Growth of Plants in Solution Culture Containing Low Levels of Chromium

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ABSTRACT

Chromium was not required for normal growth of romaine lettuce (Lactuca sativa L. subsp. longifolia), tomato (Lycopersicon esculentum Mill.), or bean (Phaseolus vulgaris L.) in solution culture containing $3.8 \times 10^{-4} \mu M$ Cr. Plants grown on this purified nutrient solution contained an average of 22 ng Cr/g dry weight. Duckweed (Lemma sp.) grew and reproduced normally on a dilute nutrient solution containing $3.8 \times 10^{-4} \mu M$ Cr.

The objective of this study was to determine if Cr is an essential element for higher plant growth. Recent reviews (18, 22, 24) have summarized the known effects of Cr in biological systems and the evidence suggesting that Cr is essential for animals and man. Several workers have reported stimulatory effects of Cr on plant growth; however, an absolute requirement of Cr for normal plant growth has not been established (9, 19). In this experiment, if essentiality could not be demonstrated, then Cr concentrations in the nutrient solution and plant tissue above which Cr is not essential for higher plants could be established.

MATERIALS AND METHODS

Double distilled, deionized water and reagent grade chemicals were used throughout the experiment. All HNO₃ was glass-distilled before use. All glassware and plastic materials were washed with detergent, rinsed in distilled water, soaked in 10% HCl overnight, and finally rinsed several times in distilled water. Cheesecloth and glass wool were extracted in a Soxhlet extractor first with dilute HNO₃ and then with water. Great care was used throughout the study to prevent or minimize Cr contamination of the purified systems.

Chromium Analysis. A modification of the method of Cary and Allaway (6) was used to determine Cr in plant tissues and nutrient solutions. The addition of AgNO₃ to the digestion mixture and consequent filtering step were omitted from the procedure. Recoveries of carrier-free $^{60}$CrCl₃ added to several of the samples ranged from 90 to 103%. It was necessary to purify HClO₄ by glass distillation under vacuum at 110 to 115 C.

Purification of Nutrient Salts. Stock solutions of KNO₃, CaCl₂, and MgSO₄ salts were purified by forming the Cr-2,4-pentanedione complex, extracting the complex with chloroform and then removing residual chloroform with ether. The salts were prepared in 4-liter batches of 2 M concentration. Fifty ml of 2,4-pentanedione were added, and the mixture was refluxed in a boiling flask fitted with a condenser and boiling stick for 6 hr. The solutions were cooled and extracted six times with chloroform. At this point, removal of carrier-free $^{60}$CrCl₃ added to some of the solutions was complete (>99.5%). The above complexing and extraction procedure was repeated. The solutions were then extracted twice with 100-ml portions of ether and then boiled to dispel the residual ether.

Stock solutions of NH₄H₂PO₄ and NH₄NO₃ were prepared by neutralizing H₃PO₄ or HNO₃ with NH₄OH to pH 5.0 and 7.0, respectively.

Primary standard grade iron wire was dissolved in a small volume of HNO₃. The solution was then made to 75 ml with 7 M LiCl and was extracted with 25 ml of 4-methyl-2-pentanone. The 4-methyl-2-pentanone was removed by evaporation, and the residue was dissolved in water. Iron content was determined, and a stoichiometric amount of disodium ethylene-dinitritotetraacetate was added.

The micronutrient stock solution was prepared from reagent grade salts.

The nutrient culture solution, modified after that proposed by Johnson et al. (16), had the following composition expressed in μmoles/l: KNO₃, 6000; CaCl₂, 4000; NH₄H₂PO₄, 2000; NH₄NO₃, 4000; MgSO₄, 1000; H₂BO₃, 25; MnSO₄·H₂O, 2.0; ZnSO₄·7H₂O, 2.0; CuSO₄·5H₂O, 0.5; H₂MoO₄, 0.5; FeEDTA, 50. The Cr-treated nutrient solution contained 0.96 μM Cr/l added to the nutrient solution as an equimolar mixture of CrCl₃ and Na₂CrO₄. This level of Cr has been shown to be nontoxic to plant growth (10, 23).

Plant Culture. Recirculated air was used in the growth chambers. The air intakes and the bottoms of the growth chambers were covered with fiberglass furnace filters. Aeration for the culture solutions was provided by filtering compressed air through commercial traps and filters followed by two containers filled with glass wool. Tygon and butyl rubber tubing was used to transport the filtered air to the glass aeration tubes in each culture container. Plastic gloves were worn whenever it was necessary to handle the plants. The cultures were supported by a plastic grid placed on top of a wooden table. To avoid contamination between treatments, the Cr treatments and O-Cr treatments were placed at opposite ends of the growth chamber.

