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Investigating the in vivo calcium transport path to developing potato tuber using ^{45}Ca: a new concept in potato tuber calcium nutrition

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Calcium is believed to be transported with water in the xylem. Consistent with this proposal, low-transpiring organs such as potato *Solanum tuberosum* tubers are known to suffer from calcium deficiency. Although roots on tubers and stolons have been shown to supply water to tubers, there is no direct evidence for the calcium transport pathway to tubers. Both a xylem and a phloem transport pathway have been suggested. We investigated in vivo calcium transport to developing potato, cv. Dark Red Norland and cv. Russet Burbank, tubers using ^{45}Ca in a controlled environment facility. Whole plant split pot experiments allowed the placement of ^{45}Ca either in the main (basal) root or the tuber and stolon areas of the pot. The results showed that ^{45}Ca was transported to the shoot with the transpiration stream from both areas but was not re-translocated to tubers or the main (basal) root system even 57 days after ^{45}Ca application. Radioactivity could only be detected in the tuber when ^{45}Ca was fed to the stolon and tuber area. When ^{45}Ca was fed to specific tubers, radioactivity was detected in the aerial shoot; however, no activity was detected in other tubers or the main (basal) roots. In another set of experiments, roots on a stolon near a tuber were precisely fed ^{45}Ca and Safranin O. The radioactive signal exactly overlapped the water transport pathway in the tuber marked with Safranin O dye, suggesting that water and calcium can be simultaneously transported from stolon roots to the tuber. No transport of ^{45}Ca across the tuber periderm was detected 8 days after ^{45}Ca was applied to the tuber periderm. This indicated that no significant transport of calcium occurs from the soil across the periderm. Our results provide evidence that: (1) calcium is not re-translocated via the phloem from the aerial shoot tubers and main (basal) roots; (2) the main root system does not supply calcium to the tuber; (3) calcium is not transported across the periderm to the interior tuber tissue; (4) calcium is transported to the tuber via the xylem along with water, and the roots on the stolon associated with the tuber supply water and calcium to the developing tuber; and (5) transpirational demand is a significant determinant of calcium distribution within the plant.

Introduction

Calcium is an important plant nutrient. The role of calcium in the plant life cycle is well established and has been reviewed extensively (Hanson 1984, Bush 1995, Marschner 1995, White and Broadley 2003, Hirschi 2004). Calcium ion binding pectins in the middle lamella are known to be essential for strengthening of the cell wall (Marschner 1995). Calcium bound to the outer surface of the plasma membrane maintains membrane stability and cell integrity (Hanson 1984, Palta 1996, Hirschi 2004). In addition, calcium acts as a second messenger, coupling stimuli such as stress, light and plant hormones to

a response (Bush 1995, Sanders et al. 1999, Ng and McAinsh 2003).

While calcium has a prominent role in plant growth and development, gaps exist in our understanding of how calcium is transported within the plant (White 2001, White and Broadley 2003). Long distance calcium transport from root to shoot is believed to occur in the xylem tissue (White 2001, White and Broadley 2003). From an in-depth analysis of the literature and measurement of the calcium fluxes, White (2001) presented compelling evidence for the xylem calcium transport pathway to the shoot. Furthermore, White (2001) presented evidence for an apoplastic calcium transport pathway. The movement of calcium from root to shoot was believed to occur largely via mass flow with the transpiration stream (Kirkby and Pilbeam 1984). Partitioning of xylem calcium to adaxial and abaxial epidermal cells suggests that a mass flow model of calcium movement is not adequate (Atkinson 1991). Furthermore, the concentration of calcium in the xylem sap suggests that long distance calcium transport in xylem is not primarily due to mass flow (Atkinson et al. 1992).

Calcium is reported to be phloem immobile (Ziegler 1975), suggesting that it does not redistribute in plants. Re-translocation of mineral nutrients is a normal feature throughout the life of a plant (Marschner 1995). Mineral nutrients such as nitrogen, phosphorus and potassium are considered to be mobile, as they readily move through phloem conduits, being re-translocated during periods of deficiency. Mineral nutrients such as calcium, sulfur, iron, boron and copper are generally considered to be phloem immobile, as they tend not to re-translocate from older portions of the plant when the element is deficient (Marschner 1995).

Potato, *Solanum tuberosum*, provides a very useful system in which translocation and re-translocation of calcium can be studied. While both tubers and aboveground leaves and stems are botanically part of the shoot system, the aerial portion of the shoot is exposed to a markedly different environment than the tubers. Tubers are typically surrounded by moist soil and have low transpiration rates. As a result, tubers are prone to calcium deficiency, resulting in various physiologic disorders (Bangerth 1979, Palta 1996).

Evidence has been presented for both phloem and xylem transport of calcium to tubers. Phloem transport of calcium to potato tubers has been proposed or implicated by some researchers. Nelson et al. (1990) were able to localize radioactive strontium (as a calcium analog) to phloem tissue; however, they were not able to conclude that the strontium had entered the sieve tubes of the potato stem. Davies and Millard (1985) claimed to localize ^{45}Ca to phloem tissue in potato tubers, and used the calcium/sucrose ratio in the phloem sap to calculate that calcium transport in the phloem may account for a significant proportion of the potato tuber calcium. Oparka and Davies (1988) used microscopic techniques to show that calcium is found in companion cells and phloem parenchyma; however, they also localized calcium in sieve elements. These studies are in contrast with early studies by Baker and Moorby (1969), who performed double-labeling experiments with radioactive phosphorus and strontium, and studied accumulation following soil applications. Radioactive phosphorus readily moved from the leaves to tubers, while strontium did not accumulate in tubers. The authors concluded that strontium, the calcium analog, was immobile in the phloem and that phosphorus could be redistributed through the phloem from the leaves to the tubers (Baker and Moorby 1969).

