Use of Jasmonate for Conditioning of Potato Plantlets and Microtubers in Greenhouse Production of Minitubers

K. Pruski[1,2*], T. Astatkie[3], P. Duplessis[1], T. Lewis[1], J. Nowak[2,4], and P. C. Struik[5]

[1]Crop Diversification Centre North, 17507-Fort Road, Edmonton, AB, Canada, T5B 4K3.
[2]Department of Plant and Animal Sciences, Nova Scotia Agricultural College (NSAC), P.O. Box 550, 50 Pictou Rd., Truro, NS, Canada, B2N 5E3.
[3]Department of Engineering, NSAC, P.O. Box 550, Truro, NS, Canada, B2N 5E3.
[4]Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.
[5]Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, P.O. Box 430, 6700 AK Wageningen, The Netherlands.
*Corresponding author: Tel: (902)893-6032; Fax: (902)897-9762; Email: kpruski@nsac.ns.ca

ABSTRACT

A two-year study was conducted to determine the effects of (1) jasmonic acid (JA) pre-treatment, (2) JA supplement in culture media, (3) cultivar (Amisk, Atlantic, Russet Burbank, Shepody, and Umatilla Russet), (4) light (0 h, 8 h), and (5) dormancy breaking treatment (Rindite, gibberellic acid) on greenhouse production of minitubers from microtubers and *in vitro* plantlets. The microtubers were produced under short day (8 h) light conditions and in darkness, from stock plantlets pre-treated with JA and untreated, and on tuberization media with or without JA. *In vitro* plantlets (the industry choice in nuclear seed potato production) of all five cultivars performed well, meeting the standard criteria for greenhouse production of minitubers. Production of minitubers from microtuber-derived plants of cvs Amisk, Russet Burbank, and Umatilla Russet was similar to that of plantlet-derived plants with regard to number of minitubers. Yields (weight), however, were lower than those from plantlets. Microtuber responses to JA varied with cultivar. Amisk produced the highest number of minitubers per plot from microtubers derived from JA pre-treated plantlets. Jasmonic acid-pretreated microtubers also gave significantly more minitubers in Russet Burbank and Umatilla Russet than the microtubers from other treatments. Shepody did not benefit from JA treatments and JA pre-treated Atlantic microtubers performed poorly, producing significantly lower yields of minitubers than other cultivars. Independently of cultivar, microtubers produced under 8-h photoperiod gave significantly higher yields of minitubers than microtubers produced in the dark. Dormancy release was the key factor influencing microtuber performance. Rindite proved to be a much more effective dormancy breaking treatment than gibberellin. JA conditioning of stock plants prior to tuberization is being proposed as a treatment in production of microtubers for greenhouse production of minitubers.

INTRODUCTION

In vitro plantlets are commonly used for speeding up multiplication of disease-free plant material in elite seed potato programs, including greenhouse production of seed tubers, minitubers (also called nuclear tubers), and *in vitro* production of microtubers (Jones 1988; Lommen 1995; Struik and Wiersema 1999). Although minitubers became the primary choice of nuclear propagules by seed potato growers, several researchers believe that microtubers also have a good potential to be integrated into the seed programs (Lillo 1989; Struik and Lommen 1990; Lommen 1995; Khuri and Moorby 1996; Nasiruddin and Blake 1997; Kim et al. 1999; Struik and Wiersema 1999). Microtubers are considered an alternative to plantlets in germplasm storage and exchange (Estrada et al. 1986), but their use in the production of minitubers in greenhouses and/or pre-elite tubers in the field is still controversial (Ranalli et al. 1994; Ranalli 1997; Coleman et al. 2001) and not adopted by the industry. Most of the commercial production of minitubers is still based on tissue culture plantlets. Limited

Accepted for publication 19 February 2003.
ADDITIONAL KEY WORDS: Gibberellic acid, *in vitro* tuberization, jasmonic acid, microtubers, minitubers, Rindite, *Solanum tuberosum* L.

information on the production of microtubers at commercial scale, suitability of cultivars to specific microtuberization methods, and on their greenhouse and field performance comparisons to plantlets (Joung et al. 1993; Lê 1999; Dobránszki et al. 1999) is probably the most critical drawback. Both propagules, plantlets and microtubers, have the same disadvantage in that they require special attention after planting. Moreover, the microtubers vary in size, the length of their dormancy period, and their physiological age (Leclerc et al. 1995; Tábori et al. 1999; Coleman and Coleman 2000). Consequently, not all microtubers uniformly sprout and produce vigorous plants after planting. Adaptation of some newly developed methodologies to mass production of microtubers, i.e., in bioreactors (Yu et al. 2000), may prove that microtubers can be accepted as an alternative to plantlets.

Over the last decade several researchers observed that jasmonic acid (JA), a growth regulator produced by plants exposed to stress (Biondi et al. 2000), is highly effective in the induction of microtubers (Koda et al. 1991; Van den Berg and Ewing 1991; Pelacho and Mingo-Castel 1991; Ravnikar et al. 1992; Pruski et al. 1993; Kreft et al. 1997). In a recent study (Pruski et al. 2002), we found that JA supplement at less than 5 µM in the plantlet multiplication medium generated plantlets with sturdier stems, better developed root systems, and higher root/shoot biomass ratios compared to plantlets grown on conventional media. Nodal explants taken from JA conditioned plantlets also tuberized earlier and more uniformly, giving higher yield of microtubers than controls. A similar stimulation of tuberization was achieved when JA was supplemented directly into the microtuberization media.

The objectives of the present study were (i) to compare greenhouse performance of JA conditioned and non-conditioned plantlets and microtubers in production of minitubers, and (ii) to evaluate responses of commonly grown commercial varieties to JA conditioning, light, and dormancy-breaking treatments.

MATERIALS AND METHODS

The study was conducted in a tissue culture laboratory and greenhouses at Crop Diversification Centre North (CDCN), Edmonton, Alberta, Canada, in 1998 and 1999. Cultivars used were Atlantic (AT), Russet Burbank (RB), Shepody (SH) in 1998, and Amisk (AM), Russet Burbank (RB), Umatilla Russet (UM) in 1999.

