Influence of Supplemental Calcium Fertilization on Potato Tuber Size and Tuber Number

Senay Ozgen, Jiwan P. Palta
Dept. of Horticulture
575 Linden Drive
University of Wisconsin, USA
Madison, WI 53706
Corresponding author:
JPPalta@facstaff.wisc.edu

Matthew D. Kleinhenz
Dept. of Horticulture and Crop Science
The Ohio State University
Ohio Agricultural Research &
Development Center
1680 Madison Avenue
Wooster, OH 44691-4096, USA

Keywords: *Solanum tuberosum,* Russet Burbank, nitrogen nutrition, tuber calcium concentration, potato yield, tuberization, tuber development

Abstract

Recent studies, including those from our laboratory, have provided evidence that by improving tuber Ca level we can reduce tuber internal defects and improve its storability. The purpose of the present study was to determine the influence of supplemental Ca fertilization on tuber size and tuber number. For this purpose, plantlets of *Solanum tuberosum* cv Russet Burbank raised in tissue culture were planted in 20 L pots filled with sandy loam field soil with the pH of 6.9 and soil Ca level of 350 ppm. All treatments received the same total amount of N (at the rate of 280 kg.ha^{-1}). Four treatments were evaluated: 1) non-split N (from ammonium nitrate), 2) split N (from ammonium nitrate), 3) split N (from liquid N (50%ammonium nitrate+50% urea)) + calcium chloride, and 4) split N (from calcium nitrate). The total Ca was applied at the rate of 168 kg.ha^{-1}. Supplemental Ca was applied on a split schedule (equally split at four, six, and eight weeks after planting). Four months after planting, tubers were harvested and evaluated. In general all Ca treatments had lower tuber number and greater tuber size as compared to the non-split N control. Although total yield was unaffected the percentage of total tubers (number) as well as the percentage of the total yield from the grade A tubers was increased by all Ca applications. Present study provides evidence that tuberization signal can be influenced by Ca level in the soil. These results suggest that the Ca content of the soil can influence both potato tuber number and tuber size and that by increasing soil Ca one may increase average tuber size and decrease tuber number. We suggest that soil Ca influences tuberization by altering the hormonal balance at the stolon tip.

INTRODUCTION

Calcium is one of the most abundant elements in soil (Mengel and Kirkby, 1987) and this element is known to play several important roles in plant membrane structure and function (Marschner, 1995; Palta, 1996). Calcium contributes to maintenance of cell membrane stability and wall structure (Marschner, 1995; Palta, 1996). Calcium deficiency has been linked to many disorders especially in fruit and storage organs (Bangerth, 1979) and has been shown to improve quality and yield of these plants. For example, calcium application in the zone of peanut formation can increase the Ca content of peanut fruit and improve peanut yield and grade (Coffelt and Hallock, 1986; Walker and Csinos, 1980). Both peanuts and potatoes are underground storage organs with low rates of transpiration. Low Ca concentration often occurs in organs with low rates of transpiration, such as potato tubers. There are many potato disorders, such as brown center, hollow heart, that were thought to be related to tuber Ca level (Bangerth, 1979). A number of recent studies have shown that the Ca content of tubers may affect their susceptibility to bacterial soft rot caused by *Erwinia caratovora* (Cother and Cullis, 1992; McGuire and Kelman, 1984; Kleinhenz et al., 1995; Kratzke, 1988) and internal defects of tubers (Kleinhenz et al., 1999; Ozgen et al., 2000; Gunter et al., 2000; Karlsson et al., 2001).

Proc. XXVI IHC – Potatoes - Healthy Food for Humanity
Ed. R.Y. Yada
Acta Hort. 619, ISHS 2003
Publication supported by Can. Int. Dev. Agency (CIDA)

The role of Ca as a secondary messenger in plant cells is well established (Marme, 1982; Hepler and Wayne 1985; Poovaiah and Reddy, 1993). Balamani et al. (1986) used lanthanum (La^{3+}) to inhibit calcium uptake by potato plantlets grown in sterilized media. This resulted in the inhibition of tuberization. They were able to restore the tuberization response by adding Ca into the medium, suggesting that lack of Ca in media can inhibit tuberization. Tuberization in potato plants is controlled by environmental and nutritional factors, which are known to affect the level of endogenous growth substances (Wareing and Jennings, 1979). Short days and cool night temperatures promote tuberization whereas long days, high night temperatures, and high nitrogen fertilization delay or inhibit this process (Menzel, 1980; Sattelmacher and Marschner, 1978). Plant hormones have also been studied with respect to their influence on tuberization. Tuberization of the stolons has been found to be promoted by cytokinins (Hussey and Stacey, 1984; Palmer and Smith, 1969) and inhibited by gibberellin (Hussey and Stacey, 1984; Wareing and Jennings, 1979). Furthermore GA has been found to inhibit cytokinin-mediated tuberization of *Solanum tuberosum* (Hussey and Stacey, 1984). Ethylene has been found to inhibit tuberization (Mingo-Castel et al., 1976) and auxin (IAA) promote tuber set (Kumar and Baijal, 1979).

