

# Effects of calcium concentrations in medium on microtuberization of potato (*Solanum tuberosum* L.)

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## Abstract

It is now widely accepted that calcium (Ca) deficiency is linked to potato (*Solanum tuberosum* L.) tuber disorders and improved tuber health is expected through increased Ca availability. The purpose of this study was to optimize the Ca concentration in Murashige-Skoog (MS) basal medium for microtuber production and determine microtuber mineral composition, especially Ca content. The response of cultivars 'Bintje' and 'Russet Burbank' to the Ca concentration in the media including 3 (control), 10, 15, 20, or 25 mM was different for most parameters measured. Ca at 10 mM concentration improved overall performance of 'Bintje' but not 'Russet Burbank', compared to controls (3 mM Ca). Higher levels of Ca in the medium were somewhat toxic to both cultivars. Increased Ca concentration in the medium did not affect final shoot fresh weight (SFW) in 'Bintje' but reduced it in 'Russet Burbank' compared with control medium. Ten mM Ca increased microtuber number by 19% in both cultivars. It also increased microtuber fresh weight by 19.5% in 'Bintje' with no effect on 'Russet Burbank'. Mean tuber weight was not affected in 'Bintje' but was decreased by 17% in 'Russet Burbank'. Harvest index in both cultivars was slightly improved at 10 mM Ca but higher levels reduced this index significantly. Microtuber tissue Ca content was greatly increased by Ca treatment in both cultivars. Maximum increase was observed at 15 mM Ca (70%) and 10 mM Ca (61%) in 'Bintje' and 'Russet Burbank', respectively. Ten mM Ca also increased P (25%) and K (19%), in 'Bintje' whereas in 'Russet Burbank' only P was increased up to 20% at this level of Ca. Unlike Zn, accumulation of other microelements were affected rather differently in two cultivars. Ten mM Ca had no reducing effects on Fe, Mn and Cu accumulation in 'Bintje' whereas it had significant reducing effects in 'Russet Burbank'. It is concluded that the conventional concentration of Ca in MS medium (3 mM) is not high enough for optimum microtuberization. Hence, it appears that optimization of the Ca concentration is necessary for each cultivar.

**Keywords:** Calcium optimization; Microtuberization; MS Medium; Potato

## INTRODUCTION

Microtubers are small tubers produced *in vitro* on single-node cuttings or layered shoots under tuber inducing conditions in small stationary containers (Wang and Hu, 1982), fermentors and bioreactors (Akita and Takayama, 1988, 1993; Akita and Ohta, 1998; Hulscher *et al.*, 1996). Microtubers are now widely used for several purposes throughout the world. They can be used to produce minitubers or used for screening genotypes for all of the important tuber characteristics such as color, shape, yield and average weight (Gopal and Minocha, 1997, 1998). Microtubers are easier to transport and handle than plantlets and are less delicate, so requires less after care, when planted in a greenhouse or screenhouse (Hoque *et al.*, 1996). For more detailed information about microtubers, the reader is referred to a recent comprehensive review article by Donnelly *et al.* (2003).

More recently, microtubers have been considered as potent alternative propagule to plantlets and minitubers for direct field planting. Haverkort *et al.* (1991) and Ranalli *et al.* (1994) harvested tubers from field planted microtuber (MT) plants and reported that they yielded 50% less than those produced by conventional tubers (CT) whereas more recently, the results from 3 years experiment comparing microtuber (1-3 g) with CT (50 g) to produce tuber yield, revealed that yield from MT was 84% of that of CT, suggesting a powerful potential for using MT for field planting (Kawakami *et al.*, 2003). Therefore, the use of microtuber technology in seed tuber production, breeding programs, germplasm conservation and research has enormous potential.

Calcium plays important roles in plants including maintenance of stability and wall structure (Marschner, 1995; Palta, 1996). Calcium deficiency

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very often occurs in organs with low transpiration rate such as potato and has been linked to many disorders such as Brown Center (BC), Internal Brown Spot (IBS), hollow heart (Bangerth, 1979), susceptibility to bacterial rot caused by *Erwinia caratovora* (Cothier and Cullis, 1992; Kleinhenz *et al.*, 1995) and internal defects of tubers (Ozgen *et al.*, 2000; Karlsson *et al.*, 2001). Within any single cultivar a reduction in tuber calcium content is associated with an increase in IBS and high correlations between Ca content of the peel and incidence of IBS have been reported (Collier *et al.*, 1980). However, by comparing the response of several cultivars it is evident that there is no clear threshold below which the disorder is induced. For example, a calcium concentration of 30 g.kg<sup>-1</sup> fresh weight may be associated with a 0% to 90% incidence of IBS (Davies, 1988). Application of Ca in pot grown potato plants decreased tuber number, increased tuber Ca content with no effect on tuber yield in 'Russet Burbank' (Kleinhenz *et al.*, 1999; Ozgen and Palta, 2004). Calcium plays an important role in tuberization *in vitro*. Inhibition of its uptake by potato plantlets resulted in the inhibition of tuberization, which was restored by adding Ca to the medium (Balamani *et al.*, 1986).

