# Micropropagation Technology in Early Phases of Commercial Seed Potato Production

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## Abstract

**Research was carried out on possibilities to improve methods commonly used in commercial potato tissue culture laboratories, predominantly those in Western Canada. The study included: (1) Photoautotrophic micropropagation, (2) Short-term, low temperature (< 12 weeks) storage of plantlets, (3) Production of microtubers (in vitro tubers) and (4) Utilization of microtubers in greenhouse production of minitubers and Pre-elite tubers in the field. Effects of jasmonic acid (JA) and photoperiod on in vitro tuberization were also tested. Performance of microtubers in production of nuclear (greenhouse) Pre-elite tubers was compared with the industry standard, in vitro plantlets. Six commercial cultivars, 'Amisk', 'Atlantic', 'Russet Burbank', 'Sangre', 'Shepody' and 'Umatilla Russet', were used in the studies. Potato plantlets produced photoautotrophically were either of the same quality or better than conventional cultures. The conventional cultures benefited from $CO_2$ enrichment, over a 4 week period, by a 20% increase in number of nodes and a 50% increase in stem dry weight. Cultures stored for up to 12 weeks in a cold room (4°C) on media containing 30 $g \cdot L^{-1}$ sucrose under continuous, low red light (690 nm) at 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD maintained high quality, vigor and re-growth capacity. Significantly better production of microtubers (more tubers and weight) was observed on solid agar than on liquid media, under the 8 h photoperiod (SD) compared to no light. Microtubers produced under SD performed better in the field or in the greenhouse than microtubers produced in dark. In most studies, JA conditioned plantlets produced more uniform and larger microtubers, especially under short days conditions (8 h light). Production of microtubers in all three russet varieties tested was superior to that of Shepody and Atlantic. Results with pretreatment of JA in the field production of Pre-elite tubers were inconclusive. Microtubers of russet varieties performed the best and can be recommended for greenhouse production of minitubers but not for direct use in the field.**

## INTRODUCTION

In potato, micropropagation became an essential component of seed production systems, providing large numbers of pathogen-free plants in early phases of multiplication. Tissue cultured plantlets are the most common type of propagule used in the seed potato system around the world. Microtubers or in vitro produced tubers are another type of propagule that could also be utilized in the production of pathogen-free seed potato material. Microtubers are small (0.02-0.7 g) tubers produced from nodal cuttings or on complete plantlets using a specific growing medium (Hussey and Stacey, 1984; Estrada et al., 1986; Lillo, 1989; Struik and Lommen, 1990). Despite their advantage over in vitro plantlets in storage and germplasm exchange (Estrada et al., 1986), microtubers have not been accepted as propagules of choice by the industry (Lommen, 1995; Struik and Wiersema, 1999). Recently, several new production protocols have been developed (Struik and Wiersema, 1999). Most of them, however, require either special equipment or frequent media exchanges which increase cost and labour demand and may lead to culture infection (Ranalli, 1997; Struik and Wiersema, 1999). The

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research work presented here is focused on possibilities to improve methods used in tissue culture propagation of potato, and on commercial production of microtubers and their utilization in the early stages of seed potato programs.

## MATERIALS AND METHODS

Tissue cultured plantlets cvs 'Amisk', 'Atlantic', 'Russet Burbank', 'Sangre', 'Shepody' and 'Umatilla Russet' used in the studies were produced from the potato germplasm bank at Crop Diversification Centre North (CDCN), Edmonton, Alberta, Canada. The plantlets were multiplied as single-node explants in GA-7 Magenta vessels (Magenta Corp., Chicago, IL, USA) on hormone-free MS (Murashige and Skoog, 1962) medium (Sigma-Aldrich Canada Ltd., Oakville, Ont., Canada) with standard vitamins, sucrose 30 $g \cdot L^{-1}$, solidified with 0.6% agar (Difco, Detroit, MI, USA). The pH was 5.7 before autoclaving. Sixteen (4x4) single-node explants were placed in each vessel. Cultures were incubated for 4 weeks in an environmental chamber (Conviron S10h, Winnipeg, Manitoba, Canada) at 20 $^{o}$C +/- 1 $^{o}$C, 16 h photoperiod at 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ mixed fluorescent (F40T12 tubes, General Electric (GE), USA) and incandescent (40W, GE, USA) illumination. After 4 weeks, the plantlets were cut into nodal cuttings and placed on fresh MS medium for further multiplication. This was repeated until the required number of plantlets was achieved.