Evidence has been presented for water uptake by tubers in response to transpiration. Wiersum (1966) compared tubers and stolons developing in moist sand overlying the main root system grown in a soil mix for their ability to take up a water-soluble dye and ^{45}Ca given to the main (basal) root system. He concluded that tubers and stolons surrounded by moist sand will not accumulate ^{45}Ca or dye given to the main roots. When tubers and stolons were exposed to dry air for a period of 25 days, the dye and ^{45}Ca were transported to the tubers and stolons. From the results of these studies, it was suggested that calcium was transported via xylem to the transpiring organs. Kratzke and Palta (1985) used dye uptake experiments with field-grown intact potato plants to show that only roots closely associated with tubers supply water to tubers. Additionally, the calcium found in various portions of the potato shoot, aerial foliage and tubers was shown to be derived from different root areas on the plant (Kratzke and Palta 1986). These studies also showed that tuber calcium concentration was increased only when calcium was supplied in the tuber and stolon area of the soil, suggesting that calcium, along with water, may be transported to the tubers from the roots closely associated with the tuber. Thus, while circumstantial evidence exists, no direct evidence for the calcium transport pathway to tubers has been presented.

This study was designed to determine the path of calcium transport to the potato tuber and determine if redistribution of calcium to tubers from other parts of the plant occurs. To that end, we performed a series of precise ^{45}Ca-feeding experiments in the presence and absence of a water-soluble dye, and monitored calcium accumulation within various potato plant organs. Radioactivity could be detected in the aerial shoot when ^{45}Ca was fed to any root on the potato plant. Radioactivity could only be detected in the tuber when ^{45}Ca was fed to stolon roots

near a tuber. These results indicate that calcium is not redistributed through the phloem, and that roots associated with the tuber can supply water and calcium to it.

Materials and methods

Split pot ^{45}Ca-feeding experiments

Experiments were designed to investigate the influence of ^{45}Ca feeding of specific areas of the root zone on the accumulation of ^{45}Ca in leaves, roots and non-periderm tuber tissue. Potato plants, *Solanum tuberosum*, cv. Russet Burbank propagated in vitro were planted in coarse sand in 15-cm-diameter (2.5-l) pots and grown in a growth chamber for 26 days under a day/night cycle of 14 : 10 h and 20/15°C. The light level was 350 μmol m^{-2} s^{-1}. The intact plants with initiated stolons were removed and placed into divided 30-cm (18.9-l) pots (Lerio Corp., Mobile, AL). Growing plants in sand up to this stage allowed easy separation of main (basal) roots from stolons. The pots were separated horizontally into two sections by a 76.2-μm-thick polyethylene bag. A slit of about 0.5 cm was cut in the center of the bag, and the plant was threaded through until the main basal roots were in the bottom half of the pot, and the stolons and associated roots were in the top half of the pot (Fig. 1). The bottom and top halves of the pot were filled with Pro Mix 'HP' soil (Premier Horticulture, Red Hill, PA). The top half was watered directly from the surface and bottom with a flexible polyvinyl chloride tube placed along the inside of the pot. The lower half had drainage holes at the bottom of the pot, and a drainage hole for the top half was cut on one side of the pot. Plants were returned to the growth chamber and kept under the same environmental conditions for 36 days.

Twenty-one days prior to treatment at the start of the experiment, plants were moved to the University of Wisconsin-Madison Biotron (Madison, WI) controlled environment facility for biological research. In the Biotron, plants were grown under a day/night cycle of 14 : 10 h and 20 : 15°C. The relative humidity was 75% and light level was 350 μmol m^{-2} s^{-1}.

^{45}Ca, 3700 MBq, as $^{45}CaCl_2$ (Perkin Elmer Life Sciences, Inc., Boston, MA) was given in solution to four plants in the upper or tuber portion of the pot and to three plants in the lower portion or main (basal) root area of the pot. The upper portion of the pot was treated by evenly distributing a total volume of 500 ml over the soil surface. The lower portion was treated by placing a total volume of 375 ml through the polyvinyl chloride tube that was used to water the lower portion of the pot. One plant received no radioactive label.

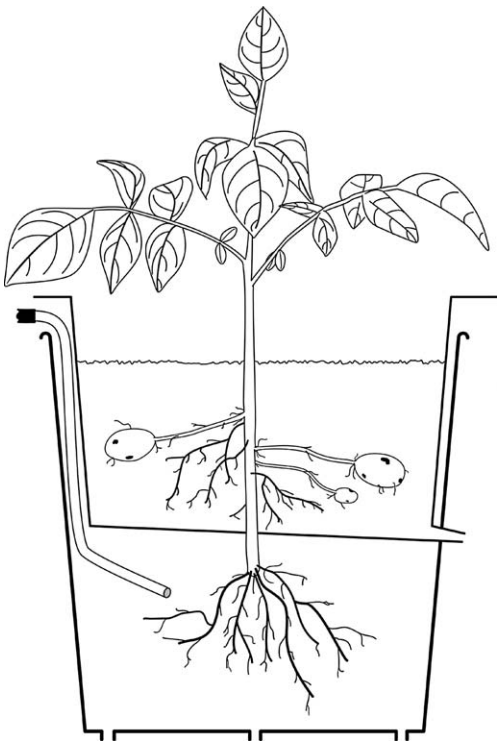

Fig. 1. A line drawing of the split pot system for precisely placing ^{45}Ca either in the main (basal) root area or in the stolon/tuber area. The main root system is depicted in the lower portion of the pot. The tuber area is depicted in the upper portion of the pot. In the tuber area, roots arise from the primary stem and stolons (attached to tubers). A drain hole was fashioned in both the upper and lower portions of the pot. A 1.88-cm-diameter Tygon tube (Fisher Scientific Co., Pittsburgh, PA) inserted into the lower pot allowed for watering and feeding of ^{45}Ca.