Plantlet Multiplication and Preparation of Stock Plants for In Vitro Tuberization

Plant material for all experiments was produced from the CDCN potato tissue culture bank. The plantlets were multiplied as single-node explants on 50 mL MS (Murashige and Skoog 1962) media with standard vitamins (Sigma-Aldrich Canada Ltd., Oakville, Ont., Canada), in GA-7 Magenta jars (Magenta Corp., Chicago, IL, USA). Sixteen (4x4) single-node explants were placed in each vessel. Sucrose (30 gL^{-1}) was used as a carbon source, and the media were solidified with 0.6 % agar (Difco, Detroit, MI, USA). The pH of the media was adjusted to 5.7 before autoclaving. Cultures were incubated for 4 wk in an environmental chamber (Conviron, Model T144, Winnipeg, Manitoba, Canada) at 20 C +/–1 C with 16-h photoperiod, 150 $\mu E m^{-2} s^{-1}$ mixed fluorescent (F40T12 tubes, General Electric [GE], USA) and incandescent (40W, GE, USA) illumination. At the end of the 4-wk period, the single-stem plantlets were cut into single-node explants and placed on fresh MS medium in GA7 Magenta jars for further multiplication. The process was repeated until the required numbers of plantlets were obtained. During the last transfer before *in vitro* tuberization experiments, single-node explants of all cultivars were divided into two groups and transferred onto agar media: (i) the same MS medium as above or (ii) the MS medium supplemented with 2.5 µM JA (Apex Organics Ltd., Leicester, UK)—this group of explants was labelled as JA pretreated (JAPret). Both groups were incubated for 4 wk under the conditions described above.

Microtuberization

Nodal explants were taken from 4-wk-old plantlets grown on media with and without JA. Apical and basal nodes were discarded. Sixteen explants were placed in each GA-7 Magenta jar on 75 mL tuberization medium (Figure 1A). Two media were used for the tuberization: (i) MS with 80 gL^{-1} sucrose (no growth regulators) or (ii) MS with 80 gL^{-1} sucrose and 2.5 µM JA (JA in Media [JAMed]). All media were solidified with 0.6% agar and pH adjusted as above. Cultures were incubated at 20 C in the dark (0 h) or under 8-h photoperiod at 50 $\mu E m^{-2} s^{-1}$ mixed fluorescent/incandescent light, each for 10 wk. At harvest, the microtubers (Figure 1B) were grouped according to size and weight, and then kept in cold storage (4 C), in sealed Petri dishes, for 12 wk.

Production of Mini (Nuclear Seed) Tubers

From Plantlets—Four replicates of 20 plantlets per cultivar were transplanted to greenhouse beds (Figure 1C) filled with 15 cm of PRO-Mix 'BX' (Premier Horticulture, Dorval, Quebec, Canada), a peat-based professional growing medium designed for cultivation of horticultural greenhouse plants (pH 5.5-6.0, electric conductivity (EC) 1.5-2.0 mmhos/cm, 75-85% sphagnum, perlite, and vermiculite). Spacing between plants was 9x9 cm. Planting was done the first week of July. Plants were grown for 16 wk under standard greenhouse conditions (24/18 C day/night temperature and with 14-h photoperiod with supplemental lighting [FT72112/CW/VHO tubes, Philips, USA] at 150 $\mu E m^{-2} s^{-1}$). One week after planting, a water-soluble 10-52-10 (NPK) fertilizer (Plant-Prod, Brampton, Ontario, Canada) at 1.5 gL^{-1} was applied to plantlets weekly, for the next 2 wk. Three weeks after planting, a water-soluble 20-20-20 (NPK) fertilizer (Plant-Prod, Brampton, ON, Canada) at 3 gL^{-1} was used every second week (total five times), for 10 wk. Between fertilizer applications, plants were watered daily during hot weather and every second day in cooler weather. Plants were sprayed with Bravo (Zeneca Agro, Calgary, AB, Canada) to prevent and control Gray mold (*Botrytis cinerea*), approximately every 2 wk from mid-July until late August. Plants were not watered during the last week before harvest of minitubers. Plots were harvested by hand, and the minitubers (Figure 1D) were sorted by size and weight (size categories: [i] 5-30 mm, [ii] 30-60 mm, and [iii] >60 mm). Minitubers were placed in a cold room at 4 C for further use.

From Microtubers—Microtubers greater than 150 mg were used in this study. To induce uniform sprouting microtubers were treated with (i) 100 ppm solution of gibberellic acid (GA_3) for 24 h (1998 and 1999 studies), or (ii) Rindite, a mix-

