Manipulation of Microtubers for Direct Field Utilization in Seed Production

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ABSTRACT

A two-year field study was conducted to determine the effects of jasmonic acid (JA), light (during *in vitro* explant production and *in vitro* tuberization phases), and dormancy-breaking treatment on performance of microtubers in the production of seed tubers (pre-elite) in five potato cultivars. Microtubers were produced under short day (8-h) conditions and in darkness, from stock plantlets pre-treated with JA and untreated, and on tuberization media with or without JA. Microtuber performance was compared to *in vitro* plantlets transplanted directly to the field. Yields of tubers from microtubers were 30% to 40% of those from plantlets. Microtubers of cultivars Amisk and Russet Burbank produced the highest yields of pre-elite tubers. Atlantic microtubers performed poorly in the field. JA pre-treatment of stock plantlets, prior to *in vitro* tuberization, enhanced seeds tuber production from microtubers in Russet Burbank and lowered in Shepody. JA presence in media during *in vitro* tuberization significantly lowered production of tubers while exposure to 8-h light resulted in microtubers performing significantly better in the field than microtubers produced in the dark. Dormancy release was the key factor influencing microtuber performance. Unlike greenhouse studies, gibberellic acid (GA_3) was more effective than Rindite. A further refinement of the production and handling methods is required before microtubers can be recommended for field production of seed tubers.

INTRODUCTION

In vitro propagation of potato by serial culture of single-node cuttings has been used for more than two decades in the rapid multiplication of disease-free material in elite seed potato programs (Goodwin et al. 1980; Jones 1988; Lommen 1995; Struik and Wiersema 1999). A plantlet is a propagule of choice, widely used by the industry for the production of minitubers, almost exclusively in greenhouses. The alternative end-product in the micropropagation process of potato is a small tuber (microtuber) produced when *in vitro* plantlets or explants are placed under tuber-inducing conditions. Microtubers have a potential to be integrated into seed potato programs (Lillo 1989; Lommen 1995; Khuri and Moorby 1996; Nasiruddin and Blake 1997; Kim et al. 1999; Struik and Wiersema 1999) and to be mass-produced in bioreactors (Yu et al. 2000). Microtubers are convenient for handling, storage, and transport of germplasm (Estrada et al. 1986) and unlike *in vitro* plantlets, do not need a hardening period in the greenhouse or in the field (Ranalli et al. 1994; Ranalli 1997; Coleman et al. 2001). Also, they may be adapted to some form of large-scale mechanized planting (Ranalli et al. 1989; Struik and Wiersema 1999).

Pruski et al. (2003) suggested that microtubers of three russet cultivars, Amisk, Russet Burbank and Umatilla Russet, could successfully be used in a commercial production of minitubers in the greenhouse. Also, jasmonic acid (JA) conditioning of stock plants, prior to taking explants for tuberization, was proposed as a treatment enhancing the quality of

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microtubers and their performance in greenhouse production of minitubers. Several authors have reported that JA, a growth regulator produced by plants exposed to stress (Biondi et al. 2000), was 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). It was also found that nodal explants taken from the JA- (2.5 µM) conditioned plantlets tuberized earlier and more uniformly, giving higher yield of microtubers than the controls (Pruski et al. 2002). Since the use of microtubers of russet varieties in production of nuclear seed in the greenhouse was successful (Pruski et al. 2003), efforts were made to establish a routine use of microtubers in the field production of seed tubers (first field generation tubers). The objectives of the present study were (i) to compare field performance of plantlets and JA-conditioned and non-conditioned microtubers (produced under 0-h and 8-h light) and (ii) to evaluate responses of commonly grown commercial varieties to the JA conditioning and microtuber dormancy release treatments.

MATERIALS AND METHODS

The study was conducted at the tissue culture laboratory and in the field at the Crop Diversification Centre North (CDCN), Alberta Agriculture Food and Rural Development, Edmonton, Alberta, Canada (53° N latitude, 113° W longitude), in 1998 and 1999. The cultivars studied were Atlantic (AT), Russet Burbank (RB), and Shepody (SH) in 1998, and Amisk – Ranger Russet (AM), Russet Burbank (RB), and Umatilla Russet (UM) in 1999. Plant material for all experiments was derived from the CDCN tissue culture bank. Multiplication of plantlets and preparation of stock plants for *in vitro* tuberization followed the same protocol as described by Pruski et al. (2003).