The photoautotrophic growth of Russet Burbank cultures was examined using single-node explants divided into two groups and transferred (apical and basal nodes were discarded) onto 50 ml agar media: (i) the same hormone-free MS medium as above, and (ii) the MS medium (with standard vitamins) but without sucrose. Sixteen (4x4) single-node explants were placed in each vessel and the vessels were capped with lids equipped with 0.22 μm filters. Both groups of cultures were incubated for 4 weeks in: (i) the same environmental growth room and under the same conditions as described above, and (ii) in an environmental growth chamber (Conviron CMP3023, Winnipeg, Manitoba, Canada) at 21/19 $^{o}$C day/night temperature, 16 h photoperiod at 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ mixed fluorescent (FT72112/CW/VHO tubes, Philips, USA) and incandescent (40W, GE, USA) illumination supplemented with 1500 $\mu l^{-1}$ $CO_2$. After 4 weeks the number of nodes (leaves), shoot length and dry weight were recorded.

In short-term (<12 weeks) storage experiments, shoot tops taken from 4 week old cultures of potato, were placed in GA-7 Magenta jars (4 shoots per jar) onto MS media +/- 30 $g \cdot L^{-1}$ sucrose and moved to cold rooms at 4 $^{o}$C +/- 1 $^{o}$C, with and without low red light illumination (690 nm, 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, provided by miniature red lights, HML-35 (LL42485), Hudson's Bay Co., ON, Canada) for 6, 9, and 12 weeks. After each storage period, shoots were subcultured on MS media of the same composition as for storage, and grown for 4weeks in a growth-room at 24/22 $^{o}$C day/night temp, 16 h photoperiod, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Length of individual shoots and their fresh weight measured after storage (Fwt1) and after 4 weeks on re-growth media (Fwt2), number of leaves per stem after storage and number of stems per culture after the re-growth were recorded. A relative growth rate (RGR) after re-growth was calculated as RGR = (Fwt2 – Fwt1)/Fwt1.

In production of microtubers, during the last transfer before in vitro tuberization experiments, single-node explants of all studied cultivars were divided into two groups and transferred onto agar media: 1) the same hormone-free MS as in the multiplication stage and 2) the MS medium supplemented with 2.5 μM JA (this group of explants was labeled as JA pre-treated (JAP)). Both groups were incubated for 4 weeks under the conditions described for multiplication stage. Effects of photoperiod, media (liquid vs. agar (solid), for only cvs 'Russet Burbank' and 'Sangre'), and JA pre-treatment of nodal cuttings on microtuber production were investigated. For in vitro tuberization, nodal explants of both groups were placed on MS tuberization media supplemented with sucrose at 80 $g \cdot L^{-1}$. Fifty ml of agar (all cultivars) and 15 ml of liquid ('Russet Burbank' and 'Sangre' only) medium was dispensed per jar. Cultures were incubated at 20 $^{o}$C with 0 (darkness), 8 and 16 h photoperiod at 50 $\mu E \cdot m^{-2}$ $s^{-1}$ mixed fluorescent/incandescent light

for 10 weeks. Every 2 weeks, 7 ml of liquid MS with 80 $g \cdot L^{-1}$ sucrose was added to each jar with cultures on liquid media. After 10 weeks microtubers were harvested, the number and weight of microtubers produced per nodal cutting was recorded. Thirty two GA7-Magenta vessels (32 reps) with 16 explants each were used in each treatment per cultivar.