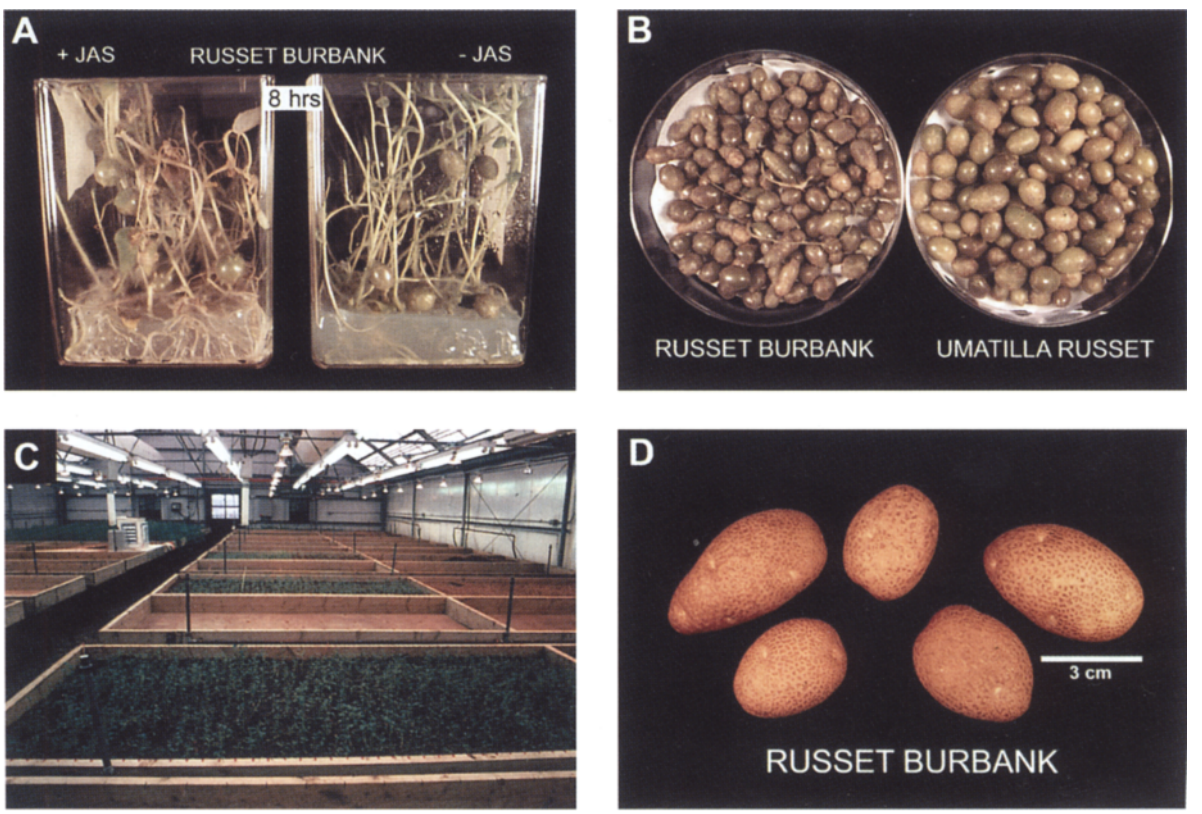

FIGURE 1.
Microtubers in the production of nuclear seed (minitubers) in the greenhouse.
A. Microtubers of Russet Burbank produced under 8-h photoperiod on media with (+JAS, left) and without (–JAS, right) 2.5 µM jasmonic acid.
B. Microtubers of Russet Burbank and Umatilla Russet.
C. Greenhouse benches for production of minitubers (CDCNorth, Edmonton).
D. Minitubers of Russet Burbank produced in the greenhouse from microtubers.

ture of ethylene chlorhydrin, ethylene bichloride and carbon tetrachloride, 7:3:1 v/v/v, respectively (Denny 1945; 1999 studies only). Petri dishes filled with microtubers were stacked in a 20-L airtight plastic container. A 50-mL beaker with the Rindite solution containing 4 mL chloroethanol (ethylene chlorhydrin), 1.6 mL dichloroethane (ethylene bichloride) and 0.56 mL carbon tetrachloride was placed in the container. Total amount of liquid was 6.16 mL (approximately 3 mL of Rindite were used per 10 L^{-1} of container volume). Microtubers were treated for 24 h at 24 C. Following the treatment, microtubers were aerated completely prior to planting. Microtubers from each tuberization treatment were planted into greenhouse beds filled with PRO-Mix 'BX' as for plantlets. Microtubers were grown under the same greenhouse conditions described above for plantlets. Microtubers received a 10-52-10 (NPK) fertilizer (the rate as for plantlets), 2 wk after planting. All other treatments, spacing, fertilization, greenhouse maintenance practices, harvest, and data collection were the same as for plantlets.

Statistical Analysis

For the greenhouse production of minitubers, a four-factor (cultivar, JA pre-treatment, JA in tuberization medium, light hours) factorial design was used in the 1998 study and a five-factor (cultivar, JA pre-treatment, JA in tuberization medium, light hours, dormancy-breaking treatment) factorial in the 1999 study. For each response (number, size, and weight of minitubers per plantlet or per microtuber), validity of the model assumptions (normal distribution and constant variance assumptions on the experimental error terms) was confirmed by examining the residuals as described in Montgomery (2001). Since for most of the responses, normality assumption was not valid under the original scale, the square root transformation was used for the analysis of variance. The results shown in the tables are back transformed to the original scale. For the responses with significant two or higher order interaction effects, Least Squares Means comparisons of all treatment combinations of the involved factors, starting with the highest order interaction, were conducted to generate letter groupings.

In 1998, the experiment that had low plant establishment rate, the CATMOD procedure in SAS with generalized logits response function (SAS Institute Inc. 1999) was used to determine the effect of JA pre-treatment, JA in the media, and light on the categorical response—establishment (whether a plant was established or not) for each cultivar tested (AT, RB, and SH). Analyses were completed using SAS (SAS Institute 1999).

RESULTS

Production of Mini (Nuclear Seed) Tubers

From Plantlets—The production of minitubers from *in vitro*-derived plantlets in 1998 and 1999, using current industrial protocols (AAFC 1996), is summarized in Table 1. All transplants, in both years, survived and grew into productive plants. No significant differences were found in total yield (kg) of minitubers between cultivars in either 1998 or 1999. In 1998, Atlantic produced the highest number of minitubers (close to four per plantlet), almost twice as many as Russet Burbank (2.5 per plantlet) and Shepody (2.2 per plantlet). In 1998, Atlantic and Russet Burbank tubers were mostly in the small and medium size categories (Table 1), whereas Shepody produced the highest number and weight of large minitubers, >60 mm diameter. In 1999, the total yield of russet varieties, Russet

TABLE 1—*Greenhouse production of minitubers from* in vitro *derived plantlets, using industry standards. Cultivars: Amisk (**AM**), Atlantic (**AT**), Russet Burbank (**RB**), Shepody (**SH**), and Umatilla Russet (**UM**).*

Cultivar	Total Number of minitubers from 20 plantlets	Number of minitubers / 20 microtubers in size class [mm]: 5-30	30-60	>60	Total Yield of minitubers (kg / 20 plantlets)	Yield of Minitubers (kg / 20 plantlets) in size class [mm]: 5-30	30-60	>60
1998 season*								
AT	78.9 a	34.1 a	42.7 a	2.0 c	1.529 a	0.207 a	1.062 a	0.185 b
RB	49.1 b	14.2 b	23.0 b	11.5 b	1.071 a	0.055 b	0.442 b	0.561 b
SH	44.3 b	5.8 c	14.2 c	24.0 a	1.816 a	0.016 c	0.229 b	1.467 a
1999 season*								
AM	65.5 a	20.7 a	21.7 a	23.0 a	2.475 a	0.067 a	0.472 b	1.935 a
RB	59.5 a	9.0 b	23.5 a	27.0 a	2.662 a	0.033 b	0.460 b	2.165 a
UM	72.5 a	13.0 ab	31.7 a	27.7 a	3.387 a	0.046 ab	0.907 a	2.433 a

Means within a column in each season followed by different letters are significantly different ($P<0.05$).
*Note that the letter groupings are for each season separately.

Burbank and Umatilla Russet, was not significantly different from that of Amisk, although their tubers were mainly in the 30- to 60-mm and >60-mm size grades (Table 1), grades preferred by the growers. In 1999, Russet Burbank produced 21% more tubers than in 1998, primarily in the largest size category. Both the total yield (53.6 vs 133.1 g per plantlet) and yield of tubers >60 mm (28.1 vs 108.3 g per plantlet) were much higher in 1999 than in 1998 (Table 1).