Plants were harvested 57 days after treatment. Material harvested included the main (basal) root from the lower portion of the pot, which was excised and rinsed well with water, four shoots with three or four fully expanded leaves, and all of the tubers from the upper portion of the pot. Tubers were peeled to remove the periderm, and the remaining tissue was diced. All tissues were dried before ashing at 550°C. Liquid scintillation cocktail, ScintSafe 30% (Fisher Scientific, Fair Lawn, NJ), was added prior to counting in the 0–252-keV range for 10 min with three cycle repeats in a Beckman LS 1801 (Beckman Instruments, Fullerton, CA) liquid scintillation counter. Data from the scintillation counter were converted to becquerels (Bq). Autoradiographs were made using Kodak BIOMAX film (Eastman Kodak Co., Rochester, NY) and processed following the manufacturer's recommendations.

A second set of experiments investigated ^{45}Ca feeding of specific areas of the root zone using *Solanum tuberosum*, cv. Dark Red Norland, propagated in vitro. Plants were grown in 15-cm pots of coarse sand for 37 days

prior to transfer to the split pot system for 47 days under a day/night cycle of 14 : 10 h and 20 : 15°C. Plants were moved to the University of Wisconsin-Madison Biotron for 45 days prior to treatments, and grown under the conditions described above. Just prior to treatment, a stolon with a tuber from six plants was uncovered and placed in a polyethylene bag filled with moist sand. For four of the plants, 3700 MBq ^{45}Ca was applied to the sand-filled bag, while the other two plants with a bagged stolon/tuber served as controls. For four separate Dark Red Norland plants, 3700 MBq ^{45}Ca was applied to the main root portion of two pots, while the other two plants served as controls. Plants were harvested 25 days after treatment and processed as previously described.

Co-transport of ^{45}Ca and dye from stolon roots to tuber

Dark Red Norland and Russet Burbank potato plants, propagated in vitro, were grown in a greenhouse until several tubers were formed. Plants were carefully excavated to expose the tubers and stolon roots very near the tubers. Stolon roots near the tubers were placed into 30-ml Dilu-Vials (Fisher Scientific Co., Pittsburgh, PA) containing 20 ml of water and ^{45}Ca as $^{45}CaCl_2$. The activity varied from 9.25 MBq ml^{-1} to 17.02 MBq ml^{-1}, depending on the experiment. Stolon roots were treated for from 1 to 6 days, and water in the vials was replenished as necessary. For some experiments, the vials also contained 0.05% (w/v) Safranin O to trace the water pathway (Kratzke and Palta 1985).

Tissue including tubers and the main stem at soil level (depending on the experiment) was harvested and processed as previously described, with the exception that autoradiographs were made using Kodak X-OMAT film (Eastman Kodak Co., Rochester, NY). Tubers were peeled before processing for determination of non-periderm tuber radioactivity.

^{45}Ca transport across the periderm

Dark Red Norland potato plants were grown as previously described at the University of Wisconsin-Madison Biotron facility. Mature tubers were harvested and washed. Tubers were positioned with their apical portion on top of a Dilu-Vial (Fisher Scientific Co., Pittsburgh, PA) containing 25 ml of water treated with 30.34 MBq ml^{-1} ^{45}Ca as $^{45}CaCl_2$ for 25 h. Control tubers were arranged in the same manner, but with no ^{45}Ca added to the water. Tubers were placed on a bench top for 8 days before peeling and sampling of internal, non-periderm, tissue. The radioactivity of thoroughly washed tubers was determined as described above.

Autoradiographs were prepared by slicing intact tubers from the stolon to the apical end. Slices were wrapped in plastic and placed on Kodak X-OMAT film (Eastman Kodak Co., Rochester, NY) for 24 h. Film was processed following the manufacturer's recommendations.

Results

Split pot experiments

Split pot experiments allowed for placement of ^{45}Ca in specific portions of the root zone (Fig. 1). These included the area where tubers developed and the main (basal) root area where tubers did not develop. Three separate experiments were conducted to track the ^{45}Ca placed in either the main root area or the tuber/stolon area (Table 1, experiment 1). Substantial radioactivity was detected in aerial shoots and main roots when ^{45}Ca was administered to the main root of Russet Burbank plants (Table 1, experiment 1). No activity was found in the Russet Burbank tubers. This result was also observed in Dark Red Norland plants (Table 1, experiment 2). When ^{45}Ca was placed in the tuber area, radioactivity was detected in tubers and aerial shoots but not in the main roots (Table 1, experiment 1). Russet Burbank plants had nearly three times more radioactivity in the aerial shoots when ^{45}Ca was placed in the main (basal) root area as opposed to the tuber area (Table 1, experiment 1). Autoradiography revealed radioactivity distributed throughout the leaves and stem when ^{45}Ca was fed to either the main root system or the tuber area (Fig. 2, Table 1, experiment 1). This included fully expanded and younger leaves (Fig. 2).

Plants of both cultivars had detectable radioactivity in the main root system only when ^{45}Ca was fed to the main root area of the pot (Table 1, experiments 1 and 2). Only background levels of radioactivity were detected in the main roots when ^{45}Ca was placed in the area of the pot containing tubers (Table 1, experiments 1 and 2) or when ^{45}Ca was placed in the plastic bag containing stolons and tubers (Table 1, experiment 3).

Radioactivity was only detected in tubers when ^{45}Ca was placed in the tuber area of the pot in Russet Burbank split pot experiments (Table 1, experiment 1). We evaluated 60 tubers from Russet Burbank plants fed ^{45}Ca in the pot tuber area and observed a wide range of radioactivity levels in these tubers. Some tubers had only background activity, whereas others had 40 times more activity than background (0.23–29.50 Bq g^{-1} DW) (Table 1, experiment 1). Background radioactivity and less variability were observed in tubers when ^{45}Ca was administered to the Russet Burbank main root area (34 tubers evaluated) and in controls (four tubers evaluated).