Microtubers are dormant following harvest and are usually cold-stored for up to 6 months before planting. Increased microtuber calcium concentration could extend storage life as it has been reported for tubers (Poovaiah, 1986). The aim of this study was to investigate the effects of supplemental medium calcium concentration on shoot fresh weight, microtuber yield parameters (total number and fresh weight, mean microtuber weight), harvest index and tissue analysis, especially calcium content in two cultivars.

## MATERIALS AND METHODS

*In vitro* plantlets of 'Binjte' and 'Russet Burbank' cultivars were obtained from the Plant Propagation Centre (Fredericton, N.B. Canada). The plantlets were micropropagated using single-node cuttings with one leaf and a lateral bud. These were grown in 25 × 150 mm test tubes containing 10 ml of MS basal salt medium and organics (Murashige and Skoog, 1962), with increased thiamine HCl (1 mg l<sup>-1</sup>), myo-inositol (100 mg l<sup>-1</sup>) and 3% sucrose. The medium was solidified with 0.7% agar (Anachemia, Montreal, QC, Canada) and adjusted to pH 5.7 prior to autoclaving at 121°C for 20 min. Cultures were incubated at 25 ± 2°C with 16/8 h day/night cycle at 65 µmolm<sup>-2</sup>s<sup>-1</sup> photon flux density (cool white fluorescent light).

For microtuberization, the 2-step procedure of Leclerc *et al.* (1994) was used. Micropropagated plantlets were transferred to pre induction medium in plastic containers containing 60 ml MS liquid medium along with 3 (control), 10, 15, 20 and 25 mM Ca, kept at 25 ± 2°C under 100 µmolm<sup>-2</sup>s<sup>-1</sup> and 16/8h day/night cycle for four weeks after then transferred to microtuber induction and growth medium on 60 ml of liquid MS medium in plastic containers containing elevated sucrose (8%), Ca treatment as in pre condition stage, reduced temperature (15°C) and short days (8/16h day/night light) for 8 weeks. The medium was renewed after 4 weeks to compensate for the rapid glucose hydrolysis

Ca treatments and cultivars were factorially arranged using a completely randomized design (CRD) with four replications and three plantlets per container. The experiment was performed twice under similar conditions. Microtubers were separated from shoots and both were weighed at the time of harvest i.e., two months after induction. Harvested microtubers were counted and weighed. Harvest index (HI) was calculated: HI = microtuber fresh weight/shoot fresh weight + microtuber fresh weight. For tissue analysis including, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu), microtubers were dried (70°C, 48h), ground, weighed, ashed (450°C, 8h) and dissolved in HCl. Data from both experiments were combined and subjected to analysis of variance using SAS (1988, version 6.03, Cary, N.C.). Means were separated using the LSD test at the 5% level.

## RESULTS

**Effects of medium Ca concentration on shoot and microtuber yields (Table 1):** Medium Ca concentration did not affect final shoot fresh weight (SFW) in 'Bintje'. However, Ca levels above 3 mM significantly reduced SFW in 'Russet Burbank'. While 10 mM medium Ca concentration significantly increased microtuber numbers for both cultivars (19% increase), as compared to controls (3 mM Ca). Microtuber numbers was not increased further at medium Ca concentrations >10 mM.

The response of the two cultivars to microtuber fresh weight production was different. In case of 'Bintje', 10 mM Ca could significantly increase fresh weight by 19.5% whereas in 'Russet Burbank', this level of Ca had no effect on shoot fresh weight. Higher levels of Ca significantly reduced microtuber fresh weight in both the cultivars. In 'Bintje', the mean

**Table 1.** Effects of calcium treatments on microtuberization of 'Bintje' and 'Russet Burbank'.

Ca (mM)	SFW (g)	Binjte				Harvest Index
		Tuber Number	Tuber Weight (g)	Mean Tuber Weight (mg)		
3	14.0±2.1bcd	11.0±0.2b	6.82±0.1c	627±60bc	0.34±0.06ab	
10	15.3±1.7b	13.1±0.4a	8.15±0.1a	620±15bc	0.36±0.01a	
15	13.9±0.7bcd	10.4±1.1b	5.90±0.5cd	570±50c	0.29±0.02bc	
20	13.1±0.2cd	11.6±0.2ab	5.49±0.4ed	473±35f	0.29±0.04bc	
25	12.6±1.0d	11.3±0.3bc	5.50±0.2d	447±20f	0.31±0.01ab	
<b>Russet Burbank</b>						
3	17.4±0.2a	8.4±0.2d	7.05±0.3b	847±15a	0.30±0.01b	
10	15.0±1.0bc	10.0±0.3c	6.97±0.2bc	699±20b	0.32±0.02ab	
15	13.0±0.1cd	8.1±1.2d	4.52±0.1e	481±45ef	0.24±0.02cd	
20	14.0±0.5bcd	8.0±1.0d	5.00±1.2de	630±60bc	0.22±0.02d	
25	10.4±1.2e	7.3±0.2d	2.84±1.0f	400±20f	0.22±0.01d	

