Transfer of calcium-45 and strontium-90 from medium to plant and their translocation in micropropagated potato

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Abstract. The uptake and translocation of calcium-45 and strontium-90 were studied in potato (*Solanum tuberosum*) cv. Sierra micropropagated plantlets and cv. Norland microtubers. The absorption of Ca-45 or Sr-90 by plantlets was not significant as the concentrations of either $CaCl_2$ or $SrCl_2$ were increased in the treatment solution. However, the percentage uptake of Ca-45 decreased with increasing concentration of these salts. Uptake by roots, stems, petioles, and leaves differed significantly ($p < 0.05$) regardless of salt concentration. When shoot-tips were immersed in the treatment solutions containing various concentrations of these salts for 161 h while the plantlets were held in a vertically inverted position, considerable amounts of radiolabel were still translocated towards the roots. The Ca-45/Sr-90 ratio assumed a wide range of values as equal amounts of each radioisotope were fed either through root- or tip-immersion. A differential uptake and translocation between the isotopes occurred for the plantlets independent of feeding mechanism. A drop of Ca-45 or Sr-90 (18.5 kBq) onto the periderm of microtubers was restricted in movement to the periderm with virtually no penetration into the inner cortical or medulary tissues. The diffusion coefficient of Sr-90 on the periderm of Norland microtubers was estimated at 6.5×10^{-8} cm^2/s.

1. INTRODUCTION

Tissue culture used in combination with the radiotracer techniques provides an ideal system to study transfer of radioisotopes from the culture medium into roots and aerial parts of plants. Micropropagated plantlets grown on defined nutrient medium not only allow the minimum quantity of a radio-labeled nutrient to be applied but the entire plantlet can be harvested, and all of its parts counted, decreasing sampling errors. The system reduces the cost of research without compromising on procedural protocol compared with nutrient transport studies involving field-grown crops. The time to complete a certain number of replications is also greatly reduced when tissue-cultured plantlets are used instead of field-grown crops. Tissue-cultured plantlets are clones that may eliminate much of the biological variability usually encountered in radioactive uptake studies with living organisms. Thus, the present research was undertaken in the hope that such a system could provide new information on the transfer of radioisotopes from the substrate into potato plants and tubers. Potato (*Solanum tuberosum*) was chosen as a test plant because of its ease of growth in culture, its importance as a major world food crop, and the relatively large surface area its tubers present to a substrate contaminated with radioisotopes.

Calcium (Ca) and strontium (Sr) belong to the second group of elements in the periodic table but the former plays an important role in plant growth and development [1]. Sr-90 has the potential of contaminating foodstuffs via uptake by roots or directly into other plant tissues. This poses a problem because Sr-90 concentrates in bones on ingestion [2-3]. Ca-45 and Sr-90 accumulation in potato tubers have been reported to be diffusion-controlled and are apparently independent of plant root absorption [4]. The literature on the uptake and translocation of Sr-90 by potato plants is rare. The radioecology of Sr-90 has been reviewed by several authors [5-6], and the release, transport, and distribution of Sr-90 after the Chernobyl disaster have been adequately described [7].

The objectives of this study were: (a) to examine the uptake and translocation of Ca-45 and Sr-90 by potato plantlets as a function of the concentration of the chloride salts of each radioisotope in the treatment solution; (b) to determine whether differential uptake occurs between these two radioisotopes when plantlet roots and shoot-tips are treated with equal amounts of each; (c) to study the movement of applied radioisotopes into microtuber tissues.

2. MATERIALS AND METHOD

Potato cvs. Norland and Sierra plantlets were micropropagated from 1-cm-long single-node cuttings with one lateral bud and subtending leaf. Culture tubes (25 X 150 mm) contained 10 ml of MS basal salt medium prepared according to conventional procedure [8]. Cultures were incubated in a growth chamber at 25°C and 40 µmol m^{-2} s^{-1} photon flux density (cool white fluorescent) with 16/8 h (day/night) cycle. The mean ± SD height and fresh weight of 1-month-old Sierra plantlets used in these experiments was 10.7 ± 0.6 cm and 3.9 ± 0.4 g, respectively. Microtubers of cv. Norland were induced using layered plantlets in high-sucrose MS medium at 15°C under 40 µmol m^{-2} s^{-1} photon flux density with 8/16 h cycle [9]. The average size (diameter) and weight of microtubers used in these experiments was 1.5 cm and 0.7 g, respectively.

Aqueous solutions of $Ca^{45}Cl_2$ and $Sr^{90}Cl_2$ (> 99% radiochemical purity) were purchased from Amersham Pharmacia Biotech, Inc., Quebec, Canada, and Isotope Products Laboratory, Burbank, California, respectively. Plantlets or microtubers were transferred to the radioisotope laboratory for the application of Ca-45 and Sr-90. The growth medium was carefully removed from roots using running water, and the plantlets were transferred to aqueous solutions of 0, 3, 9, or 18 mM $CaCl_2$ or $SrCl_2$ for uptake studies. These were of 42 and 161 h duration. Some treated plantlets were reserved for autoradiography. Others were divided into roots, stems, petioles and leaves and used for scintillation counting. Root samples were prepared following rinsing of roots 3 times in distilled water to remove loosely bound radionuclides.

The effect of plantlet orientation on uptake and translocation of Ca-45 and Sr-90 was studied by vertically inverting the plantlets while immersing the apex of the topmost true leaf (shoot-tip) into radio-labeled solutions. The semi-solid MS medium was not removed from the roots for this experiment. Following immersion, the soot-tips were carefully removed to avoid cross-contamination and the remaining plant organs were used for autoradiography or scintillation counting.

One drop (10 µl) of $Ca^{45}Cl_2$ or $Sr^{90}Cl_2$ (18.5 kBq activity) was applied onto the periderm of 3 intact Norland microtubers. After an interval of 96 h, slices were made starting at 1 mm from the application site, and these were used for autoradiography or scintillation counting.

Root samples for scintillation counting were shaken vigorously during rinsing for 2-3 min. Tissues were digested in an oven at 60°C for 2 h with 1 ml of 33% hydrogen peroxide and 66% perchloric acid (1:2 v/v). The samples were counted in a liquid scintillation spectrometer (Model LKB 1219 Rackbeta, Wallac, Turku, Finland). Sierra plantlets were also used for autoradiography using X-ray films.

Each experiment was replicated 3 to 4 times, and the mean uptake activities (± standard error of the mean (SE)) were calculated and reported. The data was analyzed using one way ANOVA provided by a statistical software package [10].

3. RESULTS AND DISCUSSION

The percentage uptake of Ca-45 decreased for all plant parts as the concentration of either $SrCl_2$ or $CaCl_2$ increased in the treatment solution. Since both Ca and Sr salts produced similar results, only the effect of one salt is shown (Fig 1a). There was a significant difference ($p < 0.05$) between the Ca-45 activities in roots, stems, petioles, and leaves regardless of salt concentration after 42 h of exposure. Roots absorbed the greatest amount and the petioles the least Ca-45. In contrast, the root percentage uptake of Sr-90 increased as the treatment solution concentration increased from zero to 3 mM but decreased at higher salt concentrations (Fig.1b). As seen with Ca-45 uptake, there was a significant ($p < 0.05$) difference between the Sr-90 activities in plantlet parts; the roots had the greatest and the petioles the least Sr-90. However, Ca-45 or Sr-90 activities in roots, stems, petioles, and leaves were not significantly different whether $SrCl_2$ or $CaCl_2$ concentrations were varied. As substrate concentrations increased, the ratio of Ca-45/Sr-90 after 42 h decreased from > 1.0 to << 1.0 for all plant parts. The Ca-45/Sr-90 ratio in all plant parts was > 1.0 for root-fed plantlets only in treatment solutions with zero Ca and Sr chloride salts. Interestingly, the Ca-45/Sr-90 ratio was consistently greater when the $CaCl_2$ concentration was increased compared with increased $SrCl_2$ in the treatment solution (Fig. 2a) although the difference was not significant at $p \leq 0.05$. The variation in the ratio may indicate that cv. Sierra discriminated in uptake and translocation between Ca-45 and Sr-90. When equal quantity of either labeled Ca-45 or Sr-90 (18.5 kBq) was added to the treatment solution, mixing of each radioisotope with its

unlabelled species occurred as the concentration of each salt increased. Plantlet uptake of unlabelled Ca correspondingly increased in competition with Ca-45, and the Ca-45/Sr-90 ratio decreased to a fraction because of higher uptake of Sr-90 compared with Ca-45 in presence of either salt.

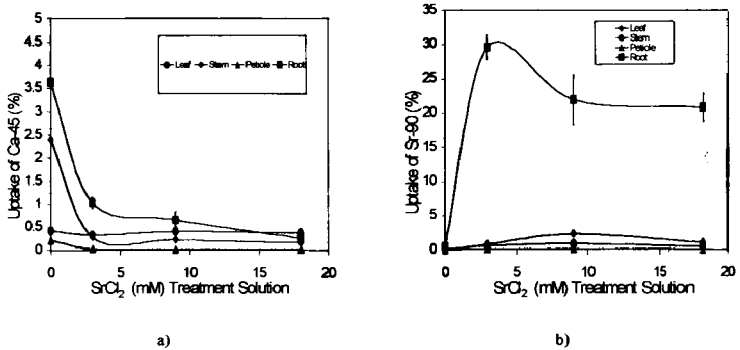

Figure 1: Percentage uptake of a) Ca-45 and b) Sr-90 as a function of $SrCl_2$ concentration by Sierra plantlets. The error bars indicate SE.

Autoradiography supported the counting results; autoradiographs showed higher absorption of Sr-90 compared with Ca-45 as the concentrations of either $CaCl_2$ or $SrCl_2$ was increased.

Shoot-tip feeding and plantlet inversion affected the Ca-45/Sr-90 ratios. Ratios reached a maximum at 3 mM for $CaCl_2$ and decreased at higher concentrations for leaves, stems, and roots. Ratios reached a maximum at 18 mM for $SrCl_2$ in all plant parts; > 1 for leaves and stems, but < 1 for roots at this concentration (Fig. 2b).

The percentage uptake and translocation of Ca-45 into leaves and stems was significantly lower ($p < 0.05$) in shoot-tip compared with root-fed plantlets in $SrCl_2$ treatment solutions. The percentage uptake and translocation of Sr-90 into leaves was significantly lower ($p < 0.05$) in shoot-tip compared with root-fed plantlets in both $CaCl_2$ and $SrCl_2$ treatment solutions.

The autoradiographic images of inverted plantlets showed no Ca-45 uptake and weak Sr-90 activity as the concentration of $CaCl_2$ increased. However, faint images of the plantlets could be seen for Ca-45 activity and strong uptake and translocation of Sr-90 occurred when $SrCl_2$ concentrations of the treatment solutions were increased. The stems showed higher activity levels than other organs. The autoradiograph images supported the counting results. Although activities in the roots were relatively small, considerable translocation occurred from the shoot-tips down towards the roots. Ca-45 and Sr-90 activities were significantly higher ($p < 0.03$) for root-fed than inverted plantlets in all plant parts in either salt except for Ca-45 activities in leaves and stems in 3 and 9 mM concentrations of $SrCl_2$. This showed that the rate of translocation in root-fed was normally higher than for inverted plantlets.