Table 1. Influence of ^{45}Ca feeding to main roots or tuber and stolon roots on the accumulation of ^{45}Ca in aerial shoot, root and tuber tissue for individual plants. Plants were grown in a split pot system such that the main basal root system was isolated from the tuber area, which contained tubers, stolons, and any associated roots. For Russet Burbank, ^{45}Ca was placed in the tuber area or on the main basal roots. Radioactivity in leaves, main roots and tubers was measured 57 days after treatment. For Dark Red Norland, ^{45}Ca was placed in a plastic bag containing moist sand and an intact tuber with any associated roots on the stolon or tuber. Radioactivity in leaves, main roots and tubers was measured 25 days after treatment. [a]The radioactivity was measured in tubers not enclosed in the polyethylene bag. Two of the tubers in the bag rotted. The data from those two plants are not included in experiment 3.

		Distribution of radioactivity (Bq g^{-1} DW)					
					Tubers		
						Activity	
Experiment/Cultivar	^{45}Ca Placement location	Plant	Aerial shoot	Main root	Number of tubers	Mean	Range
Experiment 1	Main roots	1	244	51	10	0.55	0.32–1.07
Russet Burbank		2	179	153	13	0.53	0.28–1.28
		3	155	150	11	0.52	0.35–0.75
	Tuber area	1	87	3	13	2.52	0.48–15.68
		2	131	2	22	3.45	0.33–29.50
		3	143	2	14	0.65	0.23–2.02
		4	110	2	11	1.28	0.48–5.01
	Control (no ^{45}Ca)	1	1	4	4	0.67	0.53–0.77
Experiment 2	Main roots	1	596	359	12	0.72	0.45–1.05
Dark Red Norland		2	549	3762	7	1.20	0.87–1.83
	Control (no ^{45}Ca)	1	1	6	7	0.83	0.78–0.95
		2	2	3	13	1.22	0.78–2.23
Experiment 3	Stolon and tuber roots in	1	2024	5	7	0.83	0.78–0.92[a]
Dark Red Norland	plastic bag	2	1365	6	5	0.63	0.57–0.73
		3	49	11	6	1.60	1.10–2.57
		4	39	8	9	0.88	0.75–1.00
	Control (no ^{45}Ca)	1	2	10	7	0.92	0.78–1.05
		2	3	13	5	1.43	1.08–2.05

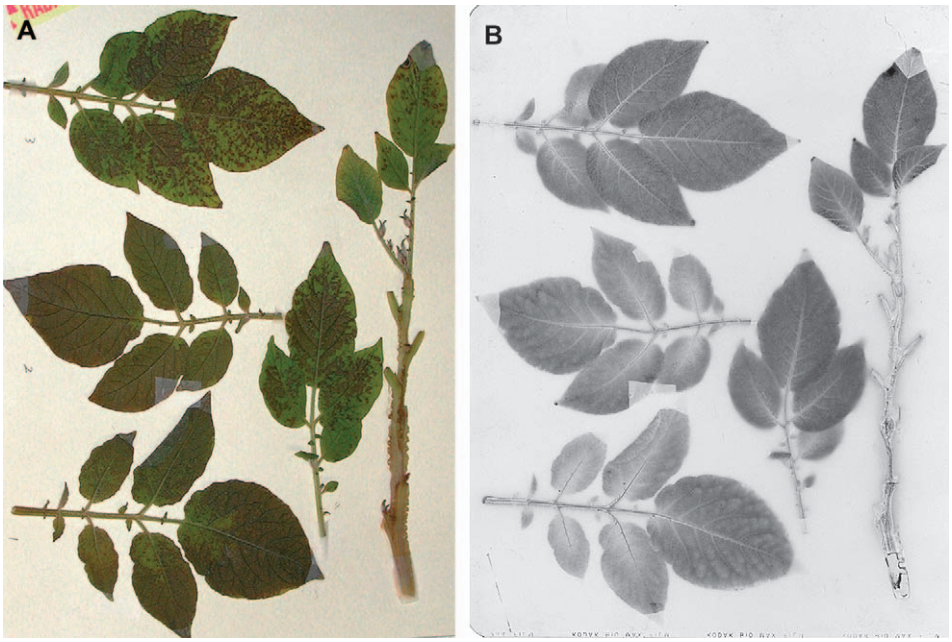

Fig. 2. Transport of ^{45}Ca from the main roots to the Russet Burbank potato plant aerial shoot. ^{45}Ca was applied to the main root area (lower portion of the pot as shown in Fig. 1). Autoradiographs were made following shoot harvest 57 days after ^{45}Ca treatment. (A) and (B) are the same shoot.

Only background radioactivity was found in Dark Red Norland tubers (Table 1, experiment 2). Dark Red Norland tubers from plants fed ^{45}Ca in the main (basal) root area of the pot had only background radioactivity levels. Likewise, when ^{45}Ca was placed in the polyethylene bag containing stolon and tuber, only background radioactivity was detected in every other tuber in the pot (Table I, experiment 3).

Co-transport of water-soluble dye and ^{45}Ca from stolon roots

Tubers and stolons, still attached to the main stem, were gently separated from the soil, and roots near tubers on the stolon were placed in distilled water (control) or water containing Safranin O and ^{45}Ca (Fig. 3A, B). Radioactivity was detected in both the main stem, at soil level, and in the tuber associated with fed stolon roots (Fig. 4). Six-fold more radioactivity was detected in the stem than in the tubers. Radioactivity levels in stems were more variable than in tubers. It was further verified, through ^{45}Ca feeding experiments in both cultivars, that roots on stolons near a tuber can supply ^{45}Ca to that tuber (Fig. 5). All Russet Burbank tubers and 80% of the Dark Red Norland tubers showed radioactivity above control levels when ^{45}Ca was fed to roots on the attached stolon. Tubers with radioactivity levels above background also had Safranin O staining in the vascular tissue, as seen in Fig. 3C–F. Autoradiography showed that the strongest tuber radioactive signal was in the vascular tissue (Fig. 3E, F), which was marked by Safranin O staining of the water transport pathway (Fig. 3C, D). The intensity of both Safranin O staining and radioactivity decreased with increasing distance from vascular tissue (Fig. 3C–F). There was no evidence of radioactivity in the external or internal phloem tissue of the tuber.

