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# Influence of Root Zone Calcium on Shoot Tip Necrosis and Apical Dominance of Potato Shoot: Simulation of This Disorder by Ethylene Glycol Tetra Acetic Acid and Prevention by Strontium

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***Abstract.*** **The important roles of calcium on plant growth and development including cell division and cell elongation is well documented. The purpose of the present study was to determine the impact of root zone calcium on the growth and health of potato apical meristem and on the maintenance of apical dominance. For this purpose, single-node potato cuttings (*Solanum tuberosum* L. cv. Dark Red Norland) were grown in sterilized modified Murashige and Skoog (MS) media containing varying concentrations of calcium (1 to 3000 μM). After 13 to 30 d of growth, plantlets were harvested and data for height of the main shoot and for the number of axillary shoots produced were recorded. Plantlets were ashed and tissue calcium concentration was determined. Shoot height was retarded with decreasing concentration of calcium in the media. Calcium deficiency induced shoot tip injury and loss of apical dominance. Tip injury was followed by the development of axillary shoots. The number of axillary shoots increased from one to 21 as calcium concentration in the media decreased from 3000 to 1 μM. At calcium concentration of 1500 μM or higher, there was a single main shoot with no axillary shoots. Addition of ethylene glycol tetra acetic acid (EGTA), a calcium chelator, to the media with 2720 μM calcium (sufficient calcium) resulted in the development of shoot injury and in the formation of axillary shoots. Calcium deficiency injury symptoms were prevented by the addition of a calcium analog, strontium, to MS media deficient in calcium. Strontium has been reported to strongly bind to plant cell walls and the inclusion of strontium prevented injury in shoots of plants grown on calcium-deficient media. These results suggest that strontium is able to mimic the role of calcium in the maintenance of cell wall integrity and supports previous studies that showed that calcium deficiency results from cell wall collapse of the subapical cells.**

Calcium plays important roles in plant growth and development. Calcium ions are essential for cell wall strength and cell–cell adhesion (Marry et al., 2006; Marschner, 1995). Calcium bound to the outer surface of the plasma membrane maintains membrane stability and cell integrity (Hanson, 1984; Hirschi, 2004; Palta, 1996). Calcium is known to be transported in xylem making transpiration the main force for calcium transport (Busse and Palta, 2006; Clarkson, 1984; Kratzke and Palta, 1986). Thus, calcium deficiency symptoms are observed in tissues with a low transpiration rate including young expanding leaves, enclosed shoot tissues, fruits, underground tubers, and in portions of the plant principally fed by the phloem rather than the xylem (White, 2001; White and Broadley, 2003).

A physiological disorder termed "shoot-tip necrosis" observed in in vitro culture of potato (*Solanum tuberosum*) has been hypothesized to be a calcium deficiency symptom (McCown and Sellmer, 1987; Sha et al., 1985). This condition is typified by browning and death of the shoot tip, loss of apical dominance, and axillary shoot development in an in vitro shoot culture. Transpiration is limited during in vitro culture by the high humidity that occurs in closed culture vessels. Therefore, uptake and transport of calcium ions, which is dependent on transpiration, is limited during in vitro culture (Williams, 1993). In addition to in vitro potato shoot culture, shoot-tip necrosis resulting from calcium deficiency has been observed in shoot cultures of *Pistacia vera* (Abousalim and Mantell, 1994). Although these authors were able to show a relationship between shoot-tip necrosis and the medium calcium concentration, they were not able to measure effects at the individual plant level owing to the interplant competition in the culture vessels.

Calcium and strontium are closely related elements and have been shown to have similar behavior in plants (Mengel et al., 2001). Early studies by Queen et al. (1963) suggested that strontium can replace calcium during plant growth. Hutchin and Vaughan (1968) showed that plant uptake and distribution of these two elements is similar but not identical. Myttenaere (1964) found that strontium is deposited in the cell wall to a much greater extent than calcium. Results from these early studies suggest that strontium may be able to mitigate calcium deficiency symptoms, especially in cell wall development. No study to our knowledge has investigated the mitigation of calcium deficiency injury in shoot cultures using strontium.