The percent uptake of Ca-45 by the leaves whose tips were immersed was the highest at zero concentration and decreased markedly as $CaCl_2$ concentration increased in the treatment solutions (Fig. 3a). Similar results were obtained when the concentrations of $SrCl_2$ in the test solutions were increased. The percent uptake of Sr-90 was considerably greater than that of Ca-45 at all $CaCl_2$ (Fig. 3b) and $SrCl_2$ (not shown) concentrations. The Ca-45 transport rate along the periderm was negligible in Norland microtubers. The entire applied activity of Ca-45 was essentially at the site of application after 96 h. The movement of Sr-90 was greater than that of Ca-45 but still low and localized to the periderm (Fig. 4). Less than 12% of the applied Sr-90 activity was detected at 0.1 cm from the application site and at 0.5 cm no activity was detected.

There was almost no penetration of Ca-45 or Sr-90 into the interior cortical tissue of the microtuber. Autoradiographic images confirmed the liquid scintillation counting results for slices of the periderm and cortical tissue as a function of distance from the application site.

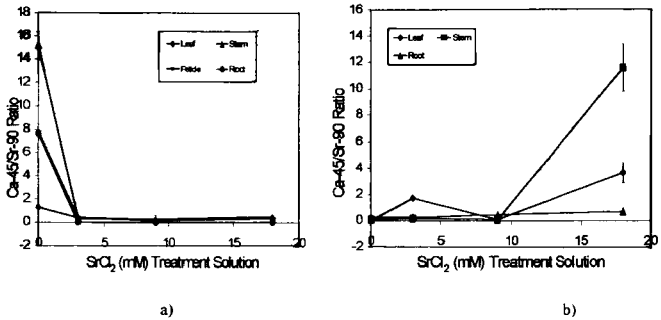

Figure 2: Ca-45/Sr-90 ratio versus $SrCl_2$ molarity a) root-fed and b) inverted and leaf-tip immersed for Sierra plantlets. The error bars indicate SE.

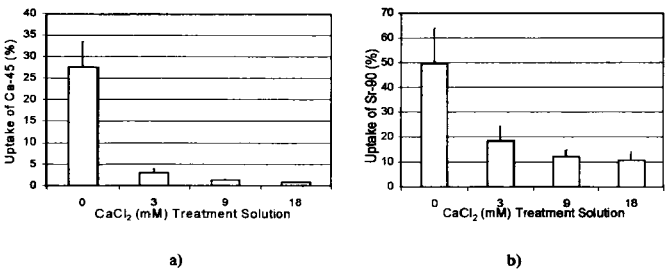

Figure 3: a) Ca-45 and b) Sr-90 uptake by non-immersed portion of leaves whose tips were immersed in treatment solutions for Sierra plantlets versus $CaCl_2$ molarity. The error bars indicate SE.

The diffusion coefficient, D, was determined for Sr-90 movement through the periderm and cortical tissue by using the Fick's second law of diffusion as shown in equation (1),

$$\partial C/\partial t = D\,\partial^2 C/\partial x^2 \qquad (1)$$

where **C** = the concentration of the diffusing substance, x = the distance traveled, and t = the time of diffusion. A solution of equation (1) can be written as [6],

$$D = (x_2^2 - x_1^2)/(4t)\ln(C_1/C_2) \qquad (2)$$

where C_1 and C_2 were determined at two arbitrary points x_1 and x_2, respectively. D was calculated by using

equation (2), and its average value for Sr-90 on potato periderm was obtained as 6.5×10^{-8} cm^2/s. For Ca-45 the D-value was much smaller and a high degree of uncertainty was therefore involved in its calculation and has not been quoted here. The diffusion coefficient of Sr-90 for Norland periderm was much lower than those reported for certain soils which varied from $0.6\text{-}8.1 \times 10^{-7}$ cm^2/s [6].