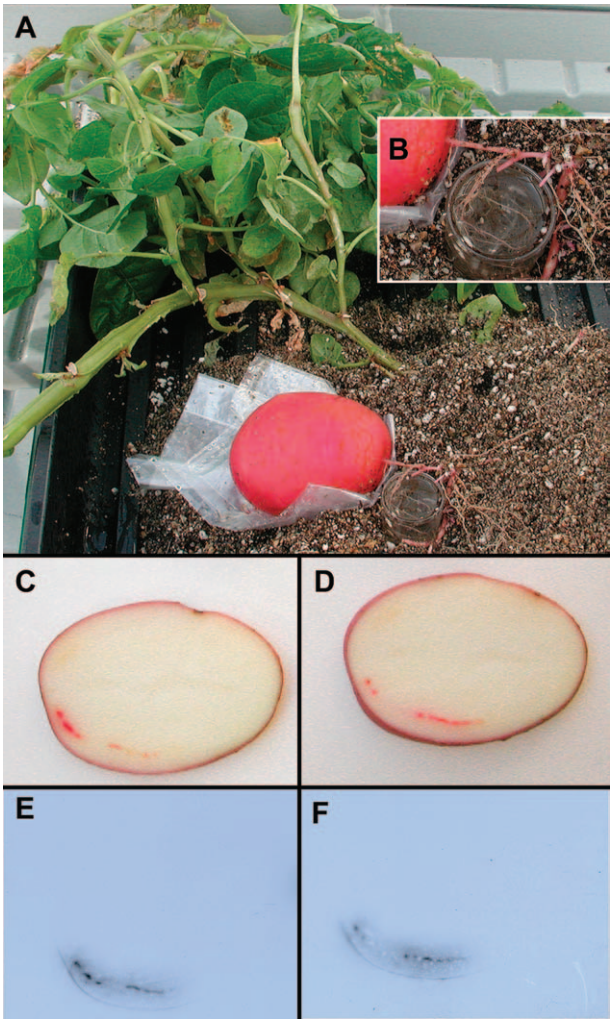

Fig. 3. Transport of ^{45}Ca and a water-soluble dye, Safranin O, from the stolon roots to a Dark Red Norland potato plant tuber. (A) and (B) Experimental set-up. (C) and (D) Tuber tissue slices showing Safranin O dye trace in the tuber xylem tissue. Autoradiographs (E) and (F) are from tissue slices (C) and (D), respectively.

^{45}Ca transport across the periderm

Intact tubers were allowed to absorb water containing ^{45}Ca by dipping them in a radioactive solution for 25 h. A longitudinal slice through the center of the labeled tuber was removed and prepared for autoradiography. A sharp boundary corresponding to the periderm between the labeled and unlabeled tissues was observed (Fig. 6). There was no evidence of radioactivity in the tuber medullary tissue even 8 days after the intact tuber was exposed to radioactivity (Fig. 6). Radioactivity in peeled tubers was at background level; however, substantial radioactivity was present in the periderm of the same tubers (Table 2). The periderm tissue contained about 120–180 times more radioactivity than background, while medullary tissue in the same tuber had background radioactivity (Table 2).

Discussion

Calcium is transported to the shoot with the transpiration stream but is not re-translocated

Calcium can be delivered to the aerial potato shoot from all roots of the potato plant. Radioactivity was detected in the aerial shoot when ^{45}Ca was placed in the main (basal) root area, in the tuber area, or directly onto roots arising from the stolon (Table 1 and Fig. 2). All potato roots are capable of calcium uptake and transport. This finding for

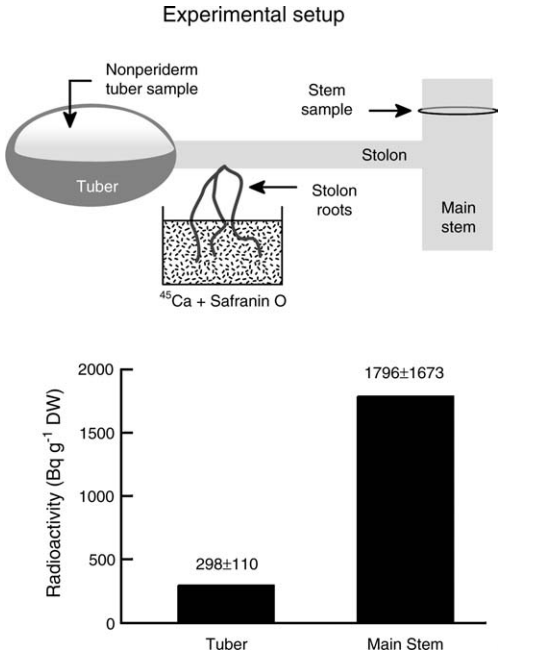

Fig. 4. Accumulation of radioactivity in the Dark Red Norland potato plant main stem at soil level and in tuber associated with stolon after feeding 17.02 MBq ml^{-1} ^{45}Ca and 0.05% (w/v) Safranin O to stolon roots for 6 days. Five stolon roots on three plants were fed ^{45}Ca. Hence, five tubers and three stems were evaluated. Mean activities are given as Bq g^{-1} DW ± SE. One control tuber came from each of four separate plants. The mean background activity in control plants was 1.10 ± 0.12 Bq g^{-1} DW ± SE and 3.45 ± 0.47 Bq g^{-1} DW ± SE in tubers and stems respectively. Control data are not shown.