From Microtubers—In 1998, the yield of minitubers from microtubers was very low, due to an incomplete breaking of their dormancy with GA prior to planting. Only 1% to 42% of planted microtubers developed into full-grown plants (Table 2), compared to 100% in 1999. Russet Burbank had the lowest percentage of plants established in the greenhouse (only up to 26%), whereas Atlantic and Shepody had the highest (up to 40% and 42%, respectively). Most of the full-grown plants were produced from microtubers derived from stock plantlets pre-treated with JA (Atlantic, Russet Burbank) and on media containing JA (Shepody). The light treatment was beneficial for plant establishment of Russet Burbank, except for the JA in media-only treatment (Table 2). The lowest establishment rate had the plants from microtubers produced in dark with no JA.

Out of the tested cultivars, Shepody produced the highest number of minitubers in 30- to 60-mm and >60-mm categories. Microtubers produced either from JA pre-treated stock plants or on JA containing media gave 55% to 65% more minitubers than no-JA control in Shepody, although the differences were not significant (Table 3). The same trend was observed in total yields (weight) with this cultivar. Since yields in 1998 were very low in all three cultivars and in all treatments, only total yields are included in the table for comparison. Similar to the production from plantlets, Atlantic minitubers were mostly small, with 65% to 75 % in the 5- to 30-mm size category. Atlantic did not produce minitubers >60 mm. Russet Burbank, however, gave more minitubers from the microtubers produced without JA-pretreatment than

TABLE 2—*Proportion (%) of established plants from microtubers in the greenhouse. JA pre-treatment*JA media*Light interaction was significant (P<0.001). Mean (%) separation performed within each cultivar. Cultivars: Atlantic (**AT**), Russet Burbank (**RB**), Shepody (**SH**), 1998 season.*

JA Pre-treated	JA in media	Light (hrs)	% of established plants AT	RB	SH
No	No	0	2 d	1 d	16 c
No	No	8	20 b	26 a	30 b
No	Yes	0	26 b	5 c	3 d
No	Yes	8	22 b	7 c	42 a
Yes	No	0	40 a	15 bc	41 ab
Yes	No	8	12 c	23 ab	31 b
Yes	Yes	0	12 c	9 bc	13 c
Yes	Yes	8	17 bc	22 ab	29 b

Means in the same column followed by different letters are significantly different ($P<0.05$).

TABLE 3—*Greenhouse production of minitubers from* in vitro *microtubers (produced under 8h photoperiod): Effects of JA treatments during in vitro stock plant production and tuberization stages. Cultivar*JA pre-treatment * JA media interaction was significant (P<0.05). Mean separation performed within each cultivar. Cultivars: Atlantic (**AT**), Russet Burbank (**RB**), Shepody (**SH**), 1998 season.*

Cultivar	JA Pre-treated	JA in media	Total number of minitubers / 20 microtubers	Number of minitubers / 20 microtubers in size class [mm]: 5-30	30-60	> 60	Total yield of minitubers (kg / 20 microtubers) all sizes
AT*	No	No	1.9 a	1.5 a	0.3 a	0.0 a	0.03 b
AT	No	Yes	3.3 a	2.4 a	0.6 a	0.0 a	0.08 ab
AT	Yes	No	2.1 a	1.5 a	0.2 a	0.0 a	0.05 b
AT	Yes	Yes	4.4 a	2.9 a	0.8 a	0.0 a	0.11 a
RB*	No	No	13.4 a	11.1 a	1.9 a	0.3 a	0.16 a
RB	No	Yes	1.1 b	0.9 c	0.1 a	0.0 b	0.01 b
RB	Yes	No	4.1 a	2.3 bc	0.9 a	0.1 b	0.06 b
RB	Yes	Yes	4.6 a	3.1 ab	0.9 a	0.01b	0.06 b
SH*	No	No	4.0 a	1.2 a	1.6 a	0.7 a	0.12 a
SH	No	Yes	6.2 a	2.0 a	2.4 a	1.2 a	0.16 a
SH	Yes	No	6.6 a	1.2 a	2.8 a	1.0 a	0.18 a
SH	Yes	Yes	4.3 a	1.2 a	1.4 a	0.7 a	0.11 a

Means in the same column followed by different letters are significantly different ($P<0.05$).
*Note that the letter groupings are for each cultivar separately.

with the JA in the media (Table 3). This was also reflected in total yield.

Microtubers produced under light were significantly better propagules than those produced in darkness. This could also be attributed to diminished dormancy resulting in better emergence (Table 2). Averaged across cultivars, the total number of minitubers obtained from light-grown microtubers (per 20 microtubers) was three times higher than from dark-grown microtubers (Table 4). Total yield and number of minitubers in the 5- to 30-mm, 30- to 60-mm, and >60-mm-diameter size classes were also two to seven times higher from microtubers produced under 8-h light during *in vitro* tuberization. Shepody produced the highest total yield of minitubers followed by Russet Burbank and Atlantic (Table 3).

The production of minitubers from microtubers was much higher in 1999 than in 1998. The presence of jasmonic acid in pre-treatment and tuberization media, the cultivar, the exposure to light during tuberization, and the dormancy breaking treatment had significant effects on the number, yield (fresh weight) and size distribution of minitubers. The cultivar, JA pre-treatment, and JA in media interacted ($P<0.05$) to affect number, weight, and size distribution of minitubers (Table 5). Relative to Russet Burbank and Umatilla Russet, Amisk produced the highest number of minitubers (>3 minitubers per microtuber) derived from JA pre-treated plantlets. In Amisk and Russet Burbank, microtubers derived from explants taken from JA conditioned stock plants gave significantly more minitubers than the microtubers from other treatments. Similar results were observed in Umatilla Russet (Table 5). Amisk numbers of minitubers derived from the microtubers were comparable to numbers obtained from plantlets (65.5 minitubers from 20 plantlets (Table 1) and 61.3 from 20 microtubers derived from JA treated stock plants [Table 5]). Also, Amisk yield of 30- to 60-mm-diameter minitubers

TABLE 4—*Greenhouse production of minitubers from* in vitro *microtubers: Single effect of Light (Li) during in vitro tuberization on eight responses averaged for the three potato cultivars, Atlantic, Russet Burbank, and Shepody over all JA treatments, 1998 season.*

Li [h]	Total Number of minitubers / 20 microtubers	Number of minitubers / 20 microtubers in size class [mm]:			Total Yield of minitubers (kg / 20 microtubers)	Yield of minitubers (kg / 20 microtubers) in size class [mm]:		
		5-30	30-60	>60		5-30	30-60	>60
0	2.6 b	1.6 b	0.6 b	0.4 b	0.25 b	0.01 b	0.09 b	-*
8	7.8 a	3.7 a	2.1 a	2.9 a	0.85 a	0.08 a	0.35 a	0.18 a

Means within a column followed by different letters are significantly different ($P<0.05$).
*No minitubers >60 mm were produced by cv Atlantic; in Russet Burbank the yield of minitubers >60 mm was insignificant.

