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Hydroponic Minituber Production in Growth Room Conditions and Carry-Over Effects of the Technique on Produced Minitubers

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Abstract

The production of minitubers was implemented with a hydroponic technique in growth rooms and the carry-over effects of the technique on the characteristics of minitubers were studied. As a comparison, minitubers from in vitro plantlets were grown in a peat-based growing medium. The results show that hydroponic production of minitubers is successful in indoor conditions with the cultivars Desiree, Van Gogh and Asterix, when day-time growing temperatures of 19.4 °C-26.0 °C and night-time temperatures of 17.5 °C-22.6 °C were used. Photosynthetically active illumination was adequate at 2383-2509 μmol $m^{-2}s^{-1}$; lighting conditions consisted of 14/10-hour day/night cycles.

The cultivars Desiree and Van Gogh developed their first tuber three weeks faster than Asterix, and the minituber yield was 4.5 per plant for Desiree, 7.5 for Van Gogh and 4.0 for Asterix. When a peat-based growing medium was used, minituber yields were almost the same but the size of the minitubers was smaller than that of hydroponically produced minitubers. The results of the carry-over experiments showed that conventionally produced minitubers emerged faster, and in terms of foliage development and yielding capacity performed better than hydroponically produced minitubers.

Keywords: *Solanum tuberosum* L., minituber production, hydroponics

1. Introduction

Faster, more cost-effective technical solutions with a higher production capacity are needed for minituber and pre-basic seed potato (*Solanum tuberosum* L.) production. Production efficiency is often measured in terms of the rate of minituber production and the number of minitubers produced. It has been proposed that soilless minituber production techniques increase production volume (Rolot & Seutin, 1999). Various soilless, hydroponic and aeroponic (Corrêa et al., 2008; Ritter et al., 2001) and bioreactor-type (Kämäräinen-Karppinen et al., 2010; Akita & Ohta, 1998) production methods have already been developed and are in use. The production capacity of these new techniques is typically compared to the minituber yields achieved by conventional in vitro propagation techniques. Even though it is possible to increase conventional minituber production (Milinkovic et al., 2012; Veeken & van der Lommen, 2009) from in vitro plantlets, but it involves clearly higher production costs and relatively low tuber yield (Rolot & Seutin, 1999).

In hydroponic production, plant roots are freely suspended in nutrient solution from which they derive the necessary nutrients and trace elements. It has been shown to be possible to increase the number of minitubers produced by using the hydroponic production technique (Rolot & Seutin, 1999). The hydroponic technique has also been used with sand substrates (Novella et al., 2008). The introduction of new minituber production techniques requires the optimization of production conditions. A specific requirement for hydroponic production is to achieve a balance between the nutrient ratios within the nutrient solution, electrical conductivity (EC) value, and pH as needed for potato production (Chang et al., 2011; Novella et al., 2008; Ritter et al., 2001). Although only few studies have been conducted on the effect of EC on potato, EC values from 1.2 to 1.7 dS m^{-1} are considered normal (Chang et al., 2011; Novella et al., 2008). In the studies of Chang et al. (2011), pH fluctuations between 5.0 and 7.2 in hydroponic production correlated with the EC levels of the nutrient solutions. In addition, temperature and illumination levels should be taken into consideration in minituber production

(Levy & Veilleux, 2007; Vreugdenhil et al., 2007). Hydroponic production can be implemented with artificial lighting (Ritter et al., 2001) or by modifying natural light with supplemental illumination (Chang et al., 2012). According to Vreugdenhil et al. (2007), long periods of daylight and high temperatures inhibit tuber formation and, correspondingly, high illumination levels, low temperatures and relatively scarce nutrient reserves improve tuberization. Levy and Veilleux (2007) consider +20 °C as the optimal temperature for tuber formation and for photosynthesis of European potato cultivars. In the studies of Chang et al. (2012) on hydroponic production during the light period, temperatures were 24±4 °C and 12±2 °C by day and night, respectively.