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Wheat (Triticum aestivum cv. Lemhi 53), tomato (Lycopersicon esculentum Mill. cv. Glamar), and romaine lettuce (Lactuca sativa L. subsp. longifolia) experimental line C898C Department of Vegetable Crops, Cornell University) seeds were soaked in aerated distilled water for 24 hr. They were then germinated in the dark on cheesecloth-covered polyethylene containers which contained one-fifth nutrient solution. After 3 to 5 days the seedlings were transferred to a growth chamber with a regime of 24 C/18 C day-night temperatures, 16 hr days and about 2300 ft-c light intensity. After 1 to 2 weeks, the seedlings were transferred to 3.2-liter polyethylene containers filled with full strength nutrient solution.

Red kidney beans (Phaseolus vulgaris L.) were rinsed repeatedly with water and then placed in the dark in wet paper for germination. After 5 days the seedlings were then transferred directly to the culture containers.

Small sprouts were broken off sprouted seed potatoes (Solanum tuberosum L. cv. Bake King) and were placed in the culture containers.

Initially, the full strength nutrient solutions in the culture containers were changed after 14 days. As the plants became larger, the solutions were renewed at 10- and then 7-day intervals. After pods began to develop on the bean plants, NH4NO3 was added on alternate days between solution changes to a final concentration of 2000 μM.

The romaine lettuce was harvested after 26 to 56 days of growth, depending upon the experiment. Tomato and potato were harvested when blossoms began to appear, except for one crop of tomatoes harvested after 26 days of growth. Wheat and bean were harvested at maturity. The plants were harvested into plastic bags or glass freeze-drying flasks, frozen, and then freeze-dried in a glass flask-type freeze dryer.

After drying, the plants were placed in plastic bags and pulverized. Some of the larger stem tissue was not pulverized by this technique and only the pulverized mixture was sampled for analysis. Wheat and bean seeds were separated from the pods and chaff by hand using plastic gloves and plastic forceps.

A single cluster of duckweed (Lemma sp.) fronds was placed in a beaker with 0.1 strength purified nutrient solution. The culture beaker was covered with a larger inverted beaker and was placed in the growth chamber. When the initial cluster had divided to cover the surface of the culture solution (about 2 weeks) one cluster (2-3 fronds) was washed with the culture solution and transferred to a new culture solution. This procedure was repeated seven times. After the eighth culture had multiplied and produced a number of daughter clusters, individual clusters were transferred to eight new cultures. The cultures were paired by number of fronds and size. One culture of each pair was treated with the Cr treatment solution to give a Cr concentration of 25 μM. After 9 days of growth, the number of fronds in each culture was recorded.

RESULTS

Table I shows the yields of plant tops grown on purified nutrient solution with and without added Cr. Since the plants were not in randomized positions in the growth room, an analysis of variance was not made. In general, variation within treatment was greater than the differences between treatments. However, all of the Cr-treated potato tops were smaller than the untreated tops. The yields presented in Table I are from second generation wheat plants where the first generation was grown on purified nutrient solution. Both Cr-treated and untreated wheat plants developed abnormal root growth, presumably due to a pathogen, that did not appear to be related to Cr treatment. There were no consistent differences in yield, appearance, or growth between Cr-treated and untreated plants in any of the studies. Although one set of bean seeds produced on low Cr nutrient solution failed to germinate, there were no consistent differences between Cr-treated and untreated seeds in germination of first generation bean or second generation wheat seed.

To confirm that an unrecognized essential element was not

| Table I. Yield and Cr Content of Plant Tops Grown on Purified Nutrient Solutions With and Without Added Cr |
|-----------------|-----------------|-----------------|-----------------|
| Plant           | Cr in Nutrient Solution | No. Plants | Yield of Dried Tops | Cr in Plant Tops |
|                 | μM                |         |                 | Meant | Median | Range |
| Lettuce         | 3.8 × 10^-4      | 11      | 13.9            | 10   | <10-42 |<10-42 |
|                 | 0.96              | 11      | 15.2            | 202  | 197    | 67-336 |
| Tomato          | 3.8 × 10^-4      | 10      | 16.9            | 28   | <10-53 |<10-54 |
|                 | 0.96              | 10      | 16.3            | 123  | 145    | 62-185 |
| Potato          | 3.8 × 10^-4      | 3       | 50.8            | 21   | 20     | 12-31 |
|                 | 0.96              | 3       | 29.9            | 167  | 173    | 122-206 |
| Wheat           | 3.8 × 10^-4      | 3       | 20.5            | 32   | 36     | 19-42 |
|                 | 0.96              | 3       | 17.7            | 221  | 229    | 118-327 |
| Bean            | 3.8 × 10^-4      | 3       | 97.0            | 24   | 23     | 18-30 |
|                 | 0.96              | 2       | 98.2            | 640  | 578    | 578-702 |