Recently, we have provided a detailed description of the injury by calcium deficiency at the cellular level in potato shoot cultures (Busse et al., 2008). We demonstrated that primary injury from calcium deficiency is localized in the expanding pith cells below the apical shoot meristem and injury is characterized by the collapse of the walls of subapical cells in potato. These results suggest that strontium may be able to prevent the cell wall collapse in potato shoot cultures grown in calcium-deficient media.

In the present study, we provide further evidence for the role of calcium in shoot tip necrosis by 1) simulating the injury symptoms by including EGTA in the tissue culture media containing sufficient calcium; and 2) alleviating these symptoms by incorporating strontium in calcium-deficient media. By placing a single culture in each vessel, we were able to do studies at an individual plant level without interplant competition.

## Materials and Methods

*Plant material, medium, and growth conditions.* Single-node cuttings from the second and third nodes of 1-month-old micropropagated potato (cv. Dark Red Norland) plantlets were cultured in vitro on MS media (Murashige and Skoog, 1962) containing a range of calcium concentrations in culture tubes (20 × 150 mm). Sucrose (3%) and 0.5 mM myoinositol were added and pH was adjusted to 5.60 ± 0.02. Agar

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(0.7%) was added before autoclaving at (132 °C) for 15 min. Cultures were placed under continuous light with about 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux from cool white fluorescent lamps and 20 °C (Steffen and Palta, 1986).

Three separate experiments were conducted. The objective of the first experiment was to establish the root zone calcium concentrations required for the production and alleviation of calcium deficiency symptoms. The objective of the second experiment was to determine if the presence of EGTA will simulate the symptoms of calcium deficiency in media containing sufficient calcium. The objective of the third experiment was to determine if supplementing a calcium-deficient media with strontium would prevent the development of calcium deficiency symptoms. In the first experiment, single-node cuttings were cultured on MS media modified with calcium chloride (1.4, 6.8, 34, 68, 170, 850, 1360, or 3000 µM calcium) for 13 and 30 d. Each treatment had 10 replications. In the second experiment, agar media containing sufficient calcium (1360 and 3000 µM calcium) was used alone or supplemented with 5 mM EGTA to reduce calcium availability. Each treatment had five replications. In the third experiment, single-node cuttings were cultured for 28 d in agar media containing deficient calcium (68 µM calcium) and supplemented with varying amounts of strontium chloride (0, 68, 204, 340, 476, 612, 748, 884, 1020 µM). Each treatment had 17 replications. In all the experiments, media calcium, strontium, and EGTA concentrations were fixed at the beginning of the experiment and the media was not replenished.

*Observation on the shoot growth and calcium and strontium contents.* Plantlets were harvested after 2 to 4 weeks of growth in the media. Shoots from each tube were separated from their roots, weighed immediately, and the shoot length for the main shoot was recorded. The number of axillary shoots arising from the primary shoots per plantlet was counted. For this purpose, the axillary shoots visible with the naked eye (3 mm long or greater) were counted. In addition, shoots with dead tips were counted. For this purpose, shoot tips showing browning and necrotic lesions were counted as dead.

Individual plants were analyzed for calcium and strontium content following the procedure of Ozgen et al. (2006). For this purpose, the samples were dried in an oven at 70 °C, weighed, and ashed (450 °C, 6 h). The ash was then dissolved in 2 N HCl. This solution was diluted with a lanthanum chloride ($LaCl_3.XH_2O$) solution and distilled, deionized water to obtain samples with a final concentration of 0.2 N HCl and 2000 $mg \cdot L^{-1}$ of lanthanum chloride. Calcium and strontium concentrations were determined by atomic absorption spectrophotometry (Varian Model Spectraa-20; Varian Associates, Inc., Sunnyvale, CA).

