On-Farm Tissue Culture Production of Lingonberries

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Abstract

Nearly all lingonberry cultivars available commercially in the United States are from Europe and exhibit two flowering periods per season. In Alaska, the first flowering period is insignificant and results in small yields. The second flowering occurs too late in the summer to produce mature fruit. Locally collected plant materials provide for development of lingonberry cultivars adapted to regional growing conditions. While lingonberries root readily from stem cuttings, there are several problems associated with stem cutting propagation: large amounts of stock plants are necessary to provide enough stem cuttings for commercial production; plants propagated from stem cuttings rarely produce rhizomes and do not form productive matted rows; and research in Sweden has suggested that plants from stem cuttings may have a limited life span which will eventually necessitate replacement of entire fields. Tissue culture provides an alternative for rapid propagation of large quantities of local cultivars. No commercial tissue culture labs exist in Alaska, so we developed a small on-farm tissue culture laboratory that is used to propagate local material for our own needs and eventually other local growers. The most significant challenges included: minimizing culture contamination, adequate ventilation and cooling in a very small laboratory space, defining optimum light and temperature environment in a location where typical experimental design was impossible, developing a system of microshoot rooting that minimized space, and maintenance of rooted cuttings for several months in winter prior to planting. Microshoot production has been extremely successful using Woody Plant Medium and Plant Preservative Mixture biocide to minimize contamination. Rooting was accomplished in thin layers of a peat-based medium rolled jellyroll fashion in plastic film. Although microshoot production was successful, rooting failures have been unacceptably high and require additional research.

INTRODUCTION

The first cultivated fields of lingonberries, *Vaccinium vitis-idaea,* in Alaska, were established using the European cultivars, 'Red Pearl' and 'Sanna' as well as 'Regal' and 'Splendor' from Wisconsin, USA. All of these cultivars have two flowering and fruiting periods per season. In Trapper Creek, Alaska, first fruit ripening occurs in September, and the second flowering period begins in mid to late August. The first significant frost generally occurs in late September. After three years of field growth, none of the tested cultivars was deemed acceptable because the length of Alaska's summers is too short to support the development of fruit from the second, and largest flowering period. Consequently, selection from wild populations of lingonberries in Alaska was begun to identify superior strains of locally adapted plant materials. One significant problem has been the lack of propagation facilities for increasing selected strains. Stem cuttings root easily (Cross, 1984, Holloway, 1984, Holloway, 1985, Labokas et al., 1988, Lehmushovi, 1976). However, in some instances, rhizome production is non-existent or very slow (Hjalmarsson, 1993, Holloway, 1984, Holloway, 1985, Hosier et al., 1985, Lehmushovi, 1976). Tissue culture has been shown to be the superior method for vegetative

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propagation of Alaska lingonberries (Holloway, 1995). The objective of this project was to develop an on-farm tissue culture facility for rapid propagation of selected Alaska strains of lingonberries.

MATERIALS, METHODS, EXPERIMENTS

Stock Plