Shanker *et al.* (2005), citing several authors, suggest that because of its high oxidizing potential Cr(VI) could directly receive electrons from PS 1-donating sites, thus diverting reducing power from the CO_2 assimilation reactions. It should be noted, however, that the valence state of Cr is largely determined by local pH and redox conditions and that for an irradiated chloroplast, the prevalent pH and redox values clearly tend to favour the presence of Cr(III) relative to Cr(VI), the former being much less toxic. Appenroth *et al.* (2001) report that Cr(VI) also decreases photosynthetic oxygen evolution by damaging the water oxidizing centres (WOCs) associated with PS 2. One way Cr(VI) could disrupt WOCs would be by restricting chloroplast Ca and Mn availability, two ions that play essential roles in their structure and function (Yano *et al.* 2006). We found (data not shown) that the contents of Ca and Mn in leaves treated with 50 µM Cr(VI) were reduced by approximately 30 % relative to the controls and this could well contribute to the observed disruption of WOCs.

It is clear that a linear correlation exists between Cr content in the plant tissues and concentrations in the medium. The slopes of the straight lines differ with the

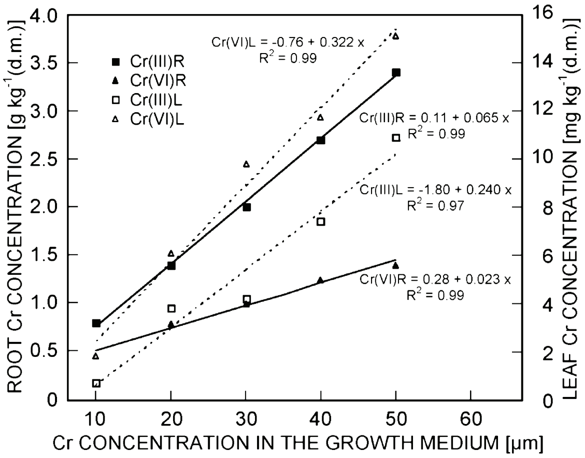

Fig. 1. Relationship between chromium concentration in tomato roots (*solid lines*) and leaves (*dashed lines*) and in the growth medium. Symbols refer to actual data points for Cr in roots and leaves, respectively. Adjusted straight lines were obtained by regression analysis. Cr(III) and Cr(VI) refer to the form in which Cr was supplied to the plants.

form of Cr supplied, with Cr(III) displaying a steeper slope than Cr(VI) in the roots and the reverse being observed in the leaves (Fig. 1). The data clearly show that Cr largely accumulates in the roots, with only a very small fraction being transported to the shoot. It is also relevant to note that a larger fraction of the absorbed Cr reaches the leaves when the metal is supplied in the hexavalent form.

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