*Statistical analysis.* As described previously, that data on the shoot tips alive were collected as two outcomes, dead or alive. These data were analyzed using the binomial option in frequency procedure to obtain 95% confidence intervals for the proportion alive (Version 9.2; SAS Institute, Inc., Cary, NC). Paired comparisons between strontium levels were made using Fisher's exact test. The Bonferroni correction was applied to account for multiple comparisons. All other data were analyzed using a completely randomized design in the PROC GLM procedure of the Statistical Analysis System (Version 8.2; SAS Institute, Inc.). Means were separated using the least significant difference test at $\alpha = 0.05$.

## Results

*Relation of shoot growth and calcium uptake to root zone calcium.* After 30 d, plantlets grown with sufficient root zone calcium (plantlets with no shoot injury symptoms)

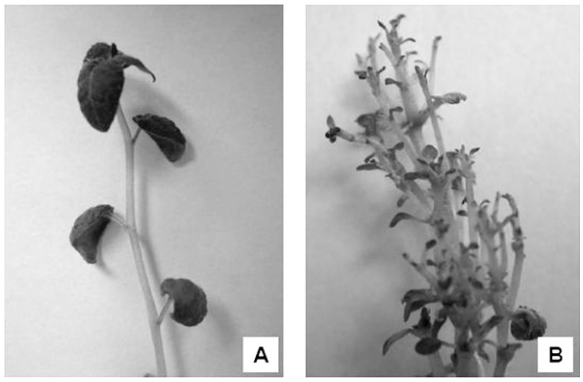


Fig. 1. Growth of 'Dark Red Norland' potato shoots for 30 days in media containing single shoots at 3000 µM calcium (Ca) (**A**) and 6.8 µM Ca (**B**). Apical dominance was lost and axillary shoot growth was stimulated at 6.8 µM Ca.

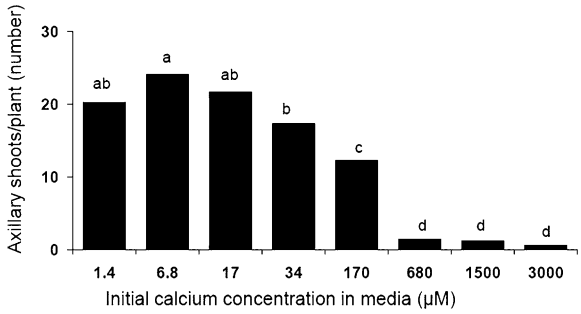


Fig. 2. Relationship between root zone calcium and number of axillary shoots per potato plant after 30 days of growth in media containing varying concentrations of calcium. Axillary shoots rising from the main stem as well as arising from the axillary shoots (secondary shoots) were counted. Mean values having same letter are not significantly different based on SAS General Linear Model procedure. Least significant difference ($\alpha = 0.05$).

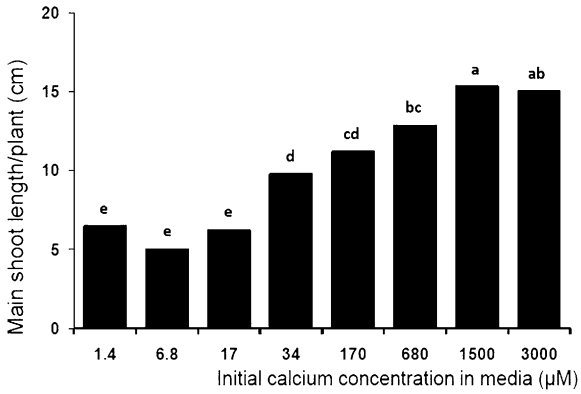


Fig. 3. Relationship between root zone calcium and the length of the main shoot of the potato plant after 30 days of growth in media containing varying concentrations of calcium. Mean values having same letter are not significantly different based on SAS General Linear Model procedure. Least significant difference ($\alpha = 0.05$).

