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The effect of chromate on the synthesis of plastid pigments and lipoquinones in *Zea mays* L. seedlings

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Abstract

Maize plants cultivated on nutrient solutions containing increasing amounts of CrO_4^{2-} (from 10^{-7} to 10^{-4} M) showed growth inhibition, strong reduction of the root system and an increase of dry matter of leaves. The highest accumulation of chromium in plant organs appeared in the roots. Chromate taken up into leaves caused changes in the content of plastid pigments and lipoquinones similar, in general, to those in senescent plants.

INTRODUCTION

Chromium is a very potent pollutant of natural and agricultural ecosystems. High levels of chromium were observed in water contaminated with industrial and municipal sewage (Kabata-Pendias and Pendias 1979). This metal is nonselectively taken up by plants but its physiological role is not known. Huffman and Allaway (1973) have shown that CrO_4^{2-} at low concentrations in nutrient solution is not required for normal growth of some plant species. At higher concentrations it has been shown to be absorbed by plants causing the reduction of growth and affecting metabolism (Sarosiek 1958, Austenfeld 1979). The toxicity of chromium for plants depends on the degree of its oxidation — Cr^{6+} is 100 times more destructive than Cr^{3+}. In general, plants tolerate soluble chromium in amounts of 0.06-50 ppm in the medium. The most sensitive plants are tobacco, maize and oats (Pratt 1966).

Abbreviations: chlf — chlorophyll; EDTA — ethylenediaminetetraacetic acid; PQA — plastoquinone A; $PQAH_2$ — plastohydroquinone A; α-T — α-tocopherol; α-TQ — α-tocopherylquinone.

Little is known on the influence of chromium on the photosynthetic apparatus in higher plants. Desmet et al. (1975) have demonstrated absorption of CrO_4^{2-} by isolated chloroplasts and its competition with methyl viologen for the same electron donor substrate. A few years later Baszyński et al. (1981) reported that chromate strongly decreased the pigment content, inhibited photosystem II activity and disorganized the fine structure of chloroplasts of *Lemna minor* L.

The aim of the present studies was to investigate the effect of CrO_4^{2-} on the synthesis of plastid pigments and lipoquinones in 2- and 4-week-old *Zea mays* L. seedlings cultivated on nutrient solutions containing increasing amounts of chromate. The uptake of Cr^{6+} by maize roots and leaves was also investigated.

MATERIAL AND METHODS

Seeds of *Zea mays* L. var. SM 480 were sterilized with 70% alcohol for 5 min., soaked for 2-3 h in distilled water and germinated on wet filter paper for 5 days at 27°C in darkness. The seedlings were then transplanted into glass vessels containing modified Hoagland's solution and maintained in a thermostated chamber under 16 hrs light ($4.5 \cdot 10^3\ \mu W \cdot cm^{-2}$) at 23°C and at a relative humidity of 90%. The modified Hoagland's solution contained in mmoles per liter: 4.6 $Ca(NO_3)_2 \cdot 4\ H_2O$, 0.5 KNO_3, 1.0 KH_2PO_4, 1.6 $MgSO_4 \cdot 7\ H_2O$, 0.1 $FeSO_4 \cdot 7\ H_2O$, 0.1 Na_2-EDTA. The micronutrients had the following composition in µmoles per liter: 100 H_3BO_3, 100 $MnSO_4 \cdot 4\ H_2O$, 5 KJ, 1 $Na_2MoO_4 \cdot 2\ H_2O$, 0.1 $CuSO_4 \cdot 5\ H_2O$, 0.1 $CoCl_2 \cdot 6\ H_2O$. Since Cr^{3+} precipitates at a pH above 5.5, only the CrO_4^{2-} form was investigated. Chromium (Cr^{6+}) in the form of K_2CrO_4 was added to the nutrient solution in the following amounts in moles per liter: 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}. The mineral supply was maintained by frequent replacement of the nutrient solution (every 3-4 days). The maize plants were harvested for the experiments after 2 and 4 weeks of growth.

Chlorophyll concentrations were measured by Arnon's method (1949). Carotenoids were chromatographed and estimated by the method of Hager and Bertenrath (1962). The plastid benzoquinones were separated on silica gel layers according to Lichtenthaler (1969). The amounts of benzoquinones were determined spectrophotometrically after Lichtenthaler (1968). Vitamin K_1 was chromatographed and estimated by the method of Lichtenthaler and Tevini (1969).

For the determination of chromium content the leaves and roots were dried at 105°C and digested in the following mixture of acids: HNO_3-

-H_2SO_4-perchloric acid 10 : 1 : 4 v/v followed by ammonium persulphate treatment. The chromium content in plant material was then estimated on a Pye-Unicam SP-9 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

In 2- and 4-week-old chromate-treated plants inhibition of growth and strong reduction of the root system were observed. In 4-week-old plants treated with 10^{-4} M CrO_4^{2-} complete necrosis of the leaves occurred. Thus, it was impossible to estimate lipoquinone and pigment levels in these plants. The uptake and accumulation of chromium in plant organs are shown in Table 1. High levels of chromium were accumulated in maize roots, especially in older seedlings. The amounts of chromium in roots at 10^{-4} M CrO_4^{2-} were about 10^3 as high as those in control plant roots (Table 1). Considerably lower amounts of the metal were translocated to the aerial part of the plants, however, these amounts as seen from the further results, caused a strong inhibition of synthesis of plastid pigments and quinones.