Microtubers produced during in vitro tuberization were used in production of nuclear tubers (greenhouse) and Pre-elite tubers in the field. Performance of microtubers was compared with the industry standard, the in vitro plantlets. Only microtubers > 150 mg were used in the studies. To induce uniform sprouting microtubers were treated with: (i) 100 ppm solution of gibberellic acid ($GA_3$) for 24 h, and (ii) Rindite, a mixture of ethylene chlorhydrin, ethylene bichloride and carbon tetrachloride, 7:3:1 v/v, respectively (Denny, 1945). For greenhouse production of minitubers, the in vitro plantlets and/or microtubers of each cultivar were transplanted to greenhouse beds filled with 15 cm of PRO-Mix 'BX' (Premier Horticulture, Dorval, Quebec, Canada), a peat-based growing medium (pH 5.5-6.0, electric conductivity (EC) 1.5 - 2.0 mmhos/cm, 75-85% sphagnum, perlite and vermiculite). Spacing between plants was 9 x 9 cm. Plants were grown for 16 weeks under standard greenhouse conditions (24/18 $^oC$ day/night temperature and with 14 h light with supplemental lighting (FT72112/CW/VHO tubes, Philips, USA) at 150 $\mu E \cdot m^{-2} \cdot s^{-1}$. One week after planting, a water-soluble 10-52-10 (NPK) fertilizer (Plant-Prod, Brampton, Ontario, Canada) at 1.5 $g \cdot L^{-1}$ was applied to plantlets weekly, for the next two weeks. Three weeks after planting, a water-soluble 20-20-20 fertilizer (Plant-Prod, Brampton, ON, Canada) at 3 $g \cdot L^{-1}$ was used every second week (total 5 times), for 10 weeks. Plants were sprayed with Bravo to prevent and control Gray mold (*Botrytis cinerea*), approximately every 2 weeks from mid-July until late August. In week 14 in the greenhouse, the plants received tap water only. Plants were not watered during the last week before harvest of minitubers. Plots were harvested by hand and the minitubers sorted by size and weight (size categories: (i) 5-30 mm, (ii) 30-60 mm, and (iii) > 60mm).

The field plots were 3m (10 feet) long with 15 cm (6 inches) between plantlets or microtubers, and 90 cm (36 inches) between rows. 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. Plots were irrigated as needed and treated with Bravo 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. The crop received a total of 1,398 corn heat units (CDCN, Edmonton weather station records) in 1998 and 1,200 in 1999. 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 Pre-elite tubers produced per plantlet and/or microtuber was recorded.

## RESULTS AND DISCUSSION

A 20% increase in the number of nodes per stem (from 7.5 to 9.4) and a 50% increase in stem dry weight were observed in cultures grown on media with sucrose and in $CO_2$ enriched atmosphere as compared to the conventionally micropropagated cultures or the cultures grown photoautotrophically on media without sucrose but in air supplemented with 1500 $\mu l \cdot L^{-1}$ $CO_2$ (Table 1). No significant differences were observed between Control (MS medium supplemented with sucrose, 30 $g \cdot L^{-1}$) and photoautotrophic cultures. Photoautotrophic cultures produced stems averaging 43.3 mm in length, with 8 nodes and weighing 9.2 mg (dry weight), similar to conventional cultures (Table 1). Increase in growth of in vitro shoots/plantlets is a result of enhanced photosynthesis which, in turn, is affected by several factors including $CO_2$ concentration (Desjardins, 1995), sucrose concentration in media (Fujiwara et al., 1992), the number of air exchanges and the lighting cycle (Hayashi et al., 1995).

In short-term (< 12 weeks) storage, sucrose (30 $g \cdot L^{-1}$) in the storage medium was critical for the potato cultures. After storage, plantlets re-grown from stock plants kept on sucrose had approximately 2.5 times higher fresh weight and almost 5 times the shoot length at four weeks in culture, compared to the plantlets re-grown from stock plants kept

on the medium without sucrose (Table 2). All RGR values calculated for potato cultures grown from the cultures stored on media without sucrose are negative. The positive RGR occur only for cultures stored on media with sucrose, irrespective of the length of the storage period (Table 2). Cultures also benefited from the light during storage, producing significantly higher RGR than those stored in dark. Miyashita et al. (1995) described beneficial effects of low intensity red light illumination (2–50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on growth of potato culture. The results indicate that growers can successfully use their existing facilities, small refrigerators and coolers with low light intensity, set at 4 $^{o}$C, for short-term storage of potatoes. The potato cultures (known to be sensitive to prolonged low temperature storage) should be frequently monitored and sub-cultured as required.