In northern conditions (64-65°N), environmental factors limit the utilization of illumination and temperature in conventional minituber production in greenhouses, and only one production period is possible when natural environmental conditions are utilized. Soilless indoor production techniques therefore provide an attractive alternative. In the present study we examined whether an indoor hydroponic production system and artificial simulation (light, temperature, humidity) of the production environment enable minituber production as an alternative to conventional method. The hydroponic production technique is not, at present, in use in minituber production in Finland and the carry-over effects of the technique on the characteristics of the produced minitubers have not been studied.

2. Method

2.1 Hydroponic Minituber Production

The hydroponic production system consisted of a microplantlet hardening table, cultivation tables with growing trays, an automatic central processing unit, nutrient solutions, and tanks with pumps (Living Foods VB, Holland, http://english.livingfoods.nl/). The production systems were placed in two growth rooms 8 m^2 and 9 m^2 in area. Each growth room had 10 hydroponic growing trays, each with a width of 225 mm, height 50 mm and length 2500 mm. Thirty microplantlets were planted in each tray 15 cm apart, with a total of 300 microplantlets per growth room and 100 microplantlets per cultivar. The study was conducted twice, in 2012 and in 2013, in both growth rooms (4 × 2 × 100 microplantlets/cultivar, total 800 plants/cultivar).

The roots of the microplantlets of Desiree, Van Gogh and Asterix grown on a regular semisolid MS medium (Murashige & Skoog, 1962) were washed with 38 °C water and transferred to hardening conditions. The hardening table had a closed nutrient solution cycle. After 13 days, the microplantlets were transferred to a cultivation table in the growth rooms (Figure 1). In the hydroponic system, the plants are cultivated in growing trays containing a nylon fabric at the bottom of the tray, which ensures optimal distribution of nutrient solution to the roots throughout the tray. The shoots of the microplantlets grow above a plastic sheet and the roots and the tubers are developed between the nylon fabric and the plastic sheet.

Figure 1. Hydroponic production trays after transfer of the microplantlets and before harvesting (right) (photo E. Virtanen)

The trays were open-ended, with the nutrient solution fed to the tray via a hose at one end and returned to a tank equipped with a pump at the other end, and then recirculated to the tray via a filter system. Three different nutrient solutions were used in the circulation system depending on the plant developmental stage. During the microplantlet rooting phase and continuing until the initiation of tuber formation, nutrient solutions varied, nitrogen level was 20-30 kg/ha as compared to field nutrient level. At the initial phase of tuber formation, solution 1 (Ca:K:Mg–0.7:1.0:0.5) was replaced with nutrient solution 2 (Ca:K:Mg–0.4:1.0:0.3) in the nutrient circulation, and solution 2 was replaced by 3 (Ca:K:Mg–1.5:1.0:0.4) during tuberization. The nutrient solutions

contained furthermore P, S, Mn, Cu, Zn, Fe and Na nutrients in their different liquid forms. The nutrient solution flow rate was 0.5 l min^{-1} and the flow cycle was one hour after the fluid flow, following a 5-minute pause. The nutrient solution pH levels 5.8 ±0.2 and EC 1.3 ±0.3 were adjusted according to the target values using an NMC-Pro controller (Netafim TM, NMC-Pro-Irrigation, Israel) to guide and control the hydroponic production system. In addition, the pH of the nutrient solution, EC and temperature were monitored daily by sensor measurements (Bluelab Combo Meter, Bluelab Corporation Limited, New Zealand, http://wwww.getbluelab.com).