TABLE 5—*Effects of JA treatments during* in vitro *explant production and tuberization stages on production of minitubers from in vitro microtubers in the greenhouse. Cultivar*JA pre-treatment * JA in media interaction was significant ($P<0.05$). Mean separation performed within each cultivar. Cultivars: Amisk (**AM**), Russet Burbank (**RB**), Umatilla Russet (**UM**), 1999 season.*

Cultivar	JA Pre-treated	JA in media	Total number of minitubers / 20 microtubers	Number of minitubers / 20 microtubers in size class [mm]: 5-30	30-60	Total yield of minitubers (kg / 20 microtubers)	Yield of minitubers (kg / 20 microtubers) size: 30-60mm
AM*	No	No	50.9 b	20.8 a	15.6 b	1.119 a	0.300 c
AM	No	Yes	55.3 ab	22.4 a	18.2 ab	1.092 a	0.417 ab
AM	Yes	No	61.3 a	24.0 a	21.6 a	1.078 a	0.446 a
AM	Yes	Yes	49.7 b	20.2 a	16.6 b	0.849 a	0.332 bc
RB*	No	No	20.4 b	4.8 b	8.7 b	0.409 b	0.160 b
RB	No	Yes	24.1 b	5.0 b	10.6 b	0.573 b	0.234 ab
RB	Yes	No	44.6 a	15.7 a	16.6 a	0.859 a	0.322 a
RB	Yes	Yes	17.9 b	3.6 b	6.9 b	0.433 b	0.140 b
UM*	No	No	35.6 b	9.5 b	15.6 b	0.826 b	0.337 b
UM	No	Yes	35.4 b	10.6 ab	15.0 b	0.729 c	0.343 b
UM	Yes	No	41.2 a	12.5 a	19.2 a	0.879 a	0.415 a
UM	Yes	Yes	33.3 b	9.2 b	16.9 b	0.701 c	0.360 b

Means in the same column followed by different letters are significantly different ($P<0.05$).
*Note that the letter groupings are for each cultivar separately.

was almost the same from plantlets (0.472 kg/20 plantlets; Table 1) as from microtubers from the JA pre-treatment (Table 5), 0.446 kg/20 microtubers; Table 5). Russet Burbank and Umatilla Russet produced fewer tubers from microtubers (RB 44.6/20 microtubers and UM 41.2/20 microtubers; Table 5) than from plantlets (RB 59.5/20 plantlets, UM 72.5/20 plantlets. Table 1). Although lower than from *in vitro* plantlets, total yields of Russet Burbank and Umatilla Russet minitubers were the highest from microtubers derived from JA-conditioned stock plants, compared to other treatments (Table 5).

Significant interactions between cultivar, light exposure during *in vitro* tuberization and jasmonate pre-treatment, and cultivar, light, and jasmonate in media were observed for minitubers 5-30 mm in diameter (Table 6). Microtubers produced under 8-h light and with or without JA pre-treatment gave higher number and yield of minitubers (5-30 mm) than the dark treatment without JA in all three cultivars. Amisk produced significantly more minitubers in this category than Russet Burbank or Umatilla Russet (Table 5).

TABLE 6—*Effects of JA and light during* in vitro *explant production and tuberization stages on yield and number of minitubers (size 5-30 mm) from microtubers in the greenhouse. Cultivar*JA pre-treatment*Light and Cultivar*Light*JA in Media interactions were significant (P<0.05). Mean separation performed within each cultivar. Cultivars: Amisk (**AM**), Russet Burbank (**RB**) and Umatilla Russet (**UM**), 1999 season.*

	Cultivar*JAP Pre-treatment*Light				Cultivar*Light*JAMedia			
Cultivar	JA Pre-treated	Light [h]	Number of minitubers size 5-30mm	Yield of minitubers 5-30mm [kg]	JA in media	Light [h]	Number of Minitubers size 5-30mm	Yield of Minitubers 5-30mm [kg]
AM*	No	0	17.6 b	0.077 a	No	0	19.1 bc	0.089 a
AM	No	8	26.0 a	0.081 a	No	8	26.0 ab	0.090 a
AM	Yes	0	16.9 b	0.063 a	Yes	0	15.6 c	0.052 b
AM	Yes	8	27.8 a	0.090 a	Yes	8	27.8 a	0.081 a
RB*	No	0	2.9 b	0.011 b	No	0	5.4 b	0.019 b
RB	No	8	7.4 a	0.024 a	No	8	14.7 a	0.047 a
RB	Yes	0	8.5 a	0.027 a	Yes	0	5.2 b	0.017 b
RB	Yes	8	8.6 a	0.030 a	Yes	8	3.4 b	0.012 b
UM*	No	0	6.5 b	0.022 b	No	0	5.9 c	0.022 b
UM	No	8	14.4 a	0.048 a	No	8	17.4 a	0.060 a
UM	Yes	0	10.4 ab	0.038 ab	Yes	0	10.8 b	0.038 b
UM	Yes	8	11.4 a	0.040 a	Yes	8	9.0 bc	0.031 b

Means in the same column followed by different letters are significantly different ($P<0.05$).
*Note that the letter groupings are for each cultivar separately.

Microtuber Dormancy Release Studies (1999 season)

Dormancy release of microtubers prior to the greenhouse plantings had a profound impact on the production of minitubers in the greenhouse (Tables 7-9).