Recently, a tuber-inducing substance termed tuberonic acid (a jasmonic acid derivative), has been postulated to function by disrupting cortical microtubules in stolon cells, allowing radial growth associated with tuberization (Koda et al.,1988; Matsuki et al., 1992). The organization of cortical arrays must be coordinated with other cellular events; the evidence suggests that phosphorylation/dephosphorylation and calcium/calmodulin system may be involved (Cyr and Palevitz, 1995). These results further suggest that Ca may influence tuberization.

A previous growth chamber experiment utilizing inert media showed tuber number and size influenced by root zone calcium (Fig. 1). Our objective in this study was to determine the influence of "in season" Ca fertilization on tuber size, tuber number and Ca content of the tuber. Field soil was used to examine the influence of soil calcium on tuberization under close to commercial production conditions.

MATERIALS AND METHODS

Plants of *Solanum tuberosum* L (cv 'Russet Burbank') were raised from micropropageted stem cuttings (Steffen et al., 1989). Uniform rooted and single stemmed micropropagated plantlets were transplanted to 0.01 m^3 size plant containers filled with Jiffy Mix (JPA, East Chicago, IL., USA). Transplants were kept covered with clear plastic for the first 2 days to minimize transplant shock and avoid desiccation. Two weeks after transplanting, plants were transferred to 20 L (30 cm diameter) pots containing 1:1 (V:V) loamy- sand soil and perlite. The soil for this purpose was collected from the top 15-20 cm layer at the University of Wisconsin - Madison Hancock Agricultural Research station. Soil analyses gave values of 2 meq/100g (CEC), 0.7 % organic matter, and nutrients 66 (P), 160 (K), 350 (Ca) and 100 (Mg) ($mg.kg^{-1}$ soil). According to this soil test N, P, and K requirements for potato production were estimated as 40 $kg.ha^{-1}$, 156 $kg.ha^{-1}$ and 156 $kg.ha^{-1}$ respectively. Based on the 5880 plants per hectare the N P K requirements per plant were calculated. The experiment was conducted in a greenhouse at the University of Wisconsin - Madison. A randomized complete block design with four nutrient treatments with 8 replicates per treatment was employed. Each replicate for every nutrient treatment consisted of one plant. Daily minimum and maximum temperatures were approximately 20/18ºC during the experimental period.

Two weeks after planting in the 20 L pots, the equivalent to 112 $kg.ha^{-1}$ nitrogen was given to all the plants as calculated above. This coincided with the emergence application for field plants. All treatments received identical amounts of total N (equivalent to 280 $kg.ha^{-1}$). Non-split N treatment received all N at 4 weeks after planting (to coincide with grower's practice of putting all N by final hilling) from ammonium nitrate (34N-0P-0K); 2). All other treatments received nearly 50% of N at 2 weeks after planting the rest of the N was given as four equal split applications from ammonium

nitrate, calcium nitrate (9:0:0:11) or liquid N (50% ammonium nitrate + 50% urea) at 4,6,8 and 10 weeks after planting.

All Ca treatments received the same total amount of Ca (at the rate of 168 $kg.ha^{-1}$) either from calcium chloride (0:0:0:27.3) or calcium nitrate (9:0:0:11) as 4 equal split applications (4,6,8 and 10 weeks after planting). All nutrients were dissolved in water and given to the plants by pouring at the top of soil. Leachate from each pot, if any, was collected and given back to the same plant.

Tubers were harvested 125 days after planting. At harvest, tubers from each pot were rinsed free of soil, weighed individually and graded (<56 g, ≥56g). After grading 5 tubers (≥ 56 g) were collected from each pot for the determination of tissue calcium concentration. Samples for tissue calcium analysis were obtained by removing a 1 cm thick longitudinal slice from the center of each tuber including the stolon attachment and apical bud. The periderm and most of the cortex were removed from the tissue with a razor blade and discarded. The remaining non-periderm tissue was prepared for Ca analyses as described by Kratzke and Palta (1986). For this purpose the samples were dried in an oven at 70 ºC, ground, weighed and ashed (450 ºC, 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 in a final concentration of 0.2 N HCl and 2000 mg L^{-1} of lanthanum chloride. Calcium concentration was determined by atomic absorption spectrophotometry (Varian model Spectraa-20, Varian Associates, Inc., Sunnyvale, California, USA).