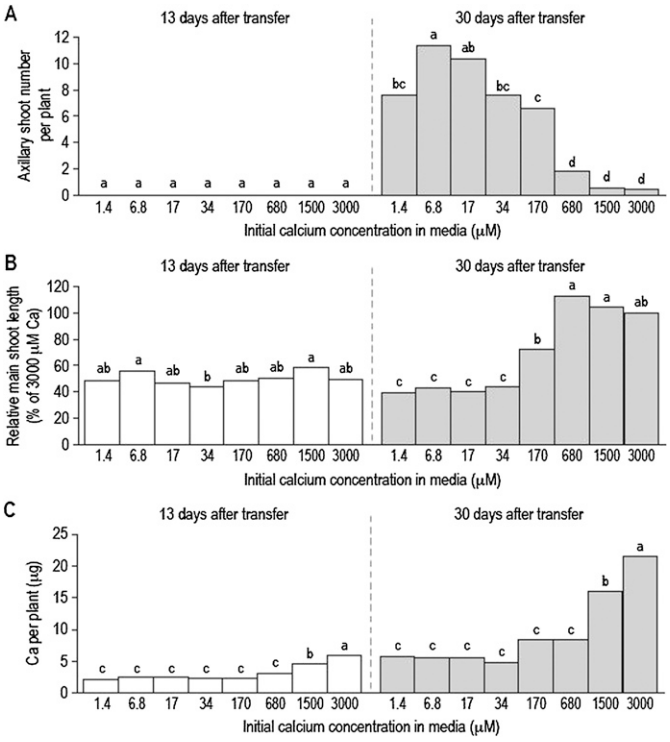


Fig. 4. Number of axillary shoots (**A**), relative shoot length [% of 3000 μM calcium (Ca) at 30 days] (**B**) and amount of Ca per potato plant (**C**) after 13 and 30 days of growth in media containing varying concentrations of Ca. Mean values having same letter are not significantly different based on SAS General Linear Model procedure. Least significant difference ($\alpha = 0.05$).

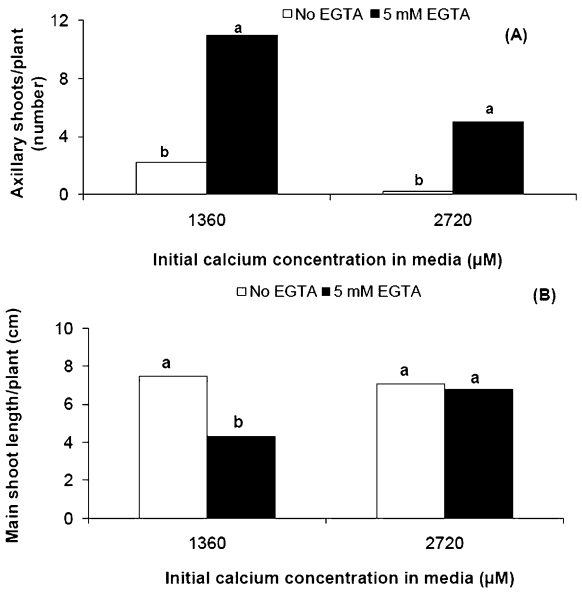


Fig. 5. Influence of 5 mM ethylene glycol tetra acetic acid in the media on axillary shoot number of potato plants growing for 30 days in media containing 1360 μM and 2720 μM calcium (**A**) and shoot length (**B**). Mean values having same letter are not significantly different based on the SAS General Linear Model procedure. Least significant difference ($\alpha = 0.05$).

had a single unbranched shoot, elongated internodes, and several relatively large leaves (Fig. 1A). In contrast, plantlet cultured with insufficient calcium media (calcium concentration at which plants developed injury symptoms) were highly branched (Fig. 1B). At root zone calcium concentrations of 34 μM and lower, the average number of axillary shoots on each plantlets was 18 or more (Fig. 2). Secondary axillary shoots were often observed arising from the primary axillary shoots. Very few axillary shoots per plant occurred at the three highest calcium concentrations tested (Fig. 2). The mean shoot length increased from 6.4 to 15.0 cm as the calcium concentration increased from 1.4 to 3000 μM (Fig. 3).