2.2 Conventional Minituber Production

Hydroponic production was compared to a conventional minituber production method, and for this reason, microplantlets were also planted in a peat-based growing medium. A total of 160 microplantlets per cultivar were planted (40 cm × 60 cm, depth 15 cm) at a distance of 10 cm from the polypropylene boxes, and peat (Kekkilä White 420 W) was used as the growing medium. The peat was fertilized with Kekkilä starter fertilizer no. 1 (NPK 14-4-20) with a pH of 5.9 and electrical conductivity 27 m Sm^{-1} and containing 1600 mg/kg water-soluble nitrogen, 500 mg kg^{-1} soluble phosphorus and 2600 mg kg^{-1} soluble potassium (dry matter). The growing boxes were irrigated from below with same solution as in the hydroponic technique.

2.3 Growth Room Conditions

It was used two growth rooms in the research; 8 m^2 and 9 m^2 in area. The walls of the rooms were constructed of plastic-coated brick. The floors were made of acrylic covered concrete. High pressure sodium lights (4 × 400 W per growth room) (Osram Vialox, Germany) with time control (Schneider Electric TAC Xenta, Sweden) were used and the lamp spectrum were the same in both growth rooms (Figure 2). Light intensity varied in both growth rooms between 16,000 and 18,000 lx regardless of the measuring points. Photosynthetically active radiation (PAR) in the foliage was 2383-2509 µmol $m^{-2}s^{-1}$ (sensor measurement wavelength 400-700 nm). In the growth rooms were implemented daytime light conditions, with 14 h (07:00 am-09:00 pm)/10 h (9:00 pm-07:00 am) day/night illumination. During the production phase, the spectrum and intensity of the light were determined for both growth rooms. The amount of illumination was measured with a HD 9021 photo-radiometer (Delta OHM SRL, Italy) equipped with RAD and PAR sensors. The RAD sensor measures radiation efficiency in lx, and the PAR sensor measures the photosynthetically active radiation in photons per square metre. Light spectrum were measured using the OL754 Portable High Accuracy UV-Visible spectroradiometer (IS-670-LED, 754-O-PMT, 754-C, Optronic Laboratories, Inc., Orlando, FL, USA).

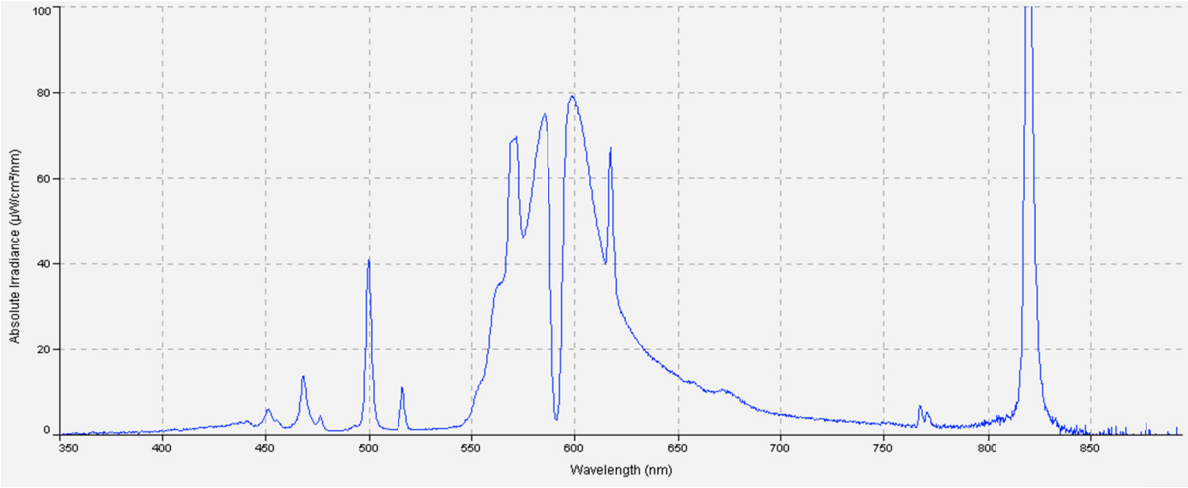

Figure 2. High-pressure sodium lamp light spectrum in the growth rooms, measured with a spectroradiometer