Field Production of Tubers

From Plantlets—Four replicates of 20 plantlets were used in each cultivar/treatment combination. In 1998, plantlets were directly planted from GA7 Magenta vessels to the field, the first week of June. In 1999, two methods of transplanting were used: (i) planting directly from the vessels to the field, and (ii) planting to Jiffy pellets (5 cm in diameter; Jiffy Products (NB) Ltd., Shippagan, NB, Canada) in the greenhouse for 1 wk prior to field transplanting, the first week of June. The field plots were 3 m (10 ft) long with 15 cm (6 in.) between plantlets, and 90 cm (36 in.) between rows (field location/land description: NW-5-TR54-54-R23-W4th meridian). Plots were tilled and rows were formed using a tuber unit potato planter. Plots were fertilized based on recommendations for a yield of 33 t per ha following soil analysis (Norwest Laboratories, Edmonton, Canada). Plots were irrigated as needed and treated with Bravo (Zeneca Agro, Calgary, AB, Canada) bi-weekly to control early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*), from mid-July until late August. Plants were topped by hand just prior to harvest in the third week of September (both 1998 and 1999 studies). The crop received a total of 1,398 corn heat units (CHU) in 1998 and 1,200 in 1999 (CDCN, Edmonton weather station records). Daily Corn Heat Unit = $(Y_{MAX} + Y_{MIN})/2$ (Y – daytime temperature). Tubers were sorted into the following categories: (i) <48 mm diameter, (ii) 48-88 mm, and (iii) >88 mm, and the number and weight of seed tubers produced per plantlet was recorded.

From Microtubers—Only microtubers greater than 150 mg were used in this study. The microtubers were stored in a cooler at 4 C for 2 months before use. To induce uniform sprouting prior to field planting, microtubers were treated with (i) a 100-ppm solution of gibberellic acid (GA_3) for 24 h (1998 and 1999 studies), and (ii) Rindite, a mixture of ethylene chlorhydrin, ethylene bichloride and carbon tetrachloride, 7:3:1 v/v/v, respectively (Denny 1945; 1999 studies only). The method followed the procedure outlined by Pruski et al. (2003). Twenty microtubers of each cultivar were planted per treatment (four replicates). In 1999, only the microtubers produced under 8-h light were used. Spacing and field maintenance practices (fertilizer rates, pest control) were as described for production of tubers from plantlets. Plants were topped by hand just prior to harvest in the third week of September (both 1998 and 1999 studies). Produced seed tubers were sorted into the three categories as described for plantlets.

Statistical Analysis

In the experiment with seed tuber production from *in vitro* plantlets a one-factor (cultivar; 1998) or a two-factor (cultivar, transplanting method; 1999) design was used. For the experiments with field production of seed tubers from microtubers, a four-factor factorial design was used in both growing seasons: in 1998, cultivar, JA pre-treatment, JA medium, light (h), and in 1999, cultivar, JA pre-treatment, JA in tuberization medium, dormancy-breaking treatment. For each response (number, size, and weight of seed tubers per plantlet or per

microtuber), validity of the model assumptions (normal distribution and constant variance of 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 means were then transformed back to the original scale and presented in tables. For the responses with significant two-way 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.

Additionally, to determine the effect of JA pre-treatment and JA in the media (all for 8 h light) for each cultivar (RB and SH) on the categorical response-establishment (whether a plant was established or not), the CATMOD procedure in SAS with generalized logits response function (SAS Institute Inc. 1999) was used in the 1998 study. The analysis indicated that the interaction effect between the two factors was significant for both cultivars. Consequently, contrasts were constructed to compare the four treatment combinations and to create letter groupings. All other analyses were completed also using SAS (SAS Institute 1999).