Table 7 summarizes a significant Cultivar*Dormancy release treatment interaction for the total number and yield of large minitubers. Since the results obtained in the 1998 studies clearly showed the necessity for the dormancy release treatment in microtubers, the 1999 experiments did not include typical control treatments (no dormancy break). Only the effects of Rindite and gibberellic acid GA_3 are compared. This also significantly saved

TABLE 7—*Minituber production from microtubers: Effects of dormancy breaking treatments applied to microtubers prior to greenhouse planting on number and yield of minitubers. Cultivar*Dormancy release treatment interaction was significant (P<0.05). Cultivars: Amisk (**AM**), Russet Burbank (**RB**), Umatilla Russet (**UM**), 1999 season.*

Cultivar	Dormancy release treatment	Total number of minitubers / 20 microtubers	Number of minitubers / 20 microtubers size: >60mm	Total Yield of minitubers (kg / 20 microtubers)	Yield of mini tubers (kg /20) microtubers) size: >60mm
AM	GA	29.2 c	5.8 c	0.611 c	0.354 c
AM	Rindite	79.4 a	14.7 a	1.458 a	0.778 a
RB	GA	9.1 d	0.8 d	0.116 d	0.040 d
RB	Rindite	44.4 b	10.1 b	1.021 b	0.598 ab
UM	GA	21.6 c	4.7 c	0.561 c	0.322 c
UM	Rindite	51.1 b	7.3 c	1.007 b	0.426 bc

Means in the same column followed by different letters are significantly different ($P<0.05$).

space in the greenhouse. Microtubers treated with Rindite produced two to five times more minitubers than those treated with gibberellic acid (GA_3) in all three varieties (Table 7). Also, total number and yield of minitubers >60 mm in diameter was significantly higher from the microtubers treated with Rindite than with GA_3. The largest difference was observed in Russet Burbank, which produced on average approximately 45 minitubers from 20 microtubers (2.22 per microtuber) treated with Rindite compared to only nine (0.45 per microtuber) from the GA_3 treatment (Table 7). On average, Amisk produced the highest total number of minitubers (close to four minitubers per planted microtuber) and the highest yield of minitubers >60 mm in diameter (Table 7).

There was a significant interaction ($P<0.05$) between Jasmonic acid in media* Light*Dormancy release treatment that affected total number, yield, and size distribution of minitubers (Table 8). Overall, Rindite treatment gave significantly higher tuber numbers and yields, irrespective of the JA presence in tuberization media and the photoperiod. Microtubers treated with GA_3 yielded significantly fewer minitubers than those treated with Rindite (Table 8). Exposure to light during the microtuber production phase had a significant effect on their performance in the greenhouse. All responses for all three cultivars were the highest for minitubers grown from microtubers produced under 8-h photoperiod with no JA in tuberization medium, coming from the Rindite dormancy release treatment (Table 8).

Table 9 presents combined effects of JA stock plants conditioning prior to *in vitro* tuberization, the exposure to light during the production of microtubers and the dormancy release treatment on greenhouse production of minitubers.

TABLE 8—*Minituber production of three russet cultivars: effects of JA in tuberization media, exposure to light during* in vitro *tuberization and dormancy breaking treatment (applied to microtubers prior to greenhouse planting) on number and yield of minitubers. JA in Media*Light(Li)*Dormancy breaking treatment interaction was significant (P<0.05), 1999 season.*

JA in Media	Li [h]	Dormancy Release Treatment	Total number of mini tubers/ 20 micro tubers	Number of minitubers / 20 microtubers in size class [mm]: 5-30	30-60	>60	Total yield of mini tubers (kg / 20 micro tubers)	Yield of minitubers (kg / 20 microtubers) in size class [mm]: 5-30	30-60	>60
No	0	GA	19.0 d	5.3 d	6.9cd	5.2bc	0.523c	0.021d	0.142cd	0.356bcd
No	0	Rindite	46.0 b	14.3b	16.7b	8.3b	0.940b	0.061b	0.357b	0.491b
No	8	GA	28.3 c	10.9bc	10.7c	4.3cd	0.539c	0.040bc	0.226c	0.264cde
No	8	Rindite	76.1 a	29.6a	30.5a	13.7a	1.445a	0.096a	0.595a	0.747a
Yes	0	GA	9.0 e	1.4e	4.1d	2.0d	0.233d	0.004e	0.094d	0.129e
Yes	0	Rindite	70.0 a	26.8a	28.8a	13.2a	1.421a	0.093a	0.609a	0.714a
Yes	8	GA	23.6cd	8.0cd	8.2c	3.5cd	0.422cd	0.027cd	0.179c	0.206de
Yes	8	Rindite	41.1 b	15.3b	14.9b	7.7b	0.842b	0.048b	0.334b	0.451bc

Means in the same column followed by different letters are significantly different ($P<0.05$).

TABLE 9—*Effects of JA pretreatment during explant production, light during* in vitro *tuberization and dormancy breaking treatment (applied to microtubers prior to greenhouse planting) on minituber production in three russet cultivars, in the greenhouse. JA pretreatment*Light(Li)*Dormancy treatment interaction was significant (P<0.05), 1999 season.*

JA Pretreatment	Li [h]	Dormancy Release Treatment	Total number of mini tubers/ 20 micro tubers	Number of minitubers / 20 microtubers in size class [mm]: 30-60	>60	Total yield of mini tubers (kg / 20 micro tubers)	Yield of minitubers (kg / 20 microtubers) in size class [mm]: 30-60	>60
No	0	GA	14.7 e	5.7 e	3.8 c	0.395 c	0.126 e	0.252 c
No	0	Rindite	47.6 c	17.8 c	8.2 b	0.959 b	0.381 c	0.488 b
No	8	GA	25.4 d	8.8 de	4.0 c	0.476 c	0.182 de	0.256 c
No	8	Rindite	60.1 ab	23.5 b	13.0 a	1.336 a	0.506 ab	0.754 a
Yes	0	GA	13.3 e	5.3 e	3.5 c	0.361 c	0.111 e	0.234 c
Yes	0	Rindite	68.3 a	27.7 a	13.3 a	1.403 a	0.585 a	0.718 a
Yes	8	GA	26.5 d	10.2 d	3.8 c	0.484 c	0.223 d	0.213 c
Yes	8	Rindite	57.1 bc	21.9 b	8.4 b	0.951 b	0.423 bc	0.444 b

Means in the same column followed by different letters are significantly different ($P<0.05$).

JAPre-treatment*Light*Dormancy release treatment interaction was significant for the total number and yield of minitubers, and for the yields in two size categories, 30-60 mm and >60 mm. All response variables for minitubers produced from Rindite treated microtubers were significantly higher than for those treated with GA_3. Exposure to light during *in vitro* tuberization phase was less pronounced in the greenhouse performance of microtubers produced from JA pretreated plantlets (Table 9) than for microtubers coming from JA containing media (Table 8). Light during the microtuberization step significantly lowered (20-40%) number and yield of minitubers coming from JA conditioned microtubers. The highest number and yield of minitubers in the greenhouse were from the microtubers from JAPret-0 h light-Rindite treatment combination (Table 9).