RESULTS

Compared to non-split N control, application of Ca either from calcium chloride or from calcium nitrate resulted in an increase in calcium concentration of the tuber non-periderm tissue (Table 1). A combination of calcium chloride and liquid N gave the highest tuber Ca concentration (Table 1). The non-periderm Ca concentration was increased from 158 ppm in non-split control to 245 ppm in calcium chloride treatment, which an increase of nearly 60 %. Although split N application appear to increase the average non-periderm Ca concentration compared to non-split N, this increase was not significant.

There were no significant differences in total tuber yield among treatments (Table 1). Plants given Ca produced fewer tubers than the non-split N treatment. (Table 1). Split application of calcium nitrate produced the lowest number of tubers per plant. Total number of tubers on average per plant was only 15 in the calcium nitrate treatment as compared to 24 and 21 for the non-split and split N treatments respectively. Mean tuber weight was higher in the Ca treated plants as compared to the non-split N control (Table 2). There was a significant difference among treatments for the proportion of tuber greater than 56 g (Table 2). Average tuber size was highest in the calcium nitrate treatment. In this treatment, mean tuber weight was about 60 g as compared to 40 g in the non-split N treatment (Table 2). In both the calcium treatments, nearly 49% of the tubers had tuber weight greater than 56g, which is equivalent to US grade A. However, only 28% of the tubers were of US grade A size in the non-split N control treatment. The split N treatment tended to produce larger tubers than the non-split N but these differences were not significant.

DISCUSSION

In this study we attempted to produce potato plants similar to field conditions using 20 L pots filled with field soil. Plants grow well and produced 800-900 g tuber yield per plant. This yield is quite equivalent to what is expected under good field conditions.

Wisconsin soils routinely show ammonium acetate-extractable Ca levels of 250-1000 $mg.kg^{-1}$ soil (Simmons and Kelling, 1987) and the soil used in this study had a Ca level of 350 $mg.kg^{-1}$. Although low for this region, Ca levels in soil used in this study exceeded those commonly thought necessary to satisfy vegetative plant demand for Ca. In our study, split application of Ca at the rate of 168 $kg.ha^{-1}$ from either calcium nitrate or

calcium chloride resulted in an increase in mean tuber Ca concentration in non-periderm tissue (Table 1). These results suggest that application of soluble forms of Ca during bulking period can enhance the Ca level of the non-periderm tissue. These results are consistent with earlier studies and support the concept that tuber calcium concentration can be significantly increased by application of soluble Ca during bulking period (Kratzke and Palta, 1985 and 1986; Kleinhenz et al., 1999; Ozgen et al., 2000; Gunter et al., 2000; Karlsson et al., 2001). In these earlier studies, tubers were shown to have tiny roots growing directly out of the tubers during tuber growth and development (Kratzke and Palta, 1985; Struckmeyer and Palta, 1986). These functional roots were demonstrated to supply water to the tubers (Kratzke and Palta, 1985). Since Ca moves in xylem along with water tuber roots could carry nutrients such as Ca to the tubers (Palta, 1996).

The present study provides evidence that soil calcium levels can alter tuber size and tuber number in potatoes (Table 1 and 2). Our results further show that both calcium nitrate and calcium chloride were effective in increasing average tuber size and decreasing tuber number. The main influence of calcium appears to be on tuber number. Since total tuber yield was not influenced (Table 1) the increase in tuber size would result if plant had fewer tubers to bulk.

The mechanism by which soil calcium may alter tuber number on a potato plant is not known. The signal for tuberization has been reported to be under complex biochemical control and involves hormones such as cytokinins and gibberellins (Escalante and Langille, 1998; Kumar and Wareing, 1974; Railton and Wareing, 1973; Smith and Palmer, 1970). Calcium is known to act as a signaling molecule that can regulate metabolism and mitigate the effect of heat and cold stresses on potatoes (Vega-Semorile et al., 1996; Tawfik et al., 1996; Kleinhenz and Palta, 2002; Palta, 1996). These and other roles of calcium have been reported, in part, to involve alteration of hormonal signals (Saunders, 1990). Thus it is possible that an increase in the calcium concentration in the soil influences the tuberization signal via changing the biochemical processes such as altering the hormonal balance at the stolon tip. Further studies are needed to elucidate the exact mechanism by which Ca may alter tuberization signal in potatoes. On a practical level our results suggest that it may be possible to alter tuber number and tuber size by manipulating the soil Ca level.