RESULTS

Field Production of Seed Tubers (Pre-elite)

From Plantlets—The production of seed tubers (first field generation tubers) from *in vitro* derived plantlets is summarized in Table 1. No significant differences were found in total yield of tubers between cultivars in either of the seasons, although the total yields in 1998 were higher than in 1999, possibly due to a higher number of corn heat units the crop received during the 1998 (1398 units compared to 1200 in 1999). Other factors, for example hours of irradiance and diurnal temperature differences between the two growing seasons, also might have influenced yields. In 1998, Atlantic and Shepody produced the highest number of seed tubers (about 30% more than Russet Burbank). Most of the tubers of Atlantic and Russet Burbank were 48 to 88 mm in diameter, and most of those of Shepody were <48 mm. Atlantic and Shepody also produced a few (<1 per 20 plantlets) large tubers, >88 mm in diameter, whereas in Russet Burbank the number of tubers and yield in this category were negligible (Table 1). No significant differences in total yields between cultivars and in the number of tubers in 48- to 88-mm category were observed in 1999 (Table 1). Surprisingly, the plug method used in 1999 during transplanting plantlets to the field did not prove effective.

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

Cultivar	Tot. number of seed tubers /20 plantlets	Number of seed tubers /20 plantlets in size class [mm]:			Total Yield of seed tubers kg/20 plantlets	Yield of seed tubers (kg/20 plantlets) in size class [mm]:		
		<48	48-88	>88		<48	48-88	>88
1998 season*								
AT	129.0 a	57.0 a	71.0 a	1.0 a	14.178 a	2.200 a	11.050 a	0.537 a
RB	80.8 b	33.6 b	47.1 c	0.1 b	12.238 a	1.604 b	10.382 b	0.061 c
SH	113.2 a	61.5 a	51.2 b	0.5 ab	13.985 a	2.195 a	11.223 a	0.330 b
1999 season*								
AM	59.6 c	20.1 c	39.3 a	No tubers	9.440 a	1.226 c	Jar 7.146a Plug 8.480a	No tubers
RB	79.3 b	41.7 b	37.5 a	No tubers	9.022a	2.241 b	Jar 7.301a Plug 4.711b	No tubers
UM	100.0 a	56.5 a	43.5 a	No tubers	11.060 a	3.295 a	Jar 8.400a Plug 6.995a	No tubers

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

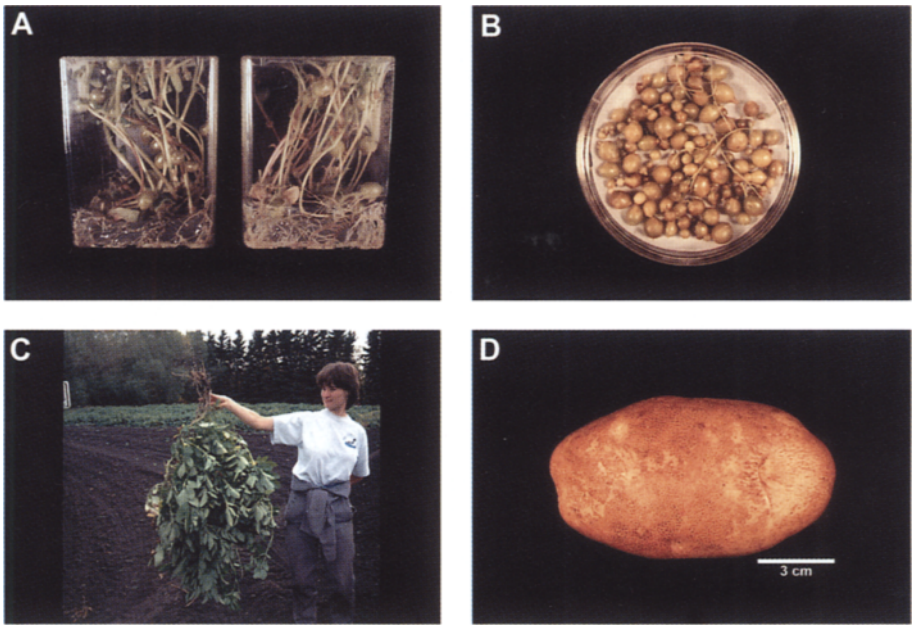

FIGURE 1.
Microtubers cv. Russet Burbank in the field production of Pre-elite tubers.