No axillary shoots were noted on any of the plantlets in Expt. 1 after 13 d (Fig. 4A). Similarly, no differences could be detected in the relative primary shoot length after 13 d of culture (Fig. 4B). A statistically significant increase in the number of axillary shoots was observed at medium calcium concentrations of 170 μM or less after 30 d of culture (Fig. 4A). However, the shoot growth was affected by root zone calcium concentration by 30 d (Fig. 4B). The plants at 170 μM and below had significantly less shoot growth as compared with the higher calcium concentrations at 30 d after transfer. The shoot growth (height) was greatest at 680 μM and higher calcium concentrations. Plants at these concentrations doubled their height between 13 and 30 d after transfer. Shoot length at 170 μM root zone calcium level was significantly greater than the ones at 34 μM and below at 30 d (Fig. 4B). Below 680 μM root zone calcium level, no differences were detected in plantlet calcium content at 13 or 30 d (Fig. 4C). There were no significant differences in either the total fresh or dry weight on a per plant basis after 30 d with any of the treatments (data not shown).

*Influence of ethylene glycol tetra acetic acid on shoot growth and calcium uptake.* The addition of 5 mM EGTA into the media increased shoot number after 30 d at both 1360 and 2720 μM calcium in the media (Fig. 5A). At 1360 μM calcium, plants growing in media without EGTA had on average approximately two axillary shoots. Addition of EGTA significantly increased the axillary shoot number to 11. At 2720 μM calcium, plants growing in media without EGTA had no axillary shoots; however, plants grown in the presence of EGTA produced only five axillary shoots at 2720 μM calcium (Fig. 5A). EGTA was effective in retarding shoot growth from 7.5 cm to 4.3 cm at 1360 μM (Fig. 5B). However, at 2720 μM calcium concentration, EGTA did not retard shoot growth.

*Influence of strontium on shoot growth and calcium uptake.* Addition of strontium to calcium-deficient media had a positive influence on the growth and viability of shoots (Tables 1 and 2; Fig. 6). The control plantlets with strontium added had the highest number of axillary shoots and fewer nodes compared with the treatments (Table 1; Fig. 6). However, addition of 68 and 204 μM strontium significantly reduced the axillary shoots (Table 1) and improved shoot tip viability (Fig. 6). Only nine of 17 control plantlets had shoot tips alive. However, all shoot tips were alive when 204 μM strontium was added to the media. This improvement in tip health by the addition of strontium was significant ($P < 0.003$). There were no significant differences in plant length, fresh weight, and dry weight per plant among treatments at 28 d (Table 1). Similar to calcium, the tissue strontium concentration increased as the media strontium concentration increased (Table 2).

## Discussion

Consistent with our recent studies (Busse et al., 2008), the results of the present study

Table 1. Influence of media strontium (Sr) concentration on dry weight, fresh weight, shoot length, number of nodes, and number axillary shoots arising from primary potato shoots.[z]

| Initial Sr concn in media (µM) | Dry shoot wt (mg/plant) | Fresh shoot wt (mg/plant) | Shoot length (cm/plant) | Node number (no./plant) | Axillary shoots[y] (no./plant) |
|---|---|---|---|---|---|
| 0 | 12.5 ab | 180 | 9.4 bc | 8.7 c | 2.4 a |
| 68 | 13.3 a | 204 | 10.5 a | 10.2 ab | 1.2 b |
| 204 | 11.4 abc | 151 | 9.3 bc | 9.8 b | 0.1 c |
| 340 | 9.9 c | 160 | 9.6 ab | 10.2 ab | 0.0 c |
| 476 | 10.6 bc | 154 | 8.7 bc | 10.3 ab | 0.2 c |
| 612 | 11.1 abc | 157 | 8.7 bc | 10.3 ab | 0.0 c |
| 748 | 12.3 abc | 185 | 9.0 bc | 10.2 ab | 0.3 c |
| 884 | 12.1 abc | 165 | 9.0 bc | 10.5 a | 0.0 c |
| 1020 | 11 abc | 158 | 8.3 c | 10.1 ab | 0.4 c |

[z]The calcium in the media was kept at a constant 68 µM (deficient level) and the strontium concentration was varied from 0 to 1020 µM. Each treatment had 17 replications. Plants were harvested at 28 d after culture. Mean values that have same letter are not significantly different based on SAS General Linear Model procedure, least significant difference ($\alpha = 0.05$). No significant differences were found for fresh weight among the various treatments.