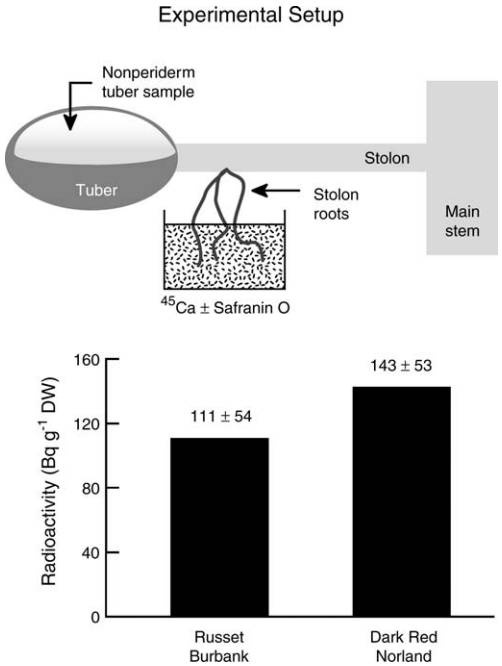

Fig. 5. Accumulation of radioactivity in Russet Burbank and Dark Red Norland potato plant tubers after feeding of ^{45}Ca to associated stolon roots. Russet Burbank data were combined from two experiments. In the first experiment, stolon roots were fed 9.25 MBq ml^{-1} ^{45}Ca for 2 days. Stolon roots in the second experiment were fed 16.28 MBq ml^{-1} ^{45}Ca and 0.05% (w/v) Safranin O for 1 day. Dark Red Norland stolon roots were fed 16.28 MBq ^{45}Ca and 0.05% (w/v) Safranin O for 6 days. The tuber tissue sampled for radioactivity was all non-periderm tissue. Background activity in control tubers from plants not fed ^{45}Ca was 2.62 ±1.13 Bq g^{-1} DW ± SE (n = 7) and 4.40 ± 0.08 Bq g^{-1} DW ± SE (n = 4) for Russet Burbank and Dark Red Norland tubers respectively.

two genotypes, Dark Red Norland and Russet Burbank, indicates that this is a common feature in potato. Genotype may play some role in the uptake and transport of calcium, since we observed more radioactivity in the aerial shoot of Dark Red Norland than in that of Russet Burbank when ^{45}Ca was placed in the main root area in split pot experiments. Variation in calcium uptake efficiency has been shown in wild potato species (Bamberg et al. 1993).

The amount of radioactivity accumulated by the shoot under the same treatment conditions in an experiment was variable (Table 1). We believe that this variability is a function of roots being present in the area to which ^{45}Ca was applied. The ^{45}Ca was not mixed throughout the soil. If roots had a lower density in some areas of the pot, less ^{45}Ca would presumably be taken up. For Dark Red Norland experiments with stolon and tubers placed in polyethylene bags, some stolon damage occurred during the experimental procedure. Tubers from two plants rotted after placement in the bags, and little radioactivity was observed anywhere in these two plants (Table 1, experiment 3).

The autoradiographic data show that ^{45}Ca is distributed throughout the shoot (Fig. 2). We observed radioactivity in both fully and partially expanded leaves. ^{45}Ca was transported to leaves and stems with different developmental ages. This is an expected result, for two reasons. First, the stem primary vascular system is a highly interconnected system. The physical redundancy would efficiently facilitate the distribution of calcium throughout the shoot. Second, calcium is believed to move in the xylem. Furthermore, water potential gradients within potato favor xylem transport of calcium to above-ground portions of the plant.

Redistribution of calcium from aerial foliage to tubers, aerial foliage to roots, or between tubers on the same plant does not occur (Table 1, experiments 1, 2 and 3). In split pot experiments in which ^{45}Ca was given to the main root area of Russet Burbank or Dark Red Norland plants, radioactivity was never detected in any of the 53 tubers examined (Table 1, experiments 1 and 2). ^{45}Ca completely bypassed tubers as it moved from the main roots to

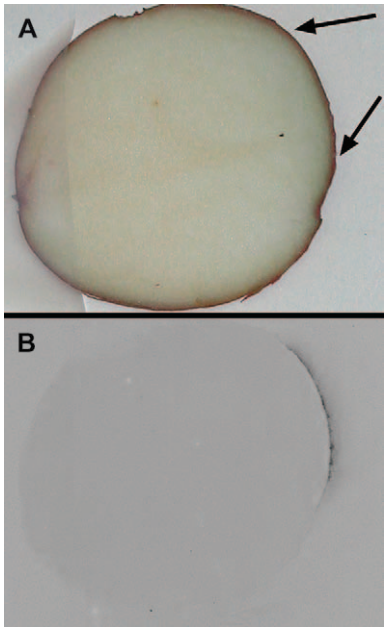

Fig. 6. Transport of ^{45}Ca across the periderm of intact Dark Red Norland tuber from the external environment. (A) Tuber slice and the site of ^{45}Ca application (between the arrows). (B) Autoradiograph of (A).

aerial shoots, and was not redistributed from aerial shoots to tubers. Movement of radioactivity from aerial shoots to tubers did not occur, despite waiting 25 days or 57 days after feeding ^{45}Ca to the Dark Red Norland or Russet Burbank main root area, respectively. When ^{45}Ca was placed in a polyethylene bag containing a Dark Red Norland stolon and tuber, only background activity was observed in the other tubers (Table 1, experiment 3). No radioactivity was detected in the main roots when ^{45}Ca was given to the Russet Burbank tuber area or Dark Red Norland tuber and stolon in a polyethylene bag.