A. Microtubers produced under 8h photoperiod in Magenta G7 vessels.
B. Microtubers prior to field planting.
C. The plant of Russet Burbank at harvest (September 24, 1999).
D. The Pre-elite tuber of Russet Burbank produced from a microtuber.

In Russet Burbank plantlets from jiffy pots yielded significantly fewer tubers than plantlets directly transplanted to the field possibly due to a poor plant establishment. In Amisk and Umatilla Russet the difference was not significant (Table 1). Umatilla Russet produced the highest number of seed tubers, 100 per 20 plantlets (20% more than Russet Burbank and 40% more than Amisk). Most of Umatilla Russet and Russet Burbank tubers were smaller than 48 mm in diameter. None of the three cultivars produced tubers larger than 88 mm (Table 1). Compared to plants grown from microtubers, most of the plants derived from plantlets produced excessive foliage with extensive brunching of the lower stems.

From Microtubers—In both the 1998 and 1999 seasons, the production of seed tubers from microtubers was low and variable (especially in 1998) in comparison to plantlets (Tables 1, 2, and 5). This may have been caused by an incomplete break of their dormancy before planting. The effects of JA addition to the pretreatment and to the tuberization media on the yield and number of seed tubers are shown in Table 2. Yields of Atlantic were consistently very low, causing difficulty in meeting normality assumptions of the statistical model. Consequently, this variety was excluded from data analysis.

Table 2 shows the effects of JA conditioning of stock plantlets from which the nodal cuttings were taken to produce microtubers, and these microtubers were later used for the production of seed tubers in the field. The JA pretreatment significantly enhanced the total number of seed tubers in Russet Burbank by approximately 40%, but lowered it in Shepody by 17% (Table 2), although in Shepody the difference was not significant. In Russet Burbank, all the responses (total number of seed tubers, number of tubers <48 mm, number of tubers 48-88 mm and yield of tubers <48 mm) were significantly higher in the JA pre-conditioned treatments than in the untreated control. The reverse was true in Shepody, although again the differences were not significant. In Russet Burbank, about 2.5 seed tubers were produced per microtuber in the JA pre-treatment vs 1.8 in untreated microtubers (Table 2). On the other hand, the effect of JA in tuberization medium on the production of seed tubers in the field was opposite to that of JA pretreatment. In Russet Burbank, the total number of seed tubers was 42% and yield was about 65% lower than those from non-JA media (Table 2). Shepody, on the other hand, was either indifferent to or benefited from the JA treatment.

The low proportion of established plants from microtubers also contributed to low yields of seed tubers in 1998. Only 40% to 63% plants developed from microtubers (Table 3) compared to 100% in 1999 and to those from *in vitro* plantlets. Shepody plants grown from microtubers produced on JA-containing media showed significantly higher percentage establishment (63%) than from the other JA treatments. The opposite was observed in Russet Burbank, where the propor-

TABLE 2—*Effects of JA treatment during* in vitro *explant production (JA pretreatment and JA in media) on production of seed tubers from microtubers in the field. Cultivar* JA pretreatment and cultivar* JA in media interactions were significant (P < 0.05). Cultivars: Russet Burbank (**RB**) and Shepody (**SH**), 1998 season.*

Cultivar	JA Pre-treatment*	Total number of seed tubers /20 micro-tubers	Number of seed tubers /20 micro-tubers in size class [mm]:		Total yield of seed tubers (kg/20 micro-tubers)	Yield of seed tubers (kg/20 micro-tubers) in size class [mm]:	
			<48	48-88		<48	48-88
RB	No	35.4 b	29.5 b	5.9 b	1.579 b	0.882 b	0.685 b
RB	Yes	50.4 a	40.1 a	10.3 a	3.180 a	1.387 a	1.506 a
SH	No	47.7 ab	36.3 ab	11.4 a	3.337 a	1.185 ab	1.720 a
SH	Yes	39.8 ab	31.2 b	8.5 ab	2.819 ab	0.891 b	1.642 a
JA in media*							
RB	No	54.3 a	41.8 a	12.4 a	3.654 a	1.732 a	1.922 ab
RB	Yes	31.4 c	27.7 c	3.7 c	1.263 c	0.855 b	0.408 c
SH	No	40.7 bc	32.3 bc	8.4 b	2.935 b	1.333 ab	1.430 b
SH	Yes	46.7ab	35.2 b	11.5 ab	3.422 ab	1.344 ab	2.078 a

Means in the same column followed by different letters are significantly different (P<0.05).
*Letter groupings separate for JA pretreatment and JA in media.