[y]Refers to axillary shoots arising from primary shoot only. The axillary shoots visible with the naked eye (3 mm long or greater) were counted.

Table 2. Influence of media strontium (Sr) concentration on the calcium and strontium uptake by potato shoots.[z]

| Initial Sr concn in media (µM) | Ca/kg DW[y] (mmoles) | Ca/plant (µg) | Sr/kg DW[y] (mmoles) | Sr/plant (µg) |
|---|---|---|---|---|
| 0 | 29.9 bc | 14.6 a | 0.0 i | 0.0 g |
| 68 | 26.8 cd | 14.4 ab | 1.6 h | 1.9 fg |
| 204 | 34.0 ab | 14.3 ab | 4.9 g | 4.6 ef |
| 340 | 35.1 a | 13.5 ab | 8.6 f | 7.5 e |
| 476 | 30.4 bc | 12.8 bc | 12.0 e | 11.2 d |
| 612 | 25.4 cd | 11.2 cd | 14.8 d | 14.5 c |
| 748 | 22.9 d | 11.2 cd | 17.0 c | 18.4 b |
| 884 | 22.7 d | 11.0 d | 19.4 b | 20.8 b |
| 1020 | 24.6 d | 10.7 d | 25.2 a | 24.2 a |

[z]The calcium (Ca) in the media was kept constant 68 µM (deficient level) and the strontium concentration was varied from 0 to 1020 µM. Each treatment had 17 replications. Plants were harvested at 28 d after culture. Shoots of two plants were combined to make one sample for calcium and strontium analysis. Mean values that have the same letter are not significantly different based on SAS General Linear Model procedure, least significant difference ($\alpha = 0.05$).

[y]Dry weight of samples.

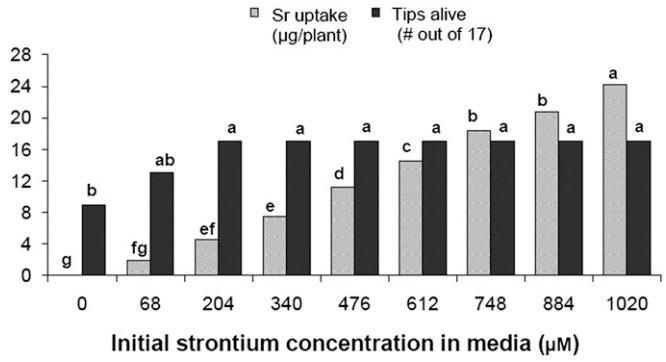


Fig. 6. Influence of media strontium concentration on strontium uptake and the viability of primary shoot tips of potato grown for 28 days in media containing 68 µM calcium (deficient level). Mean values for strontium uptake having same letter are not significantly different based on SAS General Linear Model procedure. Least significant difference ($\alpha = 0.05$). Shoot tips showing browning and necrotic lesions were counted as dead. Mean values having same letter for shoot tip alive are not statistically different based on Fisher's exact test ($\alpha = 0.05$). Each treatment had 17 replications (plantlets).

show that root zone calcium has a dramatic influence on the growth and development of potato shoots in vitro. Calcium deficiency did not result in an overall decline in growth of the plant. The fresh and dry weight was not significantly different among any of the root zone calcium treatments after 30 d (data not shown). Length of the main shoot was dramatically reduced when root zone calcium was below 680 µM. This can be explained by the observation that plant grown on calcium-deficient media (below 680 µM) developed injury to the shoot tip resulting in the death of the shoot tip. Consistent with previous reports, this death in the shoot tip was followed by axillary shoot development because of the loss of apical dominance (Busse et al., 2008; McCown and Sellmer, 1987; Sha et al., 1985).