The periderm is known to act as a protective layer to prevent water loss from the tuber and to impede the microbial infestations by soil pathogens [11]. The periderm is suberized with hydrophobic properties similar

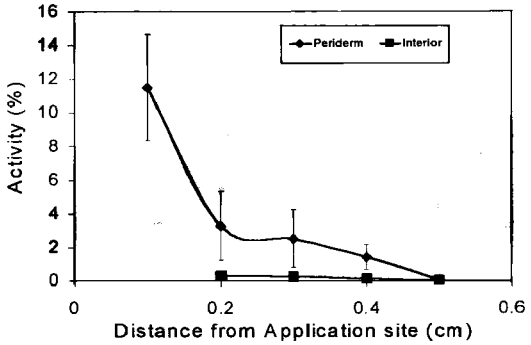

Figure 4: Migration of Sr-90 on periderm and into potato tuber of cv. Norland. The error bars indicate SE.

to wax. Our calculations indicate a very low value of D for Sr-90 and is consistent with the physical characteristics of the structure of periderm. The periderm (skin) of potato tubers is expected to prevent any significant amount of uptake of Sr-90 or Ca-45 directly via diffusion from contaminated substrates or soil solutions. These results indicate that contaminated potatoes at the level used in these experiments can be used as animal feed or even food if they are peeled since negligible amounts of these radioisotopes are transferred into the inner tissues of potato.

4. CONCLUSIONS

The uptake and translocation of Ca-45 and Sr-90 by cv. Sierra potato plantlets were significantly ($p < 0.05$) different in different plant organs irrespective of the concentrations of $CaCl_2$ or $SrCl_2$ in the treatment solutions. The Ca-45/Sr-90 ratio suggested that cv. Sierra may discriminate between the uptake of radiocalcium and radiostrontium. The vertically inverted plantlets absorbed and translocated considerable amounts of Ca-45 and Sr-90. The movement of Ca-45 in the periderm was negligible and Sr-90 was extremely slow when applied to the periderm with no appreciable penetration into the cortical tissue. The autoradiographic imagings supported the counting results. Thus, potatoes may be peeled before their use as food or feed if the substrate is lightly contaminated by radioactive fallout or accident.

Acknowledgments

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References

[1] Palta, P, J. HortScience, **31** (1996) 51-57.

[2] Nielsen, O.J. A literature review on radioactivity transfer to plants and soil, Riso Report-450, Riso National Laboratory, DK-4000, Roskilde, Denmark, 1981, pp. 78.

[3] Yera, S.T., Vallejo, V.R., Valcke, E., Colle, C, Forstel, H., Millan, R. and Jouglet, H. J. Environ. Radioactivity, **45** (1999) 191-217.

[4] Melnikova, M.K. and Baranova, Z.A. On the mechanism of Ca and Sr uptake (on the example of radiocalcium and radiostrontium) by potato tubers, Radiological Concentration Processes, Proceedings of an International Symposium, Stockholm, April 1966, B. Aberg and B.P. Hungate Eds. (Pergamon Press, New York, 1966) pp. 1040.

[5] Kulikov, V.N. and Molchanova, V.I. Continentel Radioecology, Soil and Freshwater Ecosystems, (Plenum Press, Nauka Publishers, Moscow, 1982) pp.174.

[6] Klechkovskii, M.V., Polikarpov, G.G. and Aleksakhin, M.R. Eds. Radioecology (John Wiley & Sons, New York,1973, A Halsted Press Book, Israel Program for Scientific Translations) pp.370. Translated from Russian by Kaner, N., Mills, H and translation edited by Greenberg, D.

[7] Warner F. and Harrison, M.R. Eds. Radioecology after Chernobyl, Biogeochemical Pathways of Artificial Radionuclides, (John Wiley & Sons, New York, 1993) pp.367.

[8] Murashige, T. and Skoog F. Physiologia Plantarum, **15** (1962) 473-497.

[9] Leclerc, Y., Donnelly, D.J. and Seabrook, J.E.A. Plant Cell Tiss. Org. Cult. **37** (1994) 113-120.

[10] SigmaStat, Jandel Scientific MmbH, Schimmelbuschstrasse 25, 40699 Erkrath, Germany,

[11] Peterson, R.L., Barker, W.G, and Howarth, J. M. Development and structure of tubers, Potato Physiology (Academic Press, Inc. New York, 1985) pp.123-147.