In vitro tuberization studies indicated the 8 h photoperiod was the most effective in the production of microtubers. Following the 10 week period, the highest number of microtubers per jar was produced from cv. 'Sangre' (SA) on media solidified with agar, under 8 h light (Table 3). SA produced significantly more tubers (24.7 per jar, or 1.65 per nodal explant) than 'Russet Burbank' (RB) (17.7 per jar, or 1.18 per explant), although SA's microtubers were smaller than RB's (150 vs 300 mg, respectively). The number of microtubers under 16 h photoperiod was significantly lower (6.0-9.7 for RB and 4.5-11.4 for SA) than under 8 h (8.6 - 17.7 for RB and 10.6 – 24.7 for SA) (Table 3). The average weight of RB microtubers was also the highest with 8 h light treatment and when the tuberization was carried out on solid media from cuttings pre-treated with JA (Table 3). The JA pre-treatment did not have a significant effect on the number of microtubers produced on liquid media under 8 h light. In darkness, the significant benefits of JA pre-treatments were in SA on liquid medium (average weight 259.9 mg pre-treated vs 177.5 mg for non-pre-treated). In RB, the JA pre-treatment only significantly enhanced the number of microtubers per jar on solid medium under 8 h light (Table 3). Stimulatory effects of JA on in vitro tuberization and on potato stem node cultures have been reported by Koda et al. (1991), Kreft et al. (1997) and Jackson (1999). Our further in vitro tuberization studies with other cultivars confirmed the photoperiod being the most important factor during the production of microtubers. Similar to other studies (Dóbránszki and Mandi, 1993; Nowak and Asiedu, 1992), light exposure gave significantly larger and more uniform microtubers in all tested cultivars, independent of cultivar and JA treatment. The 0 h photoperiod (dark) was found unsuitable for in vitro tuberization since both the number and weight of microtubers produced in dark treatments were significantly ('Atlantic' (AT)) and drastically ('Shepody' (SH)) lower than in treatments with 8 h light (data not shown). Production of microtubers in all three russet varieties and in SA was superior to that of SH and AT. Nowak and Asiedu (1992) reported AT being the slowest to tuberize from the six varieties they tested. Jackson (1999) concluded that it is actually the length of the dark period rather than the light period that is important in inducing in vitro tuberization. The effects of JA on production of microtubers were light and variety specific. Generally, the benefits were more pronounced in the dark than under 8 h light tuberization. JA is involved in control of leaf senescence in potato which is closely associated with tuber induction (Van den Berg and Ewing, 1991). The JA pre-treated nodal cuttings used in our experiments may have possessed the signal to quickly induce tuberization and produce more uniform and higher number of microtubers compared to untreated cuttings. Pelacho and Mingo-Castel (1991) reported JA induction of tuberization in potato stolons cultured in vitro.

Table 4 summarizes the production of nuclear seed tubers from in vitro derived plantlets in 1998 and 1999. No significant differences were found in total yield (kg) of minitubers among cultivars in either of the seasons. In 1998, AT produced the highest number of minitubers, almost twice that of RB and SH. Most of the tubers were small and medium sized (Table 4). SH produced the highest weight of > 60 mm diameter minitubers. Although the total yield of RB was not significantly different from that of the other cultivars, its tubers fell evenly into the 30-60 mm and > 60 mm sizes (Table 4). In 1999, total yield of RB minitubers doubled the 1998 figure. AM and Umatilla Russet (UM) produced about 35% of tubers in the 30-60 mm and about 30% in > 60 mm