TABLE 3—*Effect of JA pretreatment and JA media treatment on field establishment of light-produced (8-h) microtubers. JA pretreatment*JA media interaction was significant (P<0.001). Mean separation performed within each cultivar. Cultivars: Russet Burbank (**RB**), Shepody (**SH**), 1998 season.*

JA pretreated	JA in media	% of established plants RB	% of established plants SH
No	No	58 a	48 b
No	Yes	40 b	63 a
Yes	No	61 a	49 b
Yes	Yes	56 a	48 b

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

tion of established plants was the lowest for JA in media treatment (Table 3).

Exposure to light during *in vitro* tuberization had also a significant effect on the performance of microtubers in the field in both cultivars. The total number of seed tubers produced from 20 planted microtubers derived from 8-h light treatment was about 50% to 60% higher than from the microtubers produced in dark, 52.5 and 34.0, respectively (Table 4). Effects of light were even more significant for total yield, and yield and number of tubers <48 mm.

Since the 1998 results showed that microtubers produced under 8-h photoperiod gave higher yields of seed tubers in the field, only these microtubers were used in 1999. The overall production of seed tubers from microtubers in 1999 was more consistent than in 1998, mainly due to a more effective microtuber dormancy release with GA_3 and Rindite. Two two-factor inter-

TABLE 4—*Effects of light during* in vitro *tuberization on number and yield of seeed tubers in the field produced from microtubers of cultivars Russet Burbank and Shepody. Single effect of light was significant (P<0.05), 1998 season.*

Light (h)	Total number of seed tubers /20 microtubers	Number of seed tubers /20 microtubers size: <48 mm	Total yield of seed tubers (kg/20 microtubers)	Yield of seed tubers (kg/20 microtubers) size: <48 mm
0	34.0 b	26.2 b	1.476 b	0.800 b
8	52.5 a	42.4 a	2.673 a	1.372 a

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

TABLE 5—*Effects of JA treatments during* in vitro *explant production and tuberization on production of seed tubers in the field. Cultivar*JA in media, and cultivar*JA pretreatment interactions were significant (P<0.05). Cultivars: Amisk (**AM**), Russet Burbank (**RB**), Umatilla Russet (**UM**), 1999 season.*

Cultivar	JA in media	Cultivar*JA in media				JA pre-treatment	Cultivar*JA pretreatment	
		Total number of tubers /20 micro-tubers	Total yield of tubers/20 micro-tubers size: 48-88mm	Total Yield of seed tubers (kg/20 micro-tubers)	Yield of seed tubers (kg/20 micro-tubers) size: 48-88mm		Total Yield of seed tubers (kg / 20 micro-tubers)	Yield of seed tubers (kg / 20 micro-tubers) size: 48-88mm
AM	No	69.8a	14.6 a	3.871 a	1.860 a	No	3.473 a	1.576 ab
AM	Yes	55.6ab	10.8 ab	3.061 b	1.537 a	Yes	3.459 a	1.739 a
RB	No	61.4ab	10.1 ab	2.358 bc	1.115 ab	No	2.518 b	1.200 bc
RB	Yes	58.8ab	11.8 ab	2.588 bc	1.225 ab	Yes	2.428 b	1.226 b
UM	No	60.3ab	6.6 b	2.100 c	0.715 b	No	1.515 c	1.021 c
UM	Yes	42.0 b	1.9 c	0.929 d	0.053 c	Yes	1.514 c	0.976 c

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

actions (cultivar*JA in media and cultivar*JA pre-treatment) were significant for the total number and yield of seed tubers and for the number and yield of tubers in 48- to 88-mm size category (Table 5). As in 1998, with the exception of Russet Burbank, the microtubers produced with JA in the tuberization medium gave fewer seed tubers than those produced without JA. On the contrary, microtubers produced from the JA-conditioned Amisk and Russet Burbank stock plantlets gave up to 10% higher number of seed tubers in the 48- to 88-mm category (Table 5). Although these results were not statistically significant, they corresponded to the 1998 results in Russet Burbank.