Literature Cited

Balamani, V., Veluthambi, K. and Poovaiah, B.W. 1986. Effect of calcium on tuberization in potato. Plant Physiol. 80:856-858.

Bangerth, F. 1979. Calcium-related disorders of plants. Ann. Rev. Phytopathol. 17:97-122.

Coffelt, T.A. and Hallock, D.L. 1986. Soil fertility responses of Virginia type peanut cultivars. Agron. J. 78:131-137.

Cother, E.J. and Cullis, B.R. 1992. The influence of tuber position on periderm calcium content and its relationship to soft rot susceptibility. Potato Res. 35:271-277.

Cyr, R.J. and Palevitz, B.A. 1995. Organization of cortical microtubules in plant cells. Curr. Opin. Cell Biol. 7:65-71.

Gunter, C., Ozgen, S., Karlsson, B. and Palta, J.P. 2000. Calcium application at pre-emergence and during bulking may improve tuber quality and grade. HortScience 35:498.

Escalante, B.Z. and Langille, A.R. 1998. Photoperiod, temperature, gibberellin, and anti-gibberellin affect tuberization of potato stem segments in vitro. HortScience 33(4):701-703.

Hepler, P.K. and Wayne, R.O. 1985. Calcium and plant development. Ann. Rev. Plant Physiol. 36:397-439.

Hussey, G. and Stacey, N.J. 1984. Factors affecting the formation of in vitro tubers of potato (*Solanum tuberosum* L.). Ann. Bot. 53:565-578.

Karlsson, B., Palta, J.P. and Ozgen, S. 2001. Reduction of potato tuber bruising and internal defects by supplemental calcium field application. Am. J. Potato Res.78:462.

Kleinhenz, M.D. James, R.V., Stevenson, W.R. and Palta, J.P. 1995. Calcium application increases potato tuber medullary tissue calcium concentration and may reduce the incidence and severity of soft rot due to *Erwinia carotovora* pv. *atroseptica*. HortScience 30:623.

Kleinhenz, M.D., Palta, J.P., Gunter, C.C. and Kelling, K.A. 1999. Impact of source and timing of calcium and nitrogen on 'Atlantic' potato tuber calcium concentrations and internal quality. J. Am. Soc. Hort. Sci. 124(5):498-506.

Kleinhenz, M.D. and Palta, J.P. 2002. Root zone calcium modulates the response of potato plants to heat stress. Physiol. Plant 115:111-118.

Koda Y., Omer, E-S.A., Yoshihara, T., Shibata, H., Sakamura, S. and Okazawa, Y. 1988. Isolation of a tuber inducing substance from potato leaves. Plant Cell Physiol. 29:1047-1051.

Kratzke, M.G. 1988. Study of mechanism of calcium uptake by potato tubers and of cellular properties affecting soft rot. Ph.D. Thesis. The University of Wisconsin-Madison, Department of Horticulture, p.192-250.

Kratzke, M.G. and Palta, J.P. 1985. Evidence for the existence of functional roots on potato tubers and stolons: Significance in water transport to the tubers. Am. Potato J. 62:227-236.

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

Kumar, D. and Wareing, P.F. 1974. Studies on tuberization of *Solanum andigena*. New Phytol. 73:833-840.

Kumar, P. and Baijal, B.D. 1979. The role of various growth regulators on growth and development of potato (*Solanum tuberosum* L) II. Stolon development, tuber induction and yield. Agra Univ. J. Res. 28:135-140.

Marme, D. 1982. The role of calcium and calmodulin in plants. What's New Plant Physiol. 13:37-40.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, p. 285-299.

Matsuki T., Tazaki, H., Fujimori, T. and Hogetsu, T. 1992. The influences of jasmonic acid methyl ester on microtubules in potato cells and formation of potato tubers. Biosci. Biotechnol. Biochem. 56:1329-1330.

McGuire, R.G. and Kelman, A. 1984. Reduced severity of *Erwinia* soft rot in potato tubers with increased calcium content. Phytopathology 74:1250-1256.

Mengel, K. and Kirkby, E.A. 1987. Principles of plant nutrition. 4th ed. International Potash Institute, Bern, Switzerland, p. 455-480.

