## Distribution of chromium in red kidney beans (Phaseolus vulgaris L.)

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Summary. Distribution of both  $Cr^{3+}$  and  $CrO_4^{2-}$  in bean shoots followed a markedly acropetal gradient. Chemical fractionation of radiochromium accumulated in the edible bean pods indicated the greatest association (70-75%) with ionic forms (extractable by weak mineral acids).

Chromium, which is present as a soil and water pollutant due to chromium wastes released from various industrial sources, and chromium-51, a gamma-emitting activation product released in controlled or accidental discharges from nuclear installations, could enter the human foodchain through rivers, groundwater and irrigated soil. Conflicting reports on the differential absorption and uptake of trivalent chromium ions  $(Cr^{3+})$  and hexavalent chromium ions  $(CrO_4^{2-})$  have appeared during the last few years<sup>1,2</sup>; but hardly any quantitative data are available on the distribution of the 2 forms of chromium in various plant organs and the metabolic fate of root-absorbed chromium in the edible parts of the plant. These aspects have been examined in the present investigation, using <sup>51</sup>Cr as a tracer for stable chromium.

Materials and methods. Red kidney beans (Phaseolus vulgaris L.) were germinated in quartz sand and then grown in 1 l of <sup>51</sup>Cr labelled nutrient solution. The nutrient solution contained K<sup>+</sup>, 3.0; Ca<sup>2+</sup>, 8.0; Mg<sup>2+</sup>, 3.0; NO<sub>3</sub><sup>-</sup>, 10.0; SO<sub>4</sub><sup>2-</sup>, 3.0; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.0; mequivalents/l together with micronutrients Fe, Mn, Cu, Zn, B and Mo. <sup>51</sup>CrCl<sub>3</sub> and Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub>, each having a sp. act. of 50 mCi/mg Cr, constituted separate treatments and were added at an activity level of 10  $\mu$ Ci <sup>51</sup>Cr/1 (equivalent to 0.11 ng <sup>51</sup>Cr plus 200 ng stable Cr/l) to 5 replicate jars. The initial pH of the solution was adjusted to 6.0. A single bean plant was grown in each jar. The experiment was conducted in a growth room where the temperature was maintained at  $23 \pm 1$  °C, the relative humidity  $65 \pm 2\%$  and the plants were illuminated daily for 12-h periods at 1300  $\mu$ W  $\cdot$  cm<sup>-2</sup> measured at 10 cm above the top of the jars. The transpiration losses from the solution were made up daily with distilled water. The plants were harvested and separated into different tissues when the bean pods (edible tissue/part of the plant) had fully developed (6 weeks after germination). The procedure for chemical fractionation of the bean pods has been described in detail in our earlier publications<sup>3,4</sup> and is

Table 1. Distribution of  ${}^{51}Cr$  in different tissues of bean plants. Age of plants: 42 days; duration of  ${}^{51}Cr$  treatment: 32 days

Plant part	Radionuclide content (cpm/g dry wt)*		
	$^{51}Cr^{3+}$	<sup>51</sup> CrO <sub>4</sub> <sup>2</sup>	
Roots	$235 \times 10^{3}$	$201 \times 10^{3}$	
Stem	$70 \pm 5$	$51 \pm 16$	
Primary leaves	$3710 \pm 151$	$1871 \pm 282$	
Trifoliate-1	$516 \pm 48$	$583 \pm 137$	
Trifoliate-2	$323 \pm 59$	$436 \pm 110$	
Trifoliate-3	189± 19	$298 \pm 84$	
Trifoliate-4	111± 4	$139 \pm 14$	
Axillary leaves + flowers	$93 \pm 24$	$181 \pm 76$	
Pods	16± 4	$10 \pm 2$	

\* Mean values ± SE.

indicated briefly under 'results and discussion'. The final samples were assayed by gamma-ray spectrometry<sup>3,4</sup> using the photopeak of 325 keV for quantitative estimation of  ${}^{51}$ Cr.