Growth room temperatures were adjusted to 20±2 °C for daytime and 14±2 °C for the night period using AC automation (Ilmateollisuus Oy, Finland). Temperatures varied in growth room 1 between 19.4 and 26.0 °C and between 20.9 and 26.0 °C in growth room 2. Night-time temperatures were between 15.5 and 22.6 °C in both rooms. In growth room 1 daytime temperatures were 0.5 °C and night-time temperatures were 0.7 °C lower than in growth room 2. Air humidity in the growth rooms was adjusted to 60-80% using a steam generator (Vapac LE,

UK) and humidity levels were predominantly below 60%. Temperature and humidity readings were followed with data loggers (Netafim, Climate Box, Netafim, Israel).

2.4 Observations and Samplings

Plant development and tuber formation were observed according to Hack et al. (1993) using pre-marked individual plants (10 plants per growing tray), and leaf samples were taken from the same individual plants four times during the production phase. The leaf samples were taken in hydroponic production always before changing the nutrient solutions in circulation, *i.e.* on 24 May, 30 May, 14 June, and on 7 August during the harvesting phase, at which time tuber samples were also collected. Dry matter and nutrient analyses of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), sodium (Na), boron (B), copper (Cu), manganese (Mn), zinc (Zn) and iron (Fe) were conducted by laboratory of Suomen ympäristöpalvelu Oy. When plants were grown in a peat-based growing medium, samples were taken during the same developmental phases as in hydroponic production, and harvesting was conducted on 7 August and sampling and analyses were conducted as in the case of hydroponic production.

2.5 Carry-Over Study

After three months of cold storage (4 °C) minitubers sized 10-20 mm of the cultivars Van Gogh and Desiree were transferred to a greenhouse and planted in plastic growing boxes (45 cm × 60 cm) in a peat (Biolan, Novagrow) growing medium. The cultivar Asterix was not included in the carry-over study due to insufficient amount of over 10-20 mm sized minitubers. A total of 3,200 minitubers were studied (100 tubers × 2 techniques × 4 replications × 2 cultivars × 2 times). The plants were irrigated mechanically with a liquid fertilizer (Nutri S-A 0.5 ‰ solution). The greenhouse temperature was recorded by an automatic weather station (a-Weather, AWS -1.04B, Alab, Finland). The emergence dates of each individual plant were noted and the developmental stages observed according to Hack et al. (1993) with potential foliage symptoms recorded at one-week intervals. Harvesting was carried out 77 days after planting (DAP). At harvest, the number of stems, the number and weight of the tubers and the external quality of the tubers were assessed individually.

2.6 Statistical Analyses

The statistical analyses were conducted using the Mixed procedure of the SAS 9.2/SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC, USA) program, using a variance analysis model in compliance with the split plot study design. Furthermore minitubers mean±standard deviation was conducted by Duncan's multiple range test.

3. Results

3.1 Minituber Production

The foliage of Desiree and Van Gogh developed faster during hydroponic production than that of Asterix. Within 37 days from planting the microplantlets on the hardening table and transferring the plants to the trays, Desiree and Van Gogh foliage covered the entire tray. Tuber formation (*i.e.* the first stolon ends swollen to double their size) started after 41 days of minituber production in both cultivars. Asterix foliage development was almost 3 weeks behind Van Gogh and Desiree. Asterix tuber formation started 62 days after the microplantlet phase. When plants were grown in growth rooms in a peat-based growing medium, foliage development occurred almost 3 weeks later and also tuber formation started approximately 18 days later in all cultivars compared to hydroponic production.

Hydroponically-grown tubers were harvested 96 days from planting the microplantlets on the hardening table. Minituber yield was 4.5 per plant for the cultivar Desiree, 7.5 for Van Gogh and 4.0 for Asterix. The minitubers were graded in size categories < 20 mm and > 20 mm; 56.1-58.4% of the Desiree and Van Gogh cultivars and 95.3% of the Asterix cultivar were in the size category < 20 mm. The average weight of the minitubers was 11 g for the cultivar Desiree, 13 g for Van Gogh and 6 g for Asterix. In peat-based production, Desiree produced 4.4 minitubers per plant, Van Gogh 4.6 and Asterix 3.3. Peat-produced mini-tubers were all < 20 mm in size and weighed 5-9 g (Table 1).

Table 1. Comparison of hydroponic and peat minituber production methods on tuber number and tuber weight in cultivars Desiree, Van Gogh and Asterix

Method	Tuber number per plant	Tuber weight per plant	Minitubers grading (%)	
			< 20 mm	> 20 mm
Hydroponic	4.5 ± 0.2 b	11.3 ± 1.5 a	58.4	41.6
Conventional	4.4 ± 0.7 b	7.5 ± 0.8 b	98.9	1.1
Hydroponic	7.5 ± 2.1 a	13.1 ± 3.6 a	56.1	43.9
Conventional	4.6 ± 1.2 b	9.2 ± 2.7 b	97.2	2.8
Hydroponic	4.0 ± 0.9 b	6.1 ± 2.9 b	95.3	4.7
Conventional	3.3 ± 2.0 b	5.0 ± 2.2 c	100.0	0.0

Note. Values denote mean±standard deviation and different letters indicate significant differences between means by Duncan's multiple range test, p = 0.05.

There were no significant differences in the nutrient concentrations of leaf or tuber samples between hydroponic potato production and potatoes cultivated in a peat-based growing medium. Calcium concentrations were slightly (not significantly) higher in hydroponically-produced tubers compared to tubers produced using a peat-based growing medium. Correspondingly, manganese and iron concentrations were slightly (not significantly) higher in peat-produced tubers (results not shown). EC values, pH levels and temperatures in hydroponic production varied throughout the production season: EC 1.3-1.6, pH 5.8-6.2 and temperatures 19-23 °C. The pH levels in peat-based production varied pH 6.2-6.4.

3.2 Carry-Over Effects of Techniques on Minitubers

Hydroponically produced minitubers emerged slower than peat-based produced minitubers by a difference of 5 days (p = 0.000) (Figure 2), but no carry-over effect of either production method was found in foliage development, number of stems (p = 0.22) or number of tubers (p = 0.56) (Table 2). The number of stems varied from 1.6-1.7 and the number of tubers between 4.7-5.2 per plant. Although no carry-over effect of the methods was found in terms of the number of tubers, conventional peat production had a positive effect on crop yield (g/plant) (p = 0.005) (Figure 3), in cultivar Desiree. Correspondingly, the yield of hydroponically produced minitubers was 87 g/plant and conventionally peat-based 104 g/plant.

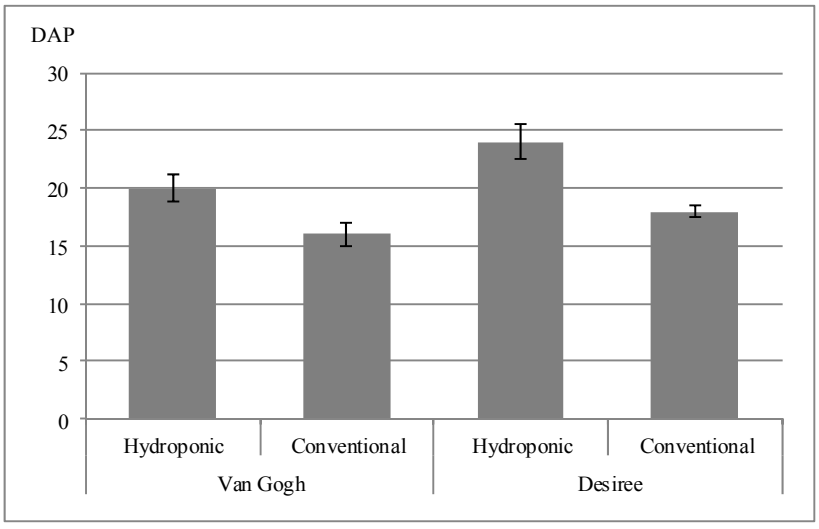

Figure 2. Hydroponically produced minitubers foliage was developed slower than by minitubers produced in peat