By observation of plants at two developmental stages and quantifying their calcium contents, we were able to relate the shoot tip injury to the calcium transported from media to the plant (Fig. 4). At 13 d after transfer, no injury was observed to the plant grown on the calcium-deficient media. This means no axillary shoot formation (Fig. 4A) as well as no retardation of shoot growth (Fig. 4B) for plants grown in calcium-deficient media (less than 680 µM). However, at 30 d, plants grown on calcium-deficient media produced significantly higher number of axillary shoots (Fig. 4A) and shoot length was retarded in these plants (Fig. 4B). We also found that at 30 d, the calcium contents in plants grown at 680 µM or less was lower than the plants grown at greater than 680 µM calcium (Fig. 4C).

Our observations of plants grown in the presence of EGTA and otherwise containing sufficient calcium (1360 µM) further supports the hypothesis that lack of calcium is associated with shoot tip injury and an increase in axillary shoots (Fig. 5). EGTA is known to be a chelator that results in the removal of calcium from plant tissues, especially the cell walls (Demarty et al., 1984; Morvan, 1983). Calcium is known to play an important role in crosslinking pectic acid fractions in the cell wall (Matoh and Koboyashi 1998). Presence of chelators such as EGTA is known to reverse the influence of extracellular calcium on many physiological processes including tuberization in potato (Balmani et al., 1986; MacIntosh et al., 1996).

Our results on the prevention on the injury by strontium in an otherwise calcium-deficient media supports the hypothesis that shoot tip injury results from a collapse of walls of the expanding subapical cells. The strontium concentration varied from 68 to 1020 µM, whereas the calcium was kept constant at a deficient level (68 µM). Under these conditions, 204 µM strontium in the media was able to prevent both shoot tip injury (Fig. 6) and the development of axillary shoots (Table 2). Calcium and strontium are closely related elements and have been shown to have similar behavior in the plants (Mengel et al., 2001). Early studies by Myttenaere (1964) found that strontium is deposited in the plant cell walls to a much greater extent than calcium. There is also evidence that strontium can replace calcium during plant growth (Queen et al., 1963). No such recent data on strontium are available. The early studies were presumably motivated by an interest in understanding the influence and fate of radioactive strontium fallout on plants. However, these early studies do provide an interesting insight on calcium deficiency in shoot cultures. We have recently provided evidence that a primary injury of calcium deficiency is localized in the expanding pith cells below the apical shoot meristem and that this injury results in a collapse of the cell walls of these expanding cell walls (Busse et al., 2008). Because strontium prevented injury in plants grown on calcium-deficient media (Fig. 6; Table 1) and strontium has been reported to strongly bind with plant cell wall (Myttenaere, 1964), we suggest that

strontium is able to mimic the role of calcium in maintenance of the cell wall integrity. Future studies using strontium might help explain the role of calcium in plant cell walls.

### Literature Cited


Abousalim, A. and S.H. Mantell. 1994. A practical method for alleviating shoot-tip necrosis symptoms in *in vitro* shoot cultures of *Pistacia* cv. Mateur. J. Hort. Sci. 69:357–365.

Balamani, V., K. Veluthambi, B.W. Poovaiah. 1986. Effect of calcium on tuberization in potato (*Solanum tuberosum* L.). Plant Physiol. 80:856–858.

Busse, J., S. Ozgen, and J.P. Palta. 2008. Influence of root zone calcium on subapical necrosis in potato shoot cultures: Localization of injury at the tissue and cellular levels. J. Amer. Soc. Hort. Sci. 133:653–662.

Busse, J.S. and J.P. Palta. 2006. Investigating the in vivo calcium transport path to developing potato tuber using $^{45}$Ca: A new concept in potato tuber calcium nutrition. Physiol. Plant. 128:313–323.