Results and discussion. Data on the distribution of  ${}^{51}Cr^{3+}$ and  ${}^{51}CrO_4{}^{2-}$  in the various tissues of bean plants grown to pod formation are shown in table 1. Data indicate massive accumulation of both forms of chromium in the roots under conditions of nutrient culture experiments. Among the aerial tissues, the <sup>51</sup>Cr content was found to be the highest in the primary leaves and decreased with each succeeding trifoliate leaf up the stem from the base to the apex; very much reduced amounts were present in the stems and pods. The observed maximum concentration of <sup>51</sup>Cr in the oldest leaves with a progressive reduction in younger tissues is indicative of an acropetal gradient in the distribution of Cr in bean plants. These results also indicate a significant lack of redistribution of chromium in the bean plants as evidenced by the marked concentration gradient of chromium of more than 2 orders of magnitude between the oldest primary leaves and the pods. The distribution pattern of chromium was similar to that of manganese but differed from that of other transition elements like iron, cobalt and zinc which are more or less uniformly distributed in the plant<sup>3</sup>.

Chemical characterization of chromium in the bean pods was undertaken to determine the metabolic fate of rootabsorbed chromium. Pods (2 g) immediately after harvest were successively extracted as indicated in table 2. Data in table 2 indicate that about 11% of <sup>51</sup>Cr<sup>3+</sup> was present in the ethanol fraction (lipids including lipophyllic pigments, free amino acids and amino sugars). The association of <sup>51</sup>Cr<sup>3+</sup> was highest (74.9%) in the extracts of weak mineral acids which comprised ionic forms including salts of organic acids, phosphates, carbonates and some protein bound forms. Further 6.7% of  ${}^{51}Cr^{3+}$  was present in pectates, proteins and polysaccharides (acetone plus soda fractions) and 7.8% in cellulose and lignin (residue). In the case of  ${}^{51}\text{CrO}_4{}^{2-}$ , 13.5% was in the ethanol fraction, 70.7% was in the acid fractions, 12.0% was associated with pectates, proteins and polysaccharides and 3.8% with cellulose and lignin. Neither  ${}^{51}Cr^{3+}$  nor  ${}^{51}CrO_4^{2-}$  was associated with the nucleic acid fraction. Previous studies on the distribution of chromium in the aerial parts of Leptospermum scoparium, a chromium accumulator plant growing on serpentine soils<sup>5</sup>, and in the leaves of cauliflower plants grown in <sup>51</sup>Crlabelled nutrient solution<sup>6</sup>, have reported the predominant association of chromium with ionic complexes, notably the

Extractants	Fraction containing	Distribution of <sup>51</sup> Cr (% of quantity in pods)		
		<sup>51</sup> Cr <sup>3+</sup>	<sup>51</sup> CrO <sub>4</sub> <sup>2-</sup>	
95% ethanol	Lipids including lipophyllic pigments, free amino acids and amino sugars	$10.62 \pm 0.06$	13.47±0.01	
0.2 M HCl				
a) Supernatant	Ionic forms including salts of organic acids, phosphates, carbonates and some protein bound forms	$69.66 \pm 0.05$	$64.63\pm0.03$	
b) Precipitate with acetone	Proteins and pectates	$2.35 \pm 0.01$	$6.42\pm0.01$	
0.5 M HClO4				
a) Supernatant	Remaining ionic forms	$5.24 \pm 0.15$	$6.04\pm0.06$	
b) Precipitate with acetone	Nucleic acids	ND	ND	
2 M NaOH	Remaining proteins and polysaccharides	$4.27 \pm 0.03$	$5.57 \pm 0.02$	
Residue	Cellulose and lignin	$7.86 \pm 0.25$	$3.87 \pm 0.23$	

The error term is the mean of triplicates. ND, not detected.

trioxalotochromate  $(Cr^{3+})$  form. The present findings on the distribution of chromium in bean plants and in the major biochemical moieties present in bean pods are of environmental significance since they are indicative of the chemical forms in which chromium occurs in the edible parts of leguminous crops grown in contaminated soils.

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- R. E. Eckert and C. Blincoe, J. Range Mgmt 23, 367 (1970). C. Myttenaere and J. M. Mousny, Plant Soil 41, 65 (1974). T. J. D'Souza and K. B. Mistry, Envir. exp. Bot. 19, 193 (1979). T. J. D'Souza and K. B. Mistry, Envir. exp. Bot. 20, 409 (1980). G. L. Lyon, P. J. Peterson and R. R. Brooks, Planta 88, 282 (1969). 5
- 6 M. Lahouti and P.J. Peterson, J. Sci. Fd Agric. 30, 136 (1979).