Calcium is reported to be immobile in the phloem (Ziegler 1975), and the lack of ^{45}Ca redistribution that we observed is consistent with these reports. The phloem mobile element phlosphorus was shown to redistribute to stolons within 90 min when ^{32}P was used as a tracer (Baker and Moorby 1969). Double-labeling experiments with ^{32}P and ^{45}Ca, using our split pot system, would be instructive and serve as a positive control for phloem transport to tubers. Our results contrast with those of Davies and Millard (1985), who contend that phloem transport could account for a significant amount of calcium being delivered to the tuber.

Table 2. Penetration of ^{45}Ca across the periderm of intact tubers from Dark Red Norland potato plants. Tubers were placed over a beaker with 25 ml of water with or without 30.34 MBq ^{45}Ca for 25 h. Tubers then were placed on a laboratory bench for 8 days prior to assaying activity.

	Radioactivity in tuber tissue (Bq g^{-1} DW)					
Treatment	Periderm			Medullary		
^{45}Ca	Tuber 1	Tuber 2	Tuber 3	Tuber 1	Tuber 2	Tuber 3
	285	232	97	1	1	1
Control	2	1	1	2	2	1

Calcium only enters tubers when fed to roots on the stolon near tubers

Our results provide the first conclusive evidence that calcium is transported to the tuber from the roots originating on the stolon in close proximity to the tuber. We found radioactivity in the tuber medullary tissue only when ^{45}Ca was placed in the tuber area or onto the stolon roots (Table 1 and Figs 4–6). This indicates that only a limited number of roots are responsible for transport of calcium to tubers. Baker and Moorby (1969) showed that radioactive strontium, a calcium analog, moved into tubers at night, when the transpirational demand of the shoot was minimal. However, they applied radioactive strontium to the soil of a potted plant and not to specific roots. Kratzke and Palta (1986) used a split pot system to separately control calcium inputs to the tuber region and basal root region. They concluded that tuber calcium content could be increased only when calcium was placed in the tuber and stolon area. We extended their findings to show specifically that roots on stolons close to tubers supply calcium to the tuber. Our results are also consistent with the selective transport of a water-soluble dye from the stolon roots to the tuber (Kratzke and Palta 1985). Kratzke and Palta (1985) demonstrated that only the roots on or near the tubers supply water to the tuber, as roots at the stem and stolon junction, junction roots and main roots did not supply water to tubers.

Our observations on the co-transport of Safranin O dye and ^{45}Ca (Fig. 3C–F) show, for the first time, that water and calcium are transported from the stolon roots to the tuber. Neither Safranin O nor radioactivity was noted in the perimedullary area containing internal phloem or in the cortical region of the tuber, where the internal and external phloem occur, respectively. Our results contrast with those of Davies and Millard (1985), who proposed that phloem transport could account for a significant amount of calcium being delivered to the tuber; we could not detect any phloem transport of ^{45}Ca even weeks after treatment.

Furthermore, our results show that calcium is not taken up directly through the periderm. Transport across the periderm to internal tuber tissue was not observed. While we saw considerable activity in the periderm, only background activity was detected in the medullary tissue.

This was observed with both autoradiographic and scintillation counting techniques (Table 2 and Fig. 3). These results show ^{45}Ca is readily adsorbed by cells within the periderm but is not transported across the periderm. Habib and Donnelly (2002) also reported that no radioactivity penetrated the periderm when tubers were exposed to ^{45}Ca for 24 h. Habib and Donnelly (2002) did find radioactivity in the central portion of the tuber after it was suspended in a ^{45}Ca solution for 5 days. A water potential of zero for 5 days would be very unlikely in nature. Kraus and Marschner (1971) concluded that tuber calcium uptake took place directly through the periderm from the soil solution. However, the autoradiograph that they presented suggested that calcium movement is largely blocked by the periderm. The suberized walls found in the periderm (Esau 1965) further suggest that transport through the periderm is not likely. Our results are also consistent with the recent proposal of calcium movement in xylem tissue (White 2001). It has been indicated by White (2001) that apoplastic delivery of calcium in the root does not cross the casparian strip in the endodermal wall. The casparian strip is composed of both lignin and suberin (Esau 1965, Schreiber et al. 1999).

Pathway of calcium transport in potato and physiologic significance

Calcium transport within a plant such as potato presents an interesting dilemma. Calcium is not redistributed through phloem tissue from the aerial shoot, which readily accumulates signal, to tubers. Consistent with this explanation, we found that even 57 days after feeding ^{45}Ca to the basal root system of Russet Burbank, only background activity could be detected in tubers, while aerial shoots had accumulated 100-fold more radioactivity than controls (Table 1, experiment 1). As calcium is only transported through the xylem tissue and is not re-translocated, movement of calcium to the tuber still presents a challenge. It does not appear that root pressure plays a large role in the transport of calcium in potato. If root pressure was a significant factor in calcium delivery to tubers, we would expect movement of radioactivity to tubers when ^{45}Ca was applied to the main (basal) root area in split pot experiments (Table 1, experiments 1 and 2). We would also have expected a more equitable distribution of radioactivity between the stem and tubers when ^{45}Ca was fed to stolon roots (Fig. 4) if root pressure played a significant role in calcium transport.

By virtue of their usual location in moist soil, tubers are low-transpiring organs. Thus, tubers will not be able to compete with aerial foliage for water uptake. The aerial potato shoot can obtain calcium from any of the roots on the plant, and this is shown in Fig. 7A, B. Calcium supplied to either the main root area or the roots on stolons near tubers can be transported to the shoot. Our data indicate that only roots near tubers on stolons can deliver calcium to tubers (Figs 3C–F and 7B). The tuber periderm does not readily allow the passage of calcium ions into the tuber interior (Fig. 6).