categories (Table 4). Total yield and number of minitubers were not significantly affected by cultivar. In all three cultivars, only 15-25% of the minitubers were 5-30 mm in diameter (Table 4). In 1998, yield of minitubers from microtubers was low, likely due to incomplete breaking of dormancy prior to planting and data are not included. The 1999 data showed that the presence of JA in pre-treatment and in tuberization media, the cultivar, and the exposure to light during tuberization all had significant effects on the number, yield (fresh wt) and size distribution of minitubers. Relative to RB and UM, AM produced the highest number of minitubers per plot (> 3 minitubers per microtuber) derived from JA pretreated plantlets (Table 5). In AM and RB, microtubers derived from explants taken from JA conditioned stock plants gave significantly more minitubers than the microtubers from other treatments. With UM, the differences were not significant (Table 5). Numbers of minitubers of AM derived from the microtubers were comparable to numbers obtained from plantlets (65.5 minitubers from 20 plantlets (Table 4) and 61.3 from 20 microtubers derived from JA treated stock plants (Table 5)). RB and UM produced fewer tubers from microtubers than from plantlets. Total yield of RB minitubers was the highest from microtubers from JA pretreatment. 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). Recently, several researchers reported that dormancy of microtubers is cultivar-dependent and is affected by the photoperiod applied during in vitro tuberization (Coleman and Coleman, 2000). We used $GA_3$ and Rindite to release microtuber dormancy prior to planting. Rindite was the most effective in greenhouse conditions. Based on our data, only the microtubers of cvs AM, RB and UM are suitable for greenhouse production of minitubers. JA pretreatment of stock plants, prior to in vitro tuberization, was effective in enhancing yields of nuclear tubers from microtubers and can be of help in the commercial laboratory/greenhouse setup.

In the field studies, in most treatments, Pre-elite tubers yields produced from microtubers were 50% of those produced from plantlets. Microtuber responses to JA varied with cultivar and generally were inconclusive; stock plantlets pretreated with JA (prior to in vitro tuberization) enhanced the Pre-elite tuber production in RB but significantly lowered it in SH. JA presence in media during in vitro tuberization significantly lowered production of Pre-elite tubers while exposure to 8 h light resulted in microtubers performing significantly better in the field than microtubers produced in the dark (data not shown).

## CONCLUSIONS

Growers may consider photoautotrophic culturing of potato in areas where the high sterility levels are difficult to maintain. Supplementing air in the growth room with 1500 $\mu l \cdot L^{-1}$ of $CO_2$ could be beneficial for potato plantlet production even on media containing sucrose since it significantly improved quality, size and biomass of produced plantlets, speeding up the multiplication. A simple refrigerator set at 4 $^{o}$C with additional low-light can be used for short-term (< 12 weeks) storage of potato cultures. The MS media should contain sucrose at 30$g \cdot L^{-1}$. JA conditioning of plantlets prior to in vitro tuberization helps to produce more uniform and larger microtubers. Microtubers of russet varieties (‘Amisk’, ‘Russet Burbank’, ‘Umatilla Russet’) can be recommended for speeding up multiplication during greenhouse production of minitubers. At this stage of knowledge, microtubers cannot be recommended for field planting.

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## Tables

Table 1. Effects of sucrose and $CO_2$ enriched atmosphere on growth of cultures of Russet Burbank. Least Squares means for $CO_2$ and Sucrose treatment combinations with Tukey's adjustment for multiple comparisons.

| Treatments $CO_2$ | Sucrose ($g \cdot L^{-1}$) | Shoot length (cm) | # of nodes per stem | Dry wt. of stems (mg) |
|---|---|---|---|---|
| Control (Air) | 0 | 18.7 c | 4.61 c | 4.8 c |
| Control (Air) | 30 | 47.9 b | 7.55 b | 9.7 b |
| $1500 \mu l \cdot L^{-1}$ | 0 | 43.3 b | 7.58 b | 9.2 b |
| $1500 \mu l \cdot L^{-1}$ | 30 | 74.1 a | 9.38 a | 14.9 a |

Means followed by the same letter are not significantly different at the 1% level of significance.