1 Chromium content of purified nutrient solutions was determined by analysis.
2 Concentrations below 10 ng Cr/g dry wt. were taken as 10 ng Cr/g dry wt. in computing the means.
3 Four values were below 10 ng Cr/g dry wt.
4 One value was below 10 ng Cr/g dry wt.

| Table II. Cr Content of Seed and Amount of Cr Supplied to the Plants by Seed or Sprouts |
|-----------------|-----------------|-----------------|
| Crop            | Cr Content      | Cr Content      |
|                 | mg/g dry wt     | mg/seed or sprout |
| Lettuce         | 75              | 0.12            |
| Tomato          | 92              | 0.28            |
| Wheat           | 52              | 1.50            |
| Bean            | 23              | 12.2            |
| First generation wheat | <10      | <0.28           |
| First generation bean | <10      | <5.30           |
| Potato sprouts  | 2               |                 |

1 Seed produced on purified nutrient solution.

Table III. Growth of Duckweed With and Without Added Cr After 8 Generations of Growth in Purified Nutrient Solution

The data are the averages of four cultures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cr in Nutrient Solution</th>
<th>Avg No. of Fronds</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cr added</td>
<td>3.8 × 10^-4</td>
<td>23</td>
</tr>
<tr>
<td>Cr added</td>
<td>0.48</td>
<td>24</td>
</tr>
</tbody>
</table>

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removed by purification of the nutrient solution or that toxic residues did not remain from the purification process, several lettuce and tomato plants were grown on unpurified nutrient solution. The growth of these plants was similar to that of the plants grown on the purified nutrient solution.

As shown in Table I, the Cr concentration in the purified nutrient solutions was about $3.8 \times 10^{-4}$ $\mu$M Cr (0.02 $\mu$g/l). The Cr content of unpurified nutrient solutions ranged from $3.8 \times 10^{-4}$ to $1.8 \times 10^{-3}$ $\mu$M Cr (0.2-1.0 $\mu$g Cr/l).

Table I also summarizes Cr analyses of the plant tops. The mean level for all plants grown on the purified nutrient solution was 22 ng Cr/g dry weight with a range of $<10$ to 53 ng Cr/g dry weight. The yields of plants containing the lowest Cr levels were not distinguishable from those of the other plants. In general, only one sample of roots from each experiment was analyzed. The Cr contents of lettuce, tomato, and potato roots grown in purified nutrient solution were similar or slightly higher than the Cr contents of the tops (mean 40 ng Cr/g dry weight; range 17-161 ng Cr/g dry weight). The Cr content of Cr-treated plant roots ranged from 400 to 20,000 ng/g dry weight, depending largely upon age of the plant at harvest.

Table II shows the Cr content of lettuce, tomato, wheat, and bean seeds, as well as potato sprouts. The first generation wheat and bean seeds were lower in Cr than the original seed. The amount of Cr supplied by the seed or sprout was minute when compared to the final Cr content of the plants shown in Table I.

Table III shows the number of fronds produced by eighth generation duckweed grown in 0.1-strength purified nutrient solution with and without added Cr. Growth appeared normal in all generations of duckweed grown on purified nutrient solution and there was no apparent response to Cr in eighth generation duckweed.

**DISCUSSION**

This study has shown that romaine lettuce, tomato, wheat, and bean plants grow normally in solution culture containing $3.8 \times 10^{-4}$ $\mu$M Cr. Background levels of Cr in the nutrient solution and environmental Cr contamination resulted in plant Cr concentrations of about 22 ng/g dry weight (Table I). Of this plant Cr, about 30% could have been supplied by the Cr in the purified nutrient solution. Interestingly, the plants receiving no Cr had root to top Cr ratios of about two, compared to >100 for Cr-treated plants. It is likely that the low root to top Cr ratio in untreated plants is due to aerial contamination. Rahn et al. (20) reported that several air samples from a rural Michigan location contained from 12 to 37 ng Cr/m$^3$. If these levels are similar to the Cr content of the air in the growth chambers, only a small amount of the total Cr in the air would need to be deposited on the plants to account for the Cr levels obtained in this experiment.