Means in each column with similar letters are not significantly different (LSD test, 5% level). Data are presented as mean ± standard errors. Total shoot fresh weight (SFW).

microtuber weight was not affected by 10 mM Ca and was reduced at 20 and 25 mM Ca by 20% and 29%, respectively. In 'Russet Burbank' different concentrations of Ca reduced the mean microtuber weight. Harvest index was not affected by Ca treatment in 'Bintje', whereas in 'Russet Burbank', Ca levels beyond 15 mM reduced it when compared with control.

**Effects of medium Ca concentration on microtuber chemical composition (Table 2):** In both cultivars, in contrast to control, addition of 10 mM Ca significantly ( $P<0.01$ ) increased phosphorus content of microtubers (25% in 'Bintje' and 20% in 'Russet Burbank's). Ten mM Ca substantially increased potassium content of microtuber by 18.5% in 'Bintje' and no changes were observed in 'Russet Burbank'. Calcium treatment had highly significant ( $P<0.01$ ) effect on Ca content of microtuber in both cultivars. In 'Bintje', Ca content

increases were 40%, 70% and 29% at 10, 15 and 20 mM Ca, respectively. In 'Russet Burbank', the increases were 60% and 21% at 10 and 15 mM, respectively. In 'Bintje', 10 and 15 mM Ca had no effects on Mg content whereas in 'Russet Burbank', all levels of Ca reduced it significantly ( $P<0.01$ ). Compared with control, Fe content of microtuber was not affected in 'Bintje', up to 20 mM but at 25 mM, significant reduction (20%) was observed. In 'Russet Burbank', only 10 and 15 mM Ca significantly reduced Fe content and at higher levels no effect was observed. Mn content of microtubers was not affected by Ca treatments in 'Bintje', whereas in 'Russet Burbank', all Ca levels reduced it. Ca treatment had no effect on Zn content in both cultivars. Cu content of microtubers was not affected at 10 mM Ca in 'Bintje' but at higher levels of Ca significant reduction was observed. In 'Russet Burbank' all levels of Ca highly reduced Cu content of microtuber tissues

**Table 2.** Effects of Ca treatments on chemical composition of microtubers.

Ca (mM)	P (%)	K (%)	Bintje					
			Ca	Mg	Fe (mg.kg <sup>-1</sup> )	Mn	Zn	Cu
3	0.51±0.05cd	2.1±0.11d	163.0±25.1d	819±40.1c	53±7.2a	39.8±3.5def	41.6±8a	3.1±0.6bc
10	0.64±0.04ab	2.49±0.08b	227.6±15.6b	836±3.6c	55±1.1a	37.1±6.2ef	49.8±4a	3.7±0.6ab
15	0.56±0.06bcd	2.23±bcde	277.0±28.1a	723±40.2cd	54±5.0a	41.0±20.1ef	40.6±6a	2.3±0.2de
20	0.52±0.01cd	2.02±ed	210.8±45.4bc	682±80.5d	49±5.0ab	30.4±10ef	45.6±9a	2.1±0.1de
25	0.56±0.15bcd	2.25±bcde	136.5±e	669±20.5d	42±1.1bcd	27.6±5.1f	41.2±8a	2.1±0.2de
<b>Russet Burbank</b>								
3	0.54±0.02bcd	2.16±0.10cde	173.6±32.5d	1070±90.6a	50±4.8ab	97.4±2.1a	48.6±7a	4.2±0.2a
10	0.65±0.03a	2.41±0.15bc	279.4±45.1a	904±56.1b	34±2.6cd	68.8±4.5b	48.6±9a	3.3±0.2bc
15	0.58±0.02ab	2.33±0.05bcd	210.8±10.8b	841±20.3bc	30±5.1d	46.4±5.6cde	41.0±8a	1.9±0.7e
20	0.52±0.04cd	2.28±0.08bcde	180.0±17.2d	904±60.2b	48±2.1abc	53.0±12.1c	48.4±6a	2.9±0.1bcd
25	0.60±0.03ab	2.16±0.09cde	163.4±25.6d	850±45.1bc	46±2.5bc	48±2.5cd	51.0±4a	2.8±0.4cd

Means in each column with similar letters are not significantly different (LSD test, 5% level). Data are presented as mean ± standard errors.