Microtuber Dormancy Release (1999 Season)

Dormancy release of microtubers prior to field planting had a significant impact on the production of seed tubers in the field (Table 6). In the field production of the seed tubers, gibberellic acid treatment of microtubers was more effective than Rindite (Table 6). For all three russet varieties, the highest number and yield of seed tubers were produced when the microtubers were soaked in 100 ppm solution of GA3 prior to field planting. The yield and the number of seed tubers were significantly higher than after Rindite exposure when the microtubers were produced on media without JA (Table 6). For microtubers coming from JA tuberization media, there were no significant differences observed between dormancy release treatments in all parameters measured. Both Rindite and GA3 treatments gave similar results (Table 6).

DISCUSSION

Data presented in this study show that the field performance of microtubers was highly dependent on the potato cultivar, jasmonic acid (JA) conditioning of plantlets prior to *in vitro* tuberization, presence of JA in tuberization media, the photoperiod during tuberization and the dormancy release treatment. Optimizing these factors for a particular cultivar will be a key to a successful use of microtubers in production of seed tubers in the field.

Similar to the greenhouse study (Pruski et al. 2003), microtubers of Amisk (Ranger Russet) and Russet Burbank produced better results in production of seed tubers in the field than the other cultivars. Although yields of pre-elite tubers from microtubers of Russet Burbank and Shepody were similar to each other in 1998 season, they were far lower than yields from transplanted plantlets (Tables 1 and 2). In 1999, a similar trend was observed in Russet Burbank where yield of pre-elite tubers produced from plantlets was three times higher than from microtubers (Tables 1 and 5). Microtuber performance of Atlantic was poor, and at this stage of knowledge, this type of a propagule cannot be recommended for field planting for this cultivar.

An incomplete dormancy release of microtubers likely caused a low proportion of established plants in the field in 1998 season (Table 3). Microtubers, after *in vitro* tuberization, are generally very dormant (Struik and Wiersema 1999; Tábori et al. 1999) and will not sprout unless stored for 4 months or

TABLE 6—*Effects of JA during* in vitro *tuberization (JA in media) and dormancy release treatments, applied to microtubers prior to field planting, on production of seed tubers from microtubers in the field, in three russet cultivars. JA in Media*Dormancy release interaction was significant (P < 0.05), 1999 season.*

JA in media	Dormancy release	Total number of seed tubers/20 microtubers	Number of seed tubers /20 microtubers in size class [mm]:		Total Yield of seed tubers (kg/20 microtubers)	Yield of seed tubers (kg/20 microtubers) size 48-88mm
			<48	48-88		
No	GA	69.3 a	56.9 a	12.4 a	3.299 a	1.562 a
No	Rindite	58.4 ab	49.9 ab	8.4 b	2.254 b	0.861 b
Yes	GA	50.7 b	44.1 b	6.5 b	1.992 b	0.680 b
Yes	Rindite	53.6 b	44.2 b	9.3 ab	2.393 b	0.801 b

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

more at low temperatures (Struik and Wiersema 1999). Using older or pre-sprouted microtubers may be beneficial, since this might increase the number of sprouts per mother tuber (thus stems per plant) or advance the growth of sprouts or stems (Struik and Lommen 1999). Given the small size of the microtubers, too many stems per microtuber can also be deleterious (Struik and Lommen 1999). With regards to dormancy release treatments, our results in the field were opposite to the results in the greenhouse described by Pruski et al. (2003). In the field, plants derived from GA_3-treated seed tubers gave a better yield than from Rindite-treated microtubers.