Table 2. Sucrose - S, Light - Li and Length of storage at 4°C – Le (S*Li*Le) interaction. Least Square means comparison of all responses.

| Treatment | | | After Storage | | | After Re-growth | | | |
|---|---|---|---|---|---|---|---|---|---|
| S | Li | Le [wks] | Fwt 1 [mg] | Shoots [mm] | Leaves [number] | Fwt 2 [mg] | Shoots [mm] | Shoots [number] | RGR |
| No | Dark | 6 | 181.6c | 42.8cd | 8.0ab | 72.0d | 9.1d | 1.0d | -0.54e |
| No | Dark | 9 | 170.5cd | 43.1cd | 7.8bcd | 64.0d | 7.0d | 1.0d | -0.58e |
| No | Dark | 12 | 158.2cd | 43.2cd | 7.0e | 64.0d | 7.0d | 1.0d | -0.56e |
| No | Light | 6 | 191.9bc | 42.6cd | 8.6a | 109.0c | 20.4c | 1.1cd | -0.21de |
| No | Light | 9 | 156.3cd | 43.5cd | 7.8bc | 69.8d | 9.0d | 1.0d | -0.52e |
| No | Light | 12 | 209.1ab | 41.6cd | 7.1de | 107.4c | 14.1cd | 1.1cd | -0.35e |
| Yes | Dark | 6 | 182.5c | 47.1ab | 8.2ab | 181.1b | 57.8ab | 1.4ab | 0.20bcd |
| Yes | Dark | 9 | 225.3ab | 49.9a | 7.4cde | 179.5b | 50.8b | 1.3bcd | -0.08cde |
| Yes | Dark | 12 | 233.7a | 47.2ab | 8.0ab | 243.3ab | 48.5b | 1.7a | 0.29bc |
| Yes | Light | 6 | 137.4d | 40.8d | 8.5a | 257.0a | 58.8a | 1.5ab | 0.96a |
| Yes | Light | 9 | 175.1cd | 44.5bc | 7.3de | 182.3b | 51.6ab | 1.3bcd | 0.45b |
| Yes | Light | 12 | 193.2abc | 47.9a | 7.9bc | 181.4b | 54.8ab | 1.6ab | 0.15bcd |

Means followed by different letters are significantly different ($P \leq 0.01$).
**RGR** – relative growth rate (Fwt2 – Fwt1)/Fwt1.

Table 3. Least Squares Means for the effect of media, JA pre-treatment **(JAP)** and photoperiod on the average number and fresh weight of microtubers per jar at the time of harvest (week 10) in Russet Burbank (RB) and Sangre (SA).

| Factor | | | Av. number of microtubers | | | Av. weight of microtubers [mg] | | |
|---|---|---|---|---|---|---|---|---|
| Cultivar | JAP | Medium | Light 0 hrs | Light 8 hrs | Light 16 hrs | Light 0 hrs | Light 8 hrs | Light 16 hrs |
| RB | Yes | Liquid | 15.2 a | 13.7 c | 9.7 a | 117.3 c | 284.8 ab | 182.4 c |
| RB | Yes | Solid | 11.5 b | 16.3 b | 6.1 b | 85.3 d | 312.8 a | 309.7 a |
| RB | No | Liquid | 11.8 b | 8.6 d | 6.0 b | 168.7 b | 280.7 ab | 239.1 ab |
| RB | No | Solid | 17.8 a | 17.7 b | 6.6 b | 70.8 d | 300.7 ab | 196.1 c |
| SA | Yes | Liquid | 9.7 b | 10.6 cd | 4.5 b | 259.9 a | 253.7 b | 240.8 ab |
| SA | Yes | Solid | 16.7 a | 24.7 a | 11.4 a | 90.2 d | 149.1 d | 286.6 a |
| SA | No | Liquid | 11.7 b | 13.5 c | 6.1 b | 177.5 b | 191.6 c | 213.4 bc |
| SA | No | Solid | 16.7 a | 22.0 a | 10.0 a | 138.8 c | 132.8 d | 278.2 a |

Means within a column followed by different letters are significantly different ($P \leq 0.05$).

Table 4. 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 | Number of minitubers from 20 plantlets | Number of minitubers size: 5-30 mm | 30-60 mm | > 60 mm | Yield of minitubers from 20 plantlets | Yield of minitubers (Kg / 20 plantlets) size: 5-30mm | 30-60 mm | > 60 mm |
|---|---|---|---|---|---|---|---|---|
| 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 ($P \leq 0.05$).
*Note that the letter groupings are for each season separately.

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 ($P \leq 0.05$). Mean separation performed within each cultivar. Cultivars: Amisk (AM), Russet Burbank (RB), Umatilla Russet (UM), 1999 season.

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

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