DISCUSSION

This study was conducted to determine the effects of jasmonate (used as a plantlet conditioner before *in vitro* tuberization or in media during *in vitro* tuberization, or both) on greenhouse production of minitubers. The objective was to enhance the number and size of minitubers produced from a greenhouse-planted microtuber in comparison to an *in vitro* plantlet.

The greenhouse performance of microtubers was cultivar dependent and was significantly affected by jasmonic acid (JA) conditioning of plantlets prior to *in vitro* tuberization, presence of JA in tuberization media, the photoperiod during tuberization (Tables 2-6) and the dormancy release treatment (Tables 7-9). Optimizing these factors for an individual cultivar may facilitate use of microtubers in the production of minitubers (nuclear tubers). As expected, plantlets (the industry standard) produced satisfactory results (Table 1), although Atlantic and Russet Burbank tubers were mostly in the small and medium size categories in 1998. The small size minitubers can be re-used as nuclear seeds. According to current regulations in Canada, seed potato producers are allowed to plant two generations of seed tubers in the greenhouse maintaining the nuclear seed status of the harvested minitubers.

The three russet cultivars, Amisk–Ranger Russet, Russet Burbank, and Umatilla Russet responded most favorably to the use of microtubers in production of nuclear seed in the greenhouse. Results with Shepody's microtubers were inconclusive and yield of minitubers from microtubers in Atlantic was very poor (Tables 2 and 3), due to an incomplete dormancy release. A more comprehensive research approach is needed to refine the system, to make the use of microtubers economically feasible in these cultivars. Microtubers are usually very dormant at harvest and will not sprout unless stored for 4 months or more at low temperatures (Struik and Wiersema 1999). Consequently, the percentage of established plants in the greenhouse was low in 1998 (Table 2), as were the yields and the number of minitubers (Table 3).

Jasmonic acid (JA), used as a conditioner in the production of microtubers, enhanced yield and size of minitubers in the greenhouse (Tables 3 and 5). Jasmonate pretreatment of stock plants prior to taking nodal explants for tuberization, was an effective inducer of microtuber formation in Russet Burbank (Pruski et al. 2002). Plantlets pretreated with JA produced more roots and more microtubers compared to non-treated controls (Figure 1A). Stimulatory effects of JAs on *in vitro* tuberization and on potato stem node cultures have also been reported by Koda et al. (1991), Ravnikar et al. (1992) and others (Pruski et al. 1993; Kreft et al. 1997; Jackson 1999). Our studies suggest that this conclusion could be extended to the performance of microtubers in the greenhouse. JA preconditioning of stock plants prior to *in vitro* tuberization increased the minituber number per microtuber planted (particularly in russet varieties), unless followed by JA in tuberization medium, in which case there was a net inhibitory effect (Table 5). However, it is not possible to explain (at this stage) what portion of the JA effect can be attributed to dormancy and what was due to other physiological effects.

Light (8 h) during tuberization was an important factor in producing microtubers that then performed well in the greenhouse. Microtubers produced in dark performed poorly (Tables 4, 6, 8, and 9). As mentioned, this could also be attributed to a better dormancy release in microtubers produced under 8-h light and consequent better emergence (Table 2). Microtubers derived from short photoperiod were greenish and seemed to be less juvenile than the tubers from dark treatments. Gopal et al. (1997) also observed that such microtubers perform far better in the field or in the greenhouse than microtubers produced in darkness. Dobránszki and Mandi (1993) reported that short days induce *in vitro* tuberization of potato shoots grown on hormone-free media. Our greenhouse observations showed a significant enhancement in performance of the microtubers produced with 8-h light (Tables 4, 6, 8, and 9).

As mentioned, microtubers of the three russet cultivars performed best in the production of minitubers in the greenhouse. Only microtubers weighing more than 0.15 g (preferably >0.2 g; the larger the better) can be planted to the greenhouse or to the field. Figure 1D shows minitubers (nuclear tubers) of Russet Burbank produced from microtubers. The three russet varieties produced more than 50% of minitubers in the 30- to 60-mm (3-6 cm) category and larger. Minitubers greater than 30 mm in size are preferred on the market.

The key factor to a successful use of microtubers along with the plantlets in the greenhouse seemed to be the dormancy release. In general, microtubers right after *in vitro* tuberization are very dormant (Struik and Wiersema 1999; Tábori et al. 1999). Recently, several researchers reported that dormancy of microtubers is cultivar-dependent and is affected by the photoperiod applied during *in vitro* tuberization (Tábori et al. 1999; Coleman and Coleman 2000). In the study reported here, we used GA_3 and Rindite to release microtuber dormancy prior to planting. Rindite proved to be most effective in greenhouse conditions (Tables 6-8). However, more studies are required to provide evidence that the product is safe to use with microtubers, since there is a controversy in the literature. In general, Rindite is not recommended for breaking dormancy of microtubers. It is considered dangerous for microtubers, since decay may easily occur after such treatment due to their small size (Ranalli 1997; Struik and Wiersema 1999). In our experiments no rot or decay of microtubers was observed due to Rindite treatment if microtubers were produced in 8 h light. Some of the microtubers produced in dark (0-h photoperiod) were slightly damaged by Rindite. Microtubers used in our studies were stored in a cooler for close to 12 wk prior to planting, which provided enough time to develop adequate skin. Moreover, immediately after harvest and before cold storage, microtubers were placed into Petri dishes and left on the laboratory bench for 24 h at room temperature to harden. Also, we only used microtubers weighing more than 0.15 g in greenhouse planting. The above conditions may have protected the microtubers from damages during Rindite application. A successful dormancy release of microtubers by Rindite was reported recently by Nasiruddin and Blake (1997) and Kim et al. (1999).

According to our data, microtubers are not yet suitable for the greenhouse production of cvs Amisk, Russet Burbank, and Umatilla Russet minitubers. Although the numbers of minitubers produced from plantlet- and/or from microtuber-derived plants were similar, yields (weight) of microtuber-derived plants were less than those of transplanted plantlets (Tables 1 and 5) and need to be addressed. JA pretreatment of stock plants prior to taking nodal explants for tuberization was effective in enhancing yields of minitubers from microtubers and can be of help in a commercial setup, where microtubers are included in the production system. Thus, we can recommend the use of JA as a plantlet/microtuber conditioner in semi-controlled environments (greenhouses). Further investigation is needed in microtuber dormancy release treatments. Rindite provided an effective microtuber dormancy break and significantly enhanced minituber production, although it would be beneficial to determine the optimal concentration(s) of this product in relation to size of the microtubers, the condition of their skin and the storage history.