Table 2. The production of minitubers was implemented with a hydroponic technique in growth-rooms and the carry-over effects of technique on characteristics of minitubers were studied

	Method		Desiree		Van Gogh	
	Hydroponic	Conventional	Hydroponic	Conventional	Hydroponic	Conventional
Emergence (days after planting)	22.5***	17.2***	24.6***	17.4***	20.4***	16.8***
Development (foliage)						
6.7.	20.0	18.0	20.0	18.0	20.0	18.0
19.7.	49.0	47.5	49.0	48.0	49.0	47.0
2.8.	60.5*	55.5*	59.0*	55.0*	62.0*	56.0*
19.8.	67.5	67.0	69.0	69.0	66.0	65.0
No. of stems	1.6	1.7	1.5	1.7	1.7	1.8
No. of tubers	4.8	5.2	4.7	5.1	4.8	5.2
Fresh wt. of tubers (g/plant)	174	208**	154	207***	194	209
Dry mattercontent of tubers (%)	22.8	24.0	20.5	22.0	25.0	26.0

Note. As a comparison, minitubers from *in vitro* plantlets (conventional) were grown in a peat-based growing medium. The cultivars were Desiree and Van Gogh. Statistically significant differences between methods or cultivars at *P = 0.05, **P = 0.01 and ***P = 0.001. There were no significant interactions between other production methods or cultivars for any other of the variables measured.

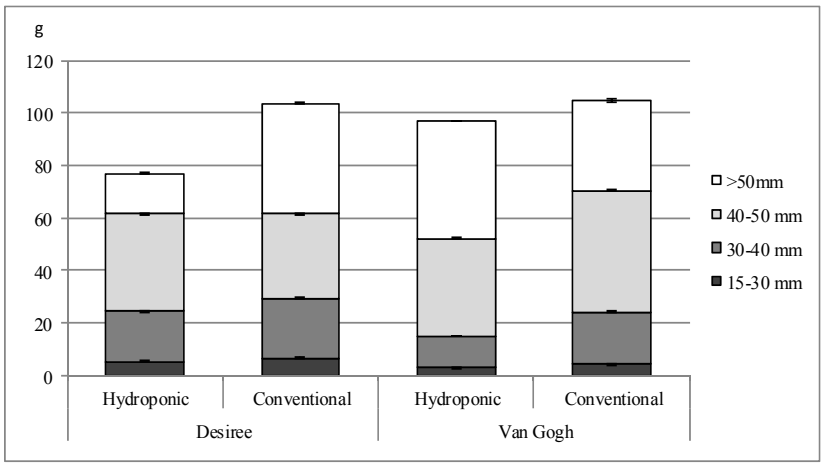

Figure 3. A carry-over effect of the production methods was found in yielding, with conventional peat production having a positive effect on yield (g/plant) (p = 0.005) and with a greater effect in cultivar Desiree than in cultivar Van Gogh

4. Discussion

It is clearly important that the methods used in minituber production are cost effective, the production characteristics of the minitubers, such as fast rate of foliage development and high yielding capacity, are also desirable. In our study Desiree and Van Gogh foliage covered the entire trays in the hydroponic system within 37 days after planting and tuber formation of the Desiree and Van Gogh cultivars started within 41 days from planting, similarly to the studies of Chang et al. (2012) (within 30-65 days, depending on the cultivar and the production technique). Yields (4.5-7.5 per plant) in this study are comparable to the hydroponic production levels obtained by Ritter et al. (2001); also with regard to tuber weight (11-13 g). Tuber formation of the late-maturing cultivar Asterix (63 days) was considerably slower than that of Desiree or Van Gogh, and also the average weight of the tubers was lower (6 g). According to Chang et al. (2008), hydroponic production techniques may not be favourable with late-maturing cultivars because the nutrient solutions may retard root and stolon growth. In the present study minituber yield was higher in hydroponic production compared to peat-based production. In conventional minituber production (a peat-based growing medium), foliage development occurred almost 3

weeks later and also tuber formation started approximately 18 days later in all cultivars compared to hydroponic production.