Table 1

Content of chromium in maize roots and leaves
(μg per g of dry matter)

Moles CrO_4^{2-} in nutrient solution	2 weeks		4 weeks	
	roots	leaves	roots	leaves
0	0.08±0.02	0.02±0.0	0.13± 0.04	0.03±0.0
10^{-7}	4.15±0.39	0.18±0.05	11.70± 1.04	0.22±0.04
10^{-6}	6.14±0.47	0.19±0.05	15.56± 2.95	0.25±0.02
10^{-5}	33.91±2.91	0.36±0.09	91.12± 6.04	0.45±0.06
10^{-4}	47.30±1.76	1.16±0.16	127.60±10.10	1.46±0.27

The values represent means ±SE of three independent replicates

Dry matter of maize leaves varied from 7.5% in control plants to 9.8 and 8.8%, respectively in 2- and 4-week-old plants treated with the highest doses of chromate.

The amounts of chlf *a* and chlf *b* were quite stable in chromate-treated 2-week-old plants (Fig. 1). Older plants showed a faster decrease of chlf *b* as compared with chlf *a* owing to the concentration of chromate (Fig. 1).

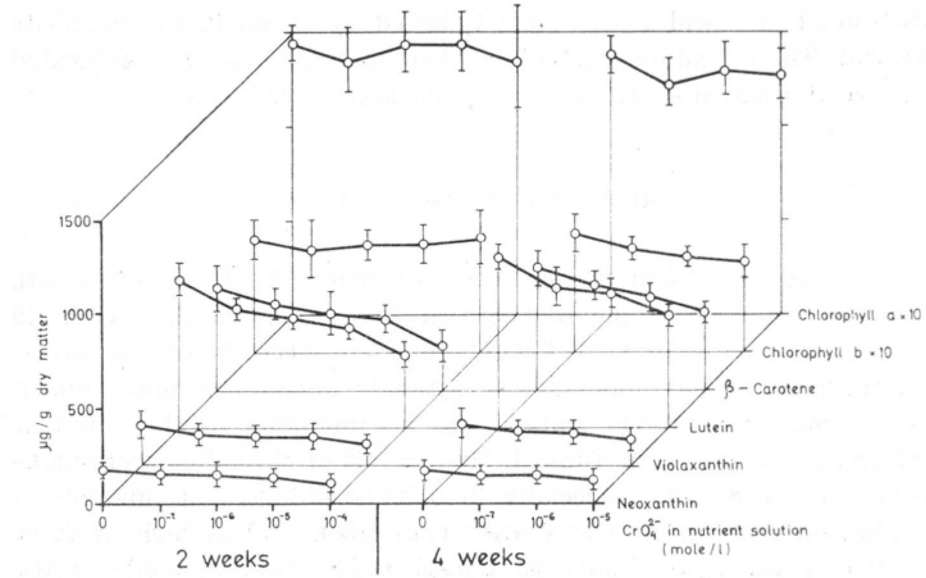

Fig. 1. Effect of CrO_4^{2-} on the synthesis of plastid pigments in maize seedlings. The values represent means ± **SE** of six independent replicates

Similar changes were observed in the carotenoids content. The content of β-carotene and xanthophylls decreased parallelly. The xanthophylls to β-carotene ratio was stable, except in maize plants cultivated 2 weeks on 10^{-4} M CrO_4^{2-} (Table 2). It is possible that chromate, a strong oxidizing agent, could oxidize some amounts of β-carotene to xanthophylls at this concentration. On the basis of the chlf $(a+b)$ to carotenoids ratios it could be assumed that the inhibition of carotenoid synthesis or its decomposition under the influence of chromate occurs faster than that of chlorophylls. Similar effects of chromate treatment were observed by Baszyński et al. (1981) for *Lemna minor* L.

Table 2

Effect of CrO_4^{2-} on the interdependent ratios of plastid pigments and lipoquinones in maize seedlings

	Moles CrO_4^{2-} in nutrient solution								
	2 weeks					4 weeks			
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	0	10^{-7}	10^{-6}	10^{-5}
Chlf *a*/*b*	2.61	2.72	2.60	2.66	2.53	2.28	2.37	2.35	2.37
Chlf $(a+b)$/carotenoids	11.7	13.2	15.6	16.6	23.7	10.3	11.1	11.9	13.7
Xanthophylls/β-carotene	2.2	2.1	2.2	2.1	2.6	2.1	2.1	2.1	2.1
PQ A/PQ AH_2	5.8	5.0	6.3	3.3	2.0	6.0	7.8	3.4	4.3
α-T/α-TQ	7.2	9.0	8.7	8.3	8.3	7.8	7.3	5.0	4.8

Figure 2 shows changes in the lipoquinones in 2- and 4-week-old maize plants, respectively, treated with CrO_4^{2-} A relatively high decrease in PQA content was observed in both groups of plants. This phenomenon was accompanied by a remarkable increase in the content of its reduced form, $PQAH_2$, particularly in 2-week-old chromate-treated plants (Fig. 2). This is partially seen in the decreasing ratio of PQA to $PQAH_2$ (Table 2).