If the purified nutrient solution was not subjected to acid digestion before determination of Cr, no Cr could be detected. Since the Cr method used depends on formation of a Cr-2,4-pentanedione complex, this suggests that the Cr present in the purified nutrient solution was not in ionic form. It may have been present in colloidal, olated, or oxalated form. Rollinson (21) pointed out that reversal of olation or oxalation, even by ligands of high coordinating ability, is low. One might expect that the availability to plants of the apparently nonreactive Cr species in the purified solution would be low.

A brief critical evaluation of the literature suggestive of essentiality of Cr for plants seems justified. After reviewing the literature, Pratt (19) concluded that the reported stimulatory effects of Cr on plant growth were generally small and erratic responses that are mostly unverified. Bertrand and his associates (2-5) reported that additions of 40 g Cr/ha to soils low in extractable Cr increased yields of several plant species as much as 40%. The leaf levels of Cr were an order of magnitude higher than those found in our experiment, and they found no change in leaf Cr content in spite of their reported yield increases. Using sand culture, Haas and Brusca (10) reported stimulatory effects of Cr on citrus and avocado. They did not report Cr analysis of nutrient solutions or plant tissue. Hewitt (13) grew plants on purified nutrient solution containing low levels of Cr and found no growth response in tomato or other species tested. He analyzed the plant tissue for Cr and found very high Cr levels (1.8 $\mu$g Cr/g dry weight). He attributed the high Cr levels in the plant tissue to the high Cr content of greenhouse dust. Turner and Rust (23) found no significant response in soybean growth when Cr was added to unpurified nutrient solutions. The Cr concentration in their plant tissue was below the detection limit of their analytical method. None of the reports of stimulation of plant growth by Cr can meet the criteria of essentiality proposed by Arnon and Stout (1), who pointed out that a yield response was not sufficient evidence for essentiality. The reported responses to Cr are difficult to explain. Possibly they are a result of a Cr effect on rhizosphere microorganisms. For example, Hervey (12) reported that as little as 0.032 $\mu$g Cr/ml inhibited growth of certain microorganisms.

In animals, Cr apparently acts as a cofactor for insulin in the cellular level (18). Since insulin action is not essential to plants, the essentiality of Cr to animals does not provide a basis for its essentiality to plants.

Of elements known to be essential for plant growth, Mo is required in the smallest quantities. Plant Mo deficiencies have been reported to occur in plants grown in nutrient solutions containing 0.1 $\mu$M Mo (13) and Mo concentrations in Mo-deficient plant tops range from about 10 to 500 ng Mo/g dry weight (15). The Cr concentrations in the nutrient solutions used in this study were two orders of magnitude lower than required Mo levels. The range in Cr content of our plant tissue (<10-53 ng Cr/g dry weight) was only slightly lower than for Mo (13) in Mo-deficient plant tissue (10-500 ng Cr/g dry weight).

The lowest concentration at which an atom or molecule can be effective in a biological system is not known. Dimman (8) suggested, on the basis of stochastic considerations, that there must be a threshold concentration for a chemical agent to induce a biological reaction. He concluded from concentrations required for various biological inhibitors to be effective and the levels of elements found in hepatocytes, that a lower level for function in the cell is around 10$^4$ atoms per cell, or about 0.1 ng/g fresh weight. However, Kliever et al. (17) calculated that the Co level producing 50% of maximal growth in Rhizobium meliloti corresponds to a concentration of 35 Co atoms per cell and 200 atoms per cell is adequate for normal growth. Recent work by Hewitt and Gundry (14) suggests that Mo is required by higher plants only for the enzyme nitrate reductase. Although plants contain relatively large amounts of this enzyme, Mo may represent (within orders of magnitude) a lower concentration limit for elemental function in higher plants. The lowest Cr levels found in the experiments presented in this paper are about 10$^4$ times higher than the levels suggested by Dimman and about 10$^4$ times higher than the levels suggested by Kliever.

The Cr levels in the purified nutrient solution and low Cr plant material were near the detection limits of the method used. Therefore, before lower Cr levels can be obtained, applicable analytical methods must be developed. Methods with low absolute detection limits include gas liquid chromatography of a halogenated Cr complex using either electron capture
(11) or mass spectrophotometric (25) detection and carbon furnace atomized atomic absorption (7). Although these methods have low absolute detection limits, they require very small samples and are not well adapted to analysis of plants. Therefore, the detection limits in terms of Cr concentrations in plants are similar to that of the method used in this study. The development of methods capable of determining Cr concentrations well below 1 ng Cr/I dry weight in nutrient solutions and 1 ng Cr/g in plant tissue may be required before further investigations of the essentiality of Cr for plants are justified.

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LITERATURE CITED