ACKNOWLEDGMENTS

Authors thank Linda Stewart, Katherine Andrew, and Leah Maskewich for technical assistance, data collection and entry. Financial support of Potato Growers of Alberta (PGA) and Alberta Agriculture Research Institute (AARI) to the project is gratefully acknowledged. Authors also thank Dr. Denis Kirkham, Canadian Food Inspection Agency, British Columbia, Canada for the outline of the procedure for Rindite application. CDCN – AAFRD provided support by allocating laboratory, growth room and greenhouse space to the project. McCain Foods Ltd. financially supported preparation and production of the manuscript.

LITERATURE CITED

AAFC (Agriculture & Agri-Food Canada). 1996. Propagation and initiation of single node cuttings of potato *in vitro*. Prepared by LK Douglass, Potato Propagation and Tissue Culture Laboratory, Potato Research Centre, AAFC, Fredericton, Canada. pp. 1-9.

Biondi S, S Fornale, KM Oksman-Caldentey, M Eeva, S Agostani, and N Bagni. 2000. Jasmonates induce over-accumulation of methylputrescine and conjugated polyamines in *Hyoscyamus muticus* L. root cultures. Plant Cell Rep 19:691-697.

Coleman WK, and SE Coleman. 2000. Modification of potato microtuber dormancy during induction and growth *in vitro* and *ex vitro*. Am J Potato Res 77:103-110.

Coleman WK, DJ Donnelley, and SE Coleman. 2001. Potato microtubers as research tools: a review. Am J Potato Res 78:47-55.

Denny FE. 1945. Synergistic effects of three chemicals in the treatment of dormant potato tubers to hasten germination. Contr Boyce Thompson Inst Pl Res 14:1-14.

Dobránszki J, and M Mandi. 1993. Induction of *in vitro* tuberization by short day period and dark treatment of potato shoots grown on hormone-free medium. Acta Biol Hung 44:411-420.

Dobránszki J, KM Tábóri, and A Ferenczy. 1999. Light and genotype effects on in vitro tuberization of potato plantlets. Potato Res 42:483-488.

Estrada R, P Tovar, and JH Dodds. 1986. Induction of *in vitro* tubers in a broad range of potato genotypes. Plant Cell Tis Organ Cult 7:3-10.

Gopal J, JL Minocha, and JS Sidhu. 1997. Comparative performance of potato crops raised from microtubers induced in the dark versus microtubers induced in light. Potato Res 40:407-412.

Jackson SD. 1999. Multiple signalling pathways control tuber induction in potato. Plant Physiol 119:1-8.

Jones ED. 1988. A current assessment of *in vitro* culture, and other rapid multiplication methods in North America and Europe. Am Potato J 65:209-220.

Joung H, JH Jeon, SW Park, and HS Kim. 1993. The impact of mass-produced potato microtubers on Korean potato agriculture. Am Potato J 70:819-820 (abstr).

Khuri S, and J Moorby. 1996. Nodal segments or microtubers as explants for *in vitro* microtuber production of potato. Plant Cell Tis Organ Cult 45:215-222.

Kim SY, JK Kim, KH Choi, YH Joung, and H Joung. 1999. Effects of Rindite on breaking dormancy of potato microtubers. Am J Potato Res 76:5-8.

Koda Y, Y Kikuta, H Tazaki, Y Tsuhino, S Sakamura, and T Yoshihara. 1991. Potato tuber-inducing activities of jasmonic acid and related compounds. Phytochem 30:1435-1438.

Kreft S, M Ravinkar, P Mesko, J Pungercar, A Umek, I Kregar, and B Strukelj. 1997. Jasmonic acid inducible aspartic proteinase inhibitors from potato. Phytochem 44:1001-1006.

Lê CL. 1999. In vitro microtuberization: an evaluation of culture conditions for the production of virus-free potatoes. Potato Res 42:489-498.

Leclerc Y, DJ Donnelly, WK Coleman, and RR King. 1995. Microtuber dormancy in three potato cultivars. Am Potato J 72:215-223.

Lillo C. 1989. A simple two-phase system for efficient in vitro tuberization in potato. Norwegian J Agric Sci 3:23-27.

Lommen WJM. 1995. Basic studies on the production and performance of potato minitubers. PhD thesis, Agriculture University of Wageningen, The Netherlands.

Montgomery DC. 2001. Design and Analysis of Experiments. Wiley, New York.

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

Nasiruddin KM, and J Blake. 1997. Effect of Rindite on storage behavior, dormancy break and sprout growth of potato microtubers (cv. Desiree). Am Potato J 74:325-330.

Pelacho AM, and AM Mingo-Castel. 1991. Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. Plant Physiol 97:1253-1255.

Pruski K, J Nowak, and T Lewis. 1993. Jasmonates and photoperiod effect on microtuber production in two potato cultivars. In Vitro Cell Develop Biol Plant 29:69 (abstr).

Pruski, K., T. Astatkie, and J. Nowak. 2002. Jasmonate effects on in vitro tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum* L.) under different media and photoperiod conditions. In Vitro Cell Develop Biol Plant 38:203-209.

Ranalli P. 1997. Innovative propagation methods in seed tuber multiplication programmes. Potato Res 40:439-453.

Ranalli P, F Bassi, G Ruaro, P del Re, M di Candilo, and G Mandolino. 1994. Microtuber and minituber production and field performance compared with normal tubers. Potato Res 37:383-391.

Ravnikar M, B Vilhar, and N Gogala. 1992. Stimulatory effects of jasmonic acid on potato node and protoplast culture. J Plant Growth Reg 11:29-33.

SAS Institute Inc. 1999. SAS Online Doc®, Version 8. SAS Institute Inc., Cary, NC.

Struik PC, and WJM. Lommen. 1990. Production, storage and use of micro- and minitubers. Proceedings of the 11th Triennial Conference of the European Association for Potato Research (EAPR), Edinburgh, UK. pp.122-133.

Struik PC, and SG Wiersema. 1999. Seed Potato Technology. Wageningen Pers, The Netherlands.

Tábóri KM, J Dobránszki, and A Ferenczy. 1999. Some sprouting characteristics of microtubers. Potato Res 42:611-617.

Van den Berg JH, and EE Ewing. 1991. Jasmonates and their role in plant growth and development with special reference to the control of potato tuberization: a review. Am Potato J 68:781-794.

Yu WC, PJ Joyce, DC Cameron and BH McCown. 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep 19:407-413.