Our results indicate that transpirational demand may be a significant determinant of where calcium is transported in a potato plant. The leaves of field-grown potatoes have been shown to have a lower water potential than tubers, especially in wet soil (Gander and Tanner 1976). Only during the evening are tuber and leaf water potentials nearly equal, although leaves almost always have a lower water potential. With neglible tuber transpirational demand, water tends to be routed to the shoot. This may explain why only roots near tubers on stolons are able to supply calcium to tubers (Table 1, experiment 1, and Figs 4 and 5). Wiersum (1966) noted transport of water-soluble dye into tubers only when tubers were in very dry sand. We observed six times more radioactivity in the stem than in the tuber when stolon roots were fed ^{45}Ca (Table 2). This is probably due to a much greater shoot transpiration rate as compared to the tuber. In support of these results, we have found that leaves and aerial stems contain 500–1000 times more calcium than the tubers on the same plant (data not shown). Win et al. (1991) used anti-transpirants to test the hypothesis that altering the leaf/tuber water potential gradient within a plant would allow greater calcium accumulation in tubers and reverse calcium-related tuber necrosis. Application of anti-transpirant increased the leaf water potential and was associated with decreased leaf calcium levels and increased tuber calcium levels. Taken together, the results of these studies and our studies indicate that calcium is transported to the tuber via the xylem, along with water, and that roots on the stolon near the tuber supply this water and calcium to the developing tuber.

Practical implication for alleviating potato tuber calcium deficiency

Our results document the pathway of calcium transport to the potato tuber through the xylem tissue and show that redistribution through the phloem tissue does not occur. This has physiologic significance for potato, since tuber quality is related to tuber calcium levels. Cultivated potato tuber calcium concentration values are reported to range from 170 to 230 $\mu g\ g^{-1}$ DW (Kleinhenz et al. 1999 Karlsson and Palta 2003, Ozgen et al. 2006). Shoot calcium concentrations in plants have been reported to be 1–50 $mg\ g^{-1}$ DW (Marschner 1995). We have found that leaves and aerial stems contain about 500–1000

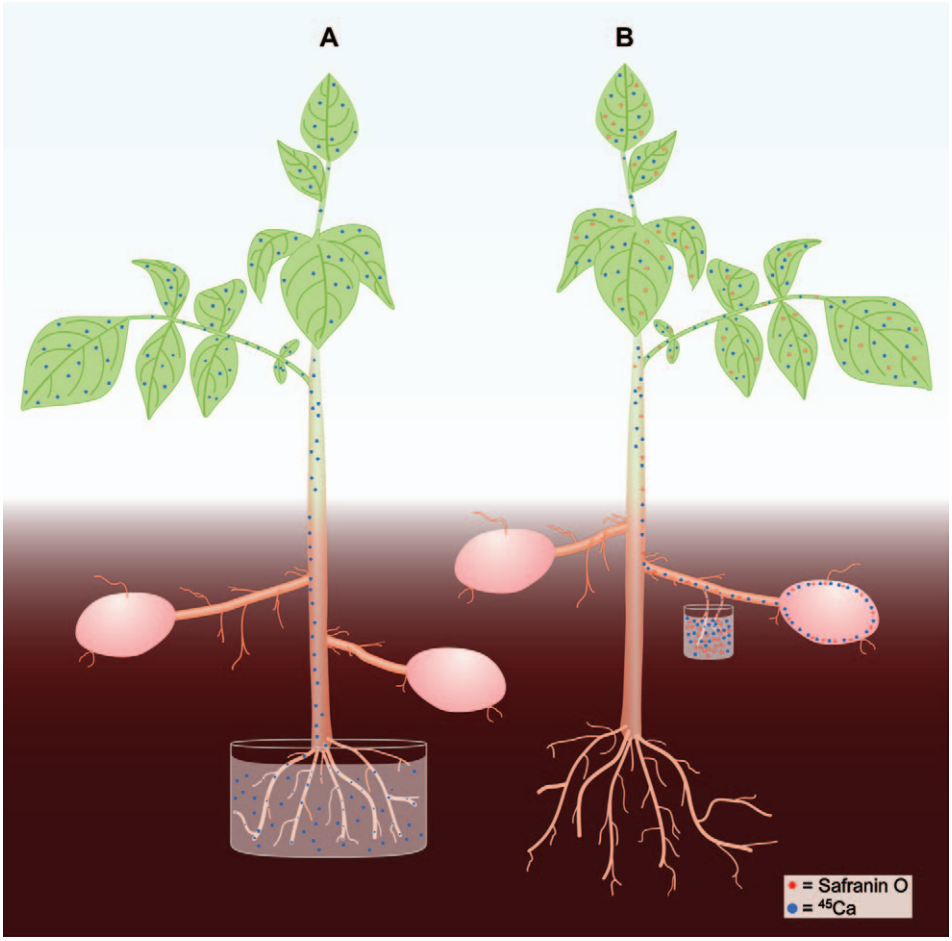

Fig. 7. Diagram showing transport path of ^{45}Ca and Safranin O dye when fed to different portions of the potato plant root zone. ^{45}Ca is represented by blue dots and Safranin O by red dots. (A) ^{45}Ca placed in the main root system is transported to the aerial shoot but not by the tubers. (B) Both ^{45}Ca and Saftranin O fed to the stolon roots are transported to the associated tuber and aerial shoot but are not transported to main root or other tubers.

times more calcium than the tubers from the same plant (data not shown). While both are botanically stem tissue, non-periderm tuber tissue is very deficient in calcium as compared to the aerial stem (Palta 1996). Lack of adequate calcium in tubers has been associated with a high incidence of physiologic defects such as hollow heart, brown center and internal brown spot (Bangerth 1979, Palta 1996). In support of this, several studies have reported improvement in tuber quality resulting from field applications of calcium during the growing season (McGuire and Kelman 1984, Tzeng et al. 1986, Kleinhenz et al. 1999). The fact that increased tuber calcium levels were found when calcium fertilizer was placed in the tuber and stolon area further support the results of the present study (Kratzke and Palta 1986). Thus, the results of our study have important implications for the placement and timing of calcium fertilizer with the goal of enhancing tuber calcium concentration.

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