One aim of this study was also to investigate whether hydroponic production technology effects on growth and yield of produced minitubers as carry-over effect compared to peat produced minitubers. In the carry-over study conventionally produced minitubers emerged and developed faster and produced higher yields than hydroponically produced minitubers. The carry-over effects indicate that more research is needed to clarify which cultivars are genetically the best suited into hydroponic production and especially comparison between early and late maturing cultivars.

In our hydroponic system, the pH and EC levels of the nutrient solutions remained at the automatically regulated levels. At its lowest, the EC was 1.3 dS m^{-1} at the time of vigorous root growth, when the pH level was a maximum of 6.2. The results are consistent with the studies of Chang et al. (2012) in which decreased EC levels and increased pH levels were found to be indicative of active root growth and rapid intake of nutrients. There were no differences in the nutrient concentrations of leaf or tuber samples between hydroponic minituber production and minitubers cultivated in conventional peat-based growing medium.

Potato does not require large amounts of illumination to photosynthesize effectively (Degamante & van der Zaag, 1988), but the amount of light and the relative proportions of wavelengths should be suitable for photosynthesis (Mathews, 2006; Yanovsky et al., 1998). In the present study, the amount of photosynthetically active illumination, 2383-2509 μmol $m^{-2}s^{-1}$, provided artificially by the growth room minituber production environment proved adequate. In addition to illumination, temperature is another key factor affecting tuber formation. According to Levy and Veilleux (2007), high night-time temperatures are more damaging than high daytime temperatures. In the present study the amount of heat produced by the high-pressure sodium lamps (4 × 400 W per growth room) could not be controlled effectively enough with the air conditioning equipment in use. This was evident, in particular, as inadequate differences between daytime and night-time temperatures in growth room temperatures of ca. 19-26 °C by day and 15-23 °C by night.

Conclusion

Our study provides significant information regarding indoor hydroponic minituber production system and artificial simulation of the production environment. In northern conditions (64-65°N), if a hydroponic production system is located in an environment, where natural light is utilized, makes it more difficult to achieve a combination of light intensity that is optimal for plant growth and tuber formation. Based on the present study, indoor hydroponic minituber production needs more research, because the carry-over effects on the characteristics of minitubers were not clear and the total carry-over yield needs to be improved to achieve at least the conventional peat-based production level. Also more studies are needed for choosing cultivars which are optimal to be used in hydroponic minituber production.

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References

Akita, M., & Ohta, Y. (1998). A simple method for mass propagation of potato (*Solanum tuberosum* L.) using a bioreactor without forced aeration. *Plant Cell Reports, 18*(3-4), 284-287. http://dx.doi.org/10.1007/s002990050572

Chang, D. C., Cho, I. C., Suh, J.-T., Kim, S. J., & Lee, Y. B. (2011). Growth and yield response of three aeroponically grown potato cultivars (*Solanum tuberosum* L.) to different electrical conductivities of nutrient solution. *American Journal of Potato Research, 88*(6), 450-458. http://dx.doi.org/10.1007/s12230-011-92116

Chang, D. C., Par, C. S., Kim, S. Y., & Lee, Y. B. (2012). Growth and tuberization of hydroponically grown potatoes. *Potato Research, 55*(1), 69-81. http://dx.doi.org/10.1007/s11540-012-9208-7

Chang, D., Park, C. S., Kim, S. Y., Kim, S. J., & Lee, Y. B. (2008). Physiological growth responses by nutrient interruption in aeroponically grown potatoes. *American Journal of Potato Research, 85*(5), 315-323. http://dx.doi.org/10.1007/s12230-008-9024-4

Corrêa, R. M., Pinto, J. E. B. P., Faquin, V., Pinto, C. A. B. P., & Reis, É. S. (2007). The production of seed potatoes by hydroponic methods in Brazil. *Fruit Veg Cereal Sci Biotech, 3*(Special Issue 1), 133-139.