Although JA, used as a conditioner in production of microtubers, proved to enhance their performance in the greenhouse production of nuclear seed (Pruski et al. 2003), the results obtained in the field were inconclusive. Stock plants pre-treated with JA before *in vitro* tuberization enhanced the seed tuber production in the field in Russet Burbank, but had no effect in Shepody (Table 2). On the other hand, JA presence in media during the production stage of microtubers, significantly lowered the proportion of established plants (Table 3) and consequently their field performance in Russet Burbank in 1998 (Table 2). In 1999, the results for Amisk and Russet Burbank followed a similar pattern, although the differences were not significant. However, Umatilla Russet microtubers produced without JA in the medium performed significantly better in the field than those from JA media (Table 5). Despite the reports in the literature indicating stimulatory effects of JA on *in vitro* tuberization and on potato stem node cultures (Koda et al. 1991; Ravnikar et al. 1992; Kreft et al. 1997; Pruski et al. 2002), based on our results, JA cannot be recommended as a microtuber conditioner for direct field planting and production of seed. The focus of future studies on utilization of microtubers should be to enhance sprouting and survival on the field.

Photoperiod (8-h light) during tuberization was an important factor in producing microtubers that later performed well in the field (Table 4). Microtubers derived from short photoperiod were greenish and seemed to be less juvenile than the tubers from dark treatments. Such microtubers perform better in the field or in the greenhouse than microtubers produced in darkness (Gopal et al. 1997; Struik and Wiersema 1999). Short days (SD) were reported to induce *in vitro* tuberization in potato shoots (grown on hormone-free media) by Dobránszki and Mandi (1993). Our observations (Table 4) showed a significantly increased production of seed tubers (1998 season) from microtubers produced in SD compared to those produced in darkness. The difference was so significant (Table 4) that in 1999 only microtubers produced in SD were used in field experiments. The photoperiod during *in vitro* tuberization is also linked to dormancy of microtubers and release from dormancy occurs faster in microtubers produced in SD (Coleman and Coleman 2000). Similar to greenhouse studies (Pruski et al. 2003), microtubers produced in dark performed poorly. Although the total number of tubers was 54% and the yield 81% higher from microtubers produced under SD than from those produced in dark (Table 4), the differences were less pronounced than in greenhouse studies (Pruski et al. 2003), where the three-fold increase in performance of SD-produced microtubers was observed in nuclear seed production.

The key factor to a successful use of microtubers in the field seemed to be the dormancy release. Recently, several researchers reported that dormancy of microtubers is cultivar dependent and, as mentioned earlier, is also affected by the

photoperiod applied during *in vitro* tuberization (Tábori et al. 1999; Coleman and Coleman 2000). In the study reported here we used gibberellic acid (GA_3) and Rindite to release microtuber dormancy prior to planting. Unlike in our greenhouse study, where Rindite proved to be very effective (Pruski et al. 2003), GA_3 gave slightly better results in the field (Table 6), particularly if used with non-JA treated microtubers. We used only microtubers >0.15 g in field plantings. Size of microtubers was reported to be critical for transplant survival by several authors (Ranalli et al. 1994; Kim et al. 1999). Also, the larger microtubers were less susceptible to Rindite damages widely described in literature (Ranalli 1997; Struik and Wiersema 1999). As in greenhouse studies, we did not observe any serious damages to microtubers treated with Rindite, if they were derived from the 8 h light tuberization treatment.

As our results suggest, the *in vitro* plantlets can be considered as a source of planting material in the field production of seed potato tubers (Table 1). In both greenhouse (Pruski et al. 2003) and field studies, transplanted plantlets were far superior to microtubers. Struik and Wiersema (1999) reported several successful studies on the use of plantlets in direct field planting to produce seed tubers. The method, however, needs more research with a focus on an effective utilization of a mechanical planter. However, microtubers did not produce satisfactory results, and at this stage of knowledge, cannot be recommended as a propagule for a commercial production of seed tubers in the field. Yields of seed tubers produced from microtubers were only 30% to 40% of those from plantlets (Tables 2, 5, 6). A further refinement of the production and handling methods is required before microtubers can be recommended for field production of seed tubers.

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