## DISCUSSION

Microtuberization *in vitro* is comparable to tuberization in field-grown plants in response to day length (Ewing and Wareing, 1978) and starch and patatin accumulation (Duncan and Ewing, 1984; Paiva *et al.*, 1983). In this study, Ca did not increase SFW in 'Bintje' but caused reduction in 'Russet Burbank'. This supports the work of Sarkar *et al.* (2005) who reported that medium Ca concentration above the levels normally added to MS medium (3 mM) had no effect on SFW increase. However, they found that 5-7 mM Ca greatly improved microplant quality of two potato genotypes. Quality was assessed based on microplant green shoots, leaf senescence and degrees of different phenotypic abnormalities such as vitrification, hyperhydricity, abnormal stem swelling, shoot tip necrosis and leaf tip burn on a 0-5 scale. Improved microplant quality was also observed in the present study and may account for the 19.5% increase in total microtuber fresh weight of 'Bintje', grown in medium with 10 mM Ca concentration. While the SFW decreased by 14% there was no decrease in microtuber fresh weight in 'Russet Burbank', grown in medium with 10 mM Ca concentration. In present study, 10 mM medium Ca concentration consistently promoted microtuberization by 19% in both cultivars. These results are in agreement with Balamani *et al.*, (1986) who inhibited tuberization in a single node leaf cutting by using the Ca-chelator, ethylene glycol tetraacetic acid (EGTA) and with the Ca ionophore A 23187. Tuberization was restored by including CaCl<sub>2</sub> in the medium, implicating Ca in the tuberization response. However, Kleinhenz *et al.*, (1999) and Ozgen and Palta (2004) found significantly reduced tuber number in cultivars 'Atlantic' and 'Russet Burbank' exposed to Ca in greenhouse pot trials. The mechanism, by which Ca may alter the tuberization response, and its apparent cultivar-specificity, is not well understood. The tuberization signal is under complex hormonal control (Ewing, 1995). There is strong evidence for a critical role of gibberellin (GA) in tuberization (Ewing, 1995; Jackson, 1999).

Response of cultivars for total microtuber weight was different, as in contrast with control, 10 mM Ca increased microtuber weight in 'Bintje' by 20% with no effect in 'Russet Burbank'. Such different response could partly be due to shoot growth (biomass) and plantlet quality. In 'Bintje', 10 mM Ca slightly increased SFW (9%) whereas this level of Ca significantly decreased it (14%) in 'Russet Burbank'. Data from *in vitro* are different from pot grown plants for 'Russet Burbank', because no yield increase was

observed from pot plants of this cultivar (Ozgen and Palta, 2003). At 10 mM Ca, mean tuber weight was not affected in 'Bintje', probably because of promotive effect of Ca which can be seen from slight improvement in SFW production and harvest index. This level of Ca negatively affected 'Russet Burbank' mainly due to lower level of SFW production (14% lower) which seems to be toxic for this cultivar (Sarkar *et al.*, 2005).

Maximum calcium concentration in microtuber tissues was obtained in 'Bintje' at 15 mM Ca (277 mg.kg<sup>-1</sup>) and in 'Russet Burbank' at 10 mM (279 mg.kg<sup>-1</sup> FW). This is probably the maximum amount of Ca that could be taken up by microtubers because during the whole course of the experiment, all Ca absorbing parts including basal roots, stolon roots, and microtubers were thoroughly immersed in the medium (Habib and Donnelly, 2002; Busse and Palta, 2003). Although we could not find any similar *in vitro* experiment to compare our results with, but these results are very consistent with the reports from pot grown plants. Ozgen and Palta (2004) recorded 245 mg.kg<sup>-1</sup> FW Ca in 'Russet Burbank' tubers treated with a moderate amount of Ca (168 kg.ha<sup>-1</sup>). Davies (1998) reported that physiological disorders such as IBS incidence percentage is negatively correlated with tuber elements; P(-0.58), Mn(-0.54), K(-0.38) and Ca(-0.34). Therefore, in increasing medium Ca, one should be careful not to negatively affect those elements accumulation in microtubers. In present study, at 10 mM Ca, microtubers of 'Bintje' accumulated more P, K and Ca with no effect on Mg whereas in 'Russet Burbank', microtubers P and Ca increased but Mg decreased by 16%. Apart from Zn, of which accumulation was not affected by calcium treatment in both cultivars, calcium affected microelement accumulation in microtubers rather differently in cultivars. In 'Russet Burbank', 10 mM Ca reduced Fe, Mn and Cu accumulation significantly whereas, in 'Bintje', no reduction was observed for those elements.

It is concluded that standard level of Ca in MS medium (3 mM) is not high enough for optimum microtuberization and microtuber chemical content and should be optimized for each cultivar. For 'Bintje', 10 mM seems to be optimum whereas in case of 'Russet Burbank', levels between 3-10 Mm should be considered.

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