Menzel, C.M. 1980. Tuberization in potato at high temperatures: Responses to gibberellin and growth inhibitors. Ann. Bot. 46:259-265.

Mingo-Castel, A.M., Smith, O.E. and Kumamoto, J. 1976. Studies on the carbon dioxide promotion and ethylene inhibition of tuberization in potato explants cultured in vitro. Plant Physiol. 57:480-485.

Ozgen, S., Gunter, C., Karlsson, B. and Palta, J.P. 2000. Supplemental application of calcium and nitrogen improves internal quality of "Russet Burbank" potatoes. HortScience 35:498.

Palmer, C.E. and Smith, O.E. 1969. Cytokinins and tuber initiation in the potato *Solanum tuberosum* L. Nature 221:279-280.

Palta, J.P. 1996. Role of calcium in plant responses to stresses: Linking basic research to the solution of practical problems. Proceedings of Colloquium: Recent advances in plant responses to stress: Bridging the gap between science and technology. Hort. Sci. 31(1):51-57.

Poovaiah, B.W. and Reddy, A.S.N. 1993. Calcium and signal transduction in plants. Crit. Rev. Plant Sci. 12(3):185-211.

Railton, I.D. and Wareing, P.F. 1973. Effects of daylength on endogenous gibberellins in leaves of *Solanum andigena*. I. Changes in levels of free acidic gibberellins-like substances. Physiol. Plant. 28:88-94.

Sattelmacher, B. and Marschner, H. 1978. Relation between nitrogen nutrition, cytokinin activity and tuberization in *Solanum tuberosum*. Physiol. Plant 44:65-68.
Saunders, M.J. 1990. Calcium and plant hormone action. Soc. Exp. Biol. 44**:**271-283.
Simmons, K.E. and Kelling, K.A. 1987. Potato responses to calcium application on several soil types. Am. Potato J. 64:119-136.
Smith, O.E. and Palmer, C.E. 1970. Cytokinin-induced tuber formation on stolons of *Solanum tuberosum*. Physiol. Plant. 23:599-606.
Steffen, K.L., Arora, R. and Palta, J.P. 1989. Relative sensitivity of photosynthesis and respiration to freeze-taw stress in herbaceous species: Importance of realistic freeze-taw protocols. Plant Physiol. 89:1372-1379.
Struckmeyer, B.E. and Palta, J.P. 1986. Anatomical evidence for the existence of roots on potato tubers and stolons. Am. Potato J. 63:57-60.
Tawfik, A.A., Kleinhenz, M.D. and Palta, J.P. 1996. Application of calcium and nitrogen for mitigating heat stress effects on potatoes. Am. Potato J. 73:261-273.
Vega-Semorile, S.E., Bamberg, J.B. and Palta, J.P. 1996. Potential for improving freezing stress tolerance of wild potato germplasm by supplemental calcium fertilization. Am. Potato J. 73:397-409.
Walker, M.E., and Csinos, A.S. 1980. Effect of gypsum on yield, grade and incidence of pod rot in five peanut cultivars. Peanut Sci. 7:109-113.
Wareing, P.F. and Jennings, A.M.V. 1979. A hormonal control of tuberization in potato. In: F. Skoog, ed, Plant Growth Substances, Proc 10th Int. Conf. Springer-Verlag, Berlin, p. 293-300..

Tables

Table 1. Treatment effect on mean tuber calcium (μg/g) , total tuber yield (g/plant) and total tuber number (#/plant). Mean values with same letter are not significantly different from each other based on SAS General Linear Model procedure. LSD(α=0.05).

	TREATMENT EFFECT ON		
Treatment	Mean Tuber Calcium (μg/g)	Total Tuber Yield (g/plant)	Total Tuber Number (#/plant)
Non-split N	157.92c	966.73a	24.0a
Split N	169.26bc	956.68a	21.12ab
Split calcium nitrate	196.13b	886.51a	15.37c
Split calcium chloride + liquid N	244.63a	920.89a	17.62bc

Table 2. Treatment effect on mean tuber weight (g) and proportion of tubers >56 g (% of total). Mean values with same letter are not significantly different from each other based on SAS General Linear Model procedure. LSD(α=0.05).