Clarkson, D.T. 1984. Calcium transport between tissue and its distribution in plant. Plant Cell Environ. 7:449–456.

Demarty, M., C. Morvan, and M. Thellier. 1984. Calcium and the cell wall. Plant, Cell and Environment 7:441–448.

Hanson, J.B. 1984. The functions of calcium in plant nutrition, p. 149–208. In: Tinker, P.B. and A. Läuchli (eds.). Advances in plant nutrition, Praeger Pub., New York, NY.

Hirschi, K.D. 2004. The calcium conundrum. Both versatile nutrient and specific signal. Plant Physiol. 136:2438–2442.

Hutchin, M.E. and B.E. Vaughan. 1968. Relation between simultaneous Ca and Sr transport rates in isolated segments of vetch, barley, and pine roots. Plant Physiol. 43:1913–1918.

Kratzke, M.G. and J.P. Palta. 1986. Calcium accumulation in potato tubers: Role of the basal roots. HortScience 21:1022–1024.

Marry, M., K. Roberts, S.J. Jopson, I.M. Huxham, M.C. Jarvis, J. Corsar, E. Robertson, and M.C. McCann. 2006. Cell–cell adhesion in fresh sugar-beet root parenchyma requires both pectin esters and calcium cross-links. Physiol. Plant. 126:243–256.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press, London, UK. p. 285–299.

Matoh, T. and M. Koboyashi. 1998. Boron and calcium, essential inorganic constituents of pectic polysaccharides in higher plant cell walls. J. Plant Res. 111:179–190.

McCown, B.H. and J.C. Sellmer. 1987. General media and vessels suitable for woody plant culture. In: Cell and tissue culture in forestry. Martinus Nijhoff Publishers, Dordrecht, The Netherlands. p. 4–16.

McIntosh, G.C., R.M. Ulloa, M. Raices, and M.T. Tellez-Inon. 1996. Changes in calcium-dependent protein kinase activity during in vitro tuberization in potato. Plant Physiol. 112:1541–1550.

Mengel, K., E. Kirkby, H. Kosegarten, and T. Appel. 2001. Principles of plant nutrition. 5th Ed. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Morvan, C. Etude de quelques propietes physichimiques de parois vegetales. Influence de la structure de leurs polysaccharides. Thesis, Rouen, pp. 1–175.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Myttenaere, C. 1964. Influence du rapport strontium–calcium sur la localisation du strontium et du calcium chez *Pisum sativum*. Physiol. Plant. 17:814–827.

Ozgen, S., B. Karlsson, and J.P. Palta. 2006. Response of potatoes (cv. Russet Burbank) to supplemental calcium applications under field conditions: Tuber calcium, yield and incidence of internal brown spot. Amer. J. Potato Res. 83:195–204.

Palta, J.P. 1996. Role of calcium in plant responses to stresses: Linking basic research to the solutions of practical problems. HortScience 31:51–57.

Queen, W.H., H.W. Fleming, and J.C. O'Kelly. 1963. Effects on *Zea mays* seedlings of a strontium replacement for calcium in nutrient media. Plant Physiol. 38:410–413.

Sha, L., B.H. MacCown, and L.A. Peterson. 1985. Occurrence and cause of shoot tip necrosis in shoot cultures. J. Amer. Soc. Hort. Sci. 110: 631–634.

Simon, E.W. 1978. The symptoms of calcium deficiency in plants. New Phytol. 80:1–15.

Steffen, K.L. and J.P. Palta. 1986. Effect of light on photosynthetic capacity during cold acclimation in a cold-sensitive and cold-tolerant potato species. Physiol. Plant. 66:353–359.

White, P.J. 2001. The pathways of calcium movement to the xylem. J. Expt. Bot. 52:891–899.

White, P.J. and M.R. Broadley. 2003. Calcium in plants. Ann. Bot. (Lond.) 92:487–511.

Williams, R.R. 1993. Mineral nutrition *in vitro*—A mechanistic approach. Aust. J. Bot. 41:237–251.