Demagante, A., & Van der Zaag, P. (1988). The response of potato (*Solanum* spp.) to photoperiod and light intensity under high temperatures. *Potato Research, 31*(1), 73-83. http://dx.doi.org/10.1007/BF02360023

Hack, H., Gall, H., Klemke, T., Klose, R., Meier, U., Stauss, R., & Witzenberger, A. (1993). The BBCH-scale for phonological growth stages of potato (*Solanum tuberosum* L.). *Proc the 12th Annual Congress of the European Association for Potato Research* (pp. 153-154). Paris.

Kämäräinen-Karppinen, T., Virtanen, E., Rokka, V. M., & Pirttilä, A. M. (2010). Novel bioreactor technology for mass propagation of potato microtubers. *Plant Cell, Tissue and Organ Culture, 101*(2), 245-249. http://dx.doi.org/10.1007/s11240-010-9679-7

Levy, D., & Veilleux, R. E. (2007). Adaptation of potato to high temperatures and salinity—A review. *American Journal of Potato Research, 84*, 487-506. http://dx.doi.org/10.1007/BF02987885

Mathews, S. (2006). Invited review: Phytochrome-mediated development in land plants: Red light sensing evolves to meet the challenges of changing light environments. *Molecular Ecology, 15*(12), 3483-3503. http://dx.doi.org/10.1111/j.1365-294X.2006.03051.x

Milinkovic, M., Horstra, C. B., Rodoni, B. C., & Nicolas, M. E. (2012). Effects of age and pretreatment of tissue-cultured potato plants on subsequent minituber production. *Potato Research, 55*, 15-25. http://dx.doi.org/10.1007/s11540-011-9203-4

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Novella, M. B., Andriolo, J. L., Bisognin, D. A., Cogo, C. M., & Bandinelli, M. G. (2008). Concentration of nutrient solution in the hydroponic production of potato minitubers. *Ciência Rural, 38*(6), 1529-1533. http://dx.doi.org/10.1590/S0103-84782008000600006

Ritter, E., Angulo, B., Riga, P., Herran, C., Relloso, J., & San Jose, M. (2001). Comparison of hydroponic and aeroponic cultivation systems for the production of potato minitubers. *Potato Research, 44*(2), 127-135. http://dx.doi.org/10.1007/BF02410099

Rolot, J. L., & Seutin, H. (1999). Soilless production of potato minitubers using a hydroponic technique. *Potato Research, 42*(3-4), 457-469. http://dx.doi.org/ 10.1007/BF02358162

Veeken, A. J. H., & van der Lommen, W. J. M. (2009). How planting density affects number and yield of potato minitubers in a commercial glasshouse production system. *Potato Research, 52*(2), 105-119. http://dx.doi.org/10.1007/s11540-008-9124-z

Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M. A., MacKerron, D. K. L., & Ross, H. A. (2007). Potato Biology and Biotechnology. *Advances and Perspectives*. Elsevier, Oxford, Amsterdam. http://dx.doi.org/10.1017/S0014479708006832

Yanovsky, M., Alconada-Magliano, T., Mazzella, M., Gatz, C., Thomas, B., & Casal, J. (1998). Phytochrome A affects stem growth, anthocyanin synthesis, sucrose-phosphate-synthase activity and neighbour detection in sunlight-grown potato. *Planta, 205*(2), 235-241. http://dx.doi.org/10.1007/s004250050316