	TREATMENT EFFECT ON	
Treatment	Mean Tuber Weight (g)	Proportion of Tubers >56 g (% of total)
Non-split N	41.26b	28.08b
Split N	48.72ab	41.88ab
Split calcium nitrate	59.50a	48.42a
Split calcium chloride + liquid N	54.40a	47.67a

Figures

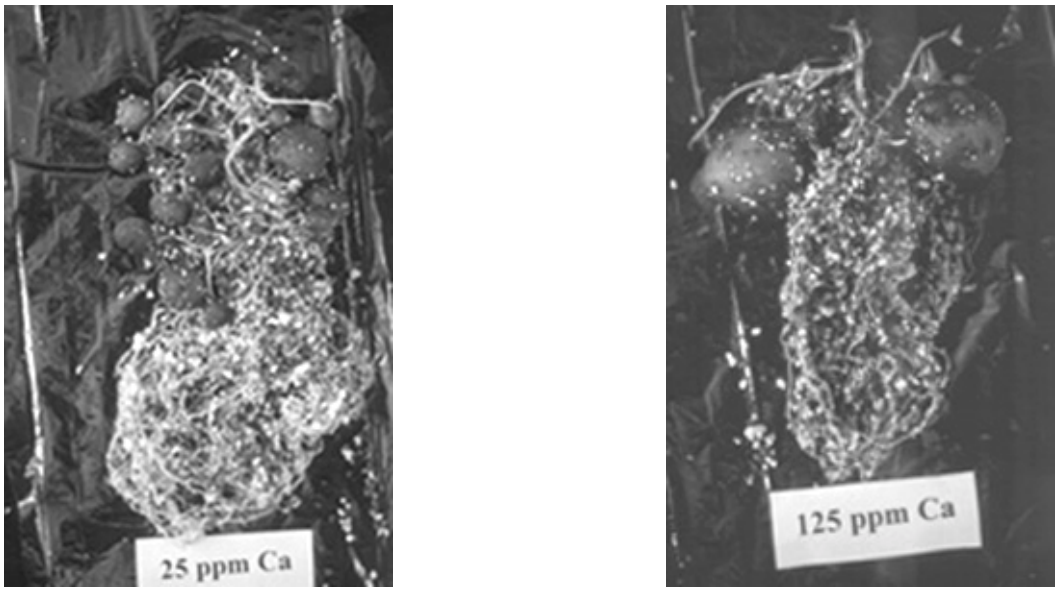

Fig. 1. A precisely controlled study using inert media and growth chamber conditions showed that tuber number and size are influenced by root zone calcium. Plants continuously drip-irrigated with a complete nutrient solution containing 25 ppm (left), 125 ppm (right) calcium from calcium chloride.

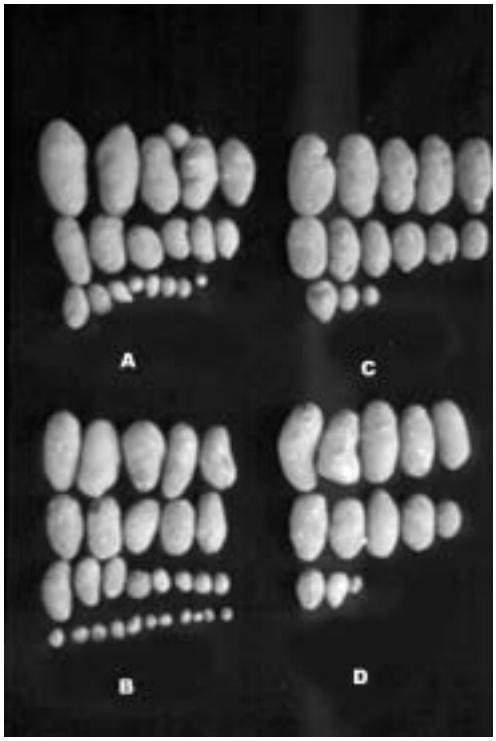

Fig. 2. The present study with field soil showed that tuber number and size are influenced by root zone calcium. All treatments received identical amounts of total N (equivalent to 280 $kg.ha^{-1}$): (*A*) Non-split N treatment received all N at 4 weeks after planting (all N by final hilling) from ammonium nitrate; (*B*) Split N treatments received N as 4 equal split applications (at 4, 6, 8, and 10 weeks after planting); (*C*) Split calcium nitrate treatment received both N and Ca (at the rate of 168 $kg.ha^{-1}$ from calcium nitrate) as 4 equal split applications (at 4, 6, 8, and 10 weeks after planting); (*D*) Split calcium chloride +liquid N treatment also received both N (from liquid N) and Ca (at the rate of 168 $kg.ha^{-1}$ from calcium chloride) as 4 equal split applications (at 4, 6, 8, and 10 weeks after planting).