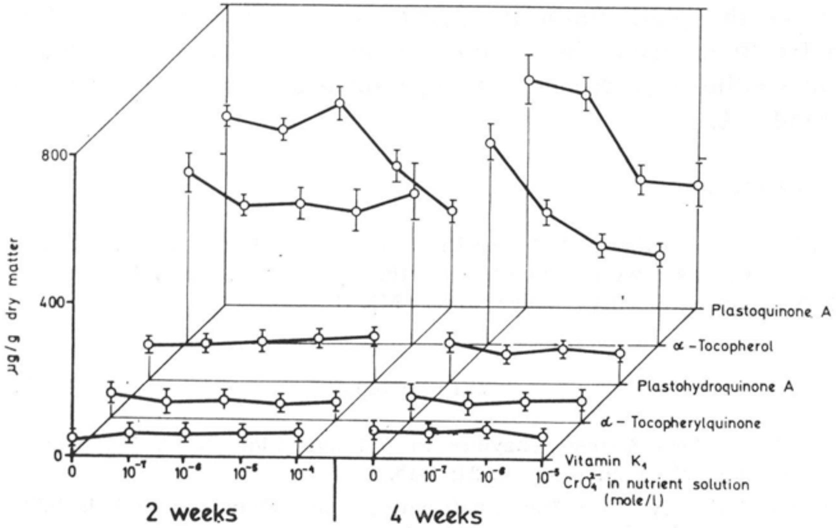

Fig. 2. Effect of CrO_4^{2-} on the synthesis of plastid lipoquinones in maize seedlings. The values represent means ± SE of six independent replicates

The content of α-T in chromate-treated 2- and 4-week-old plants was reduced at the highest CrO_4^{2-} concentrations to about 70 and 50% of that in control plants, respectively (Fig. 2). The content of α-TQ remained quite stable irrespective of the age of the plants and the concentration of chromate in the nutrient solution (Fig. 2). Therefore the decreasing ratio of α-T to α-TQ in older plants reflects a higher sensitivity of α-T to chromate treatment (Table 2). Naphtoquinone vitamin K_1 showed a slight increase after 2 weeks owing to the increasing doses of CrO_4^{2-} and was stable in 4-week-old plants (Fig. 2). Higher levels of vitamin K_1 were positively correlated with a higher content of chromium in plants as reported previously (Szkolnik 1980).

Our results confirmed the toxic effect of higher CrO_4^{2-} concentrations on plants (Turner and Rust 1971, Kabata-Pendias and Pendias 1979, Baszyński et al. 1981). Baszyński et al. (1980, 1981) reported that some heavy metals, e.g. cadmium and chromium, cause changes in the photosynthetic apparatus similar to those due to senescence. Some of our results presented here seem to confirm this observation. The high decrease in β-carotene and lutein contents as expressed per unit of dry matter and a considerably lower decrease in violaxanthin and neoxanthin content in chromate-treated maize are similar to the values reported for senescent plants (Wolińska 1975). The decrease in the ratios of PQA to $PQAH_2$ and α-T to α-TQ observed in our experiments were also reported previously for senescent plants (Król 1974). However, the decrease in PQA and α-T content is faster than that caused by senescence only. It is possible that degradation of the photosynthetic apparatus in higher plants under chromate treatment could be affected, among other things, by the inhibition of synthesis or decomposition of some chloroplast constituents, e.g. PQA and α-T.

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Wpływ jonów chromianowych na syntezę barwników i lipochinonów plastydowych w siewkach Zea mays L.

Streszczenie

Badano wpływ jonów chromianowych na syntezę barwników i lipochinonów plastydowych w liściach 2- i 4-tygodniowych siewek kukurydzy uprawianych w kulturach wodnych zawierających wzrastające stężenia jonów CrO_4^{2-} (10^{-7} - 10^{-4} M). Jony chromianowe powodowały zahamowanie wzrostu badanych roślin, silną redukcję systemu korzeniowego, a po 4 tygodniach przy stężeniu 10^{-4} M powodowały całkowite obumieranie roślin. Chrom był pobierany i akumulowany przede wszystkim w systemie korzeniowym, natomiast w liściach 2- i 4-tygodniowych siewek kukurydzy akumulowany był w ilościach kilkudziesięciokrotnie mniejszych. Obserwowano znaczny spadek zawartości zarówno barwników jak i lipochinonów plastydowych u roślin uprawianych w kulturach wodnych zawierających jony chromianowe. Zmiany w zawartości składników chloroplastowych podobne były do zmian obserwowanych w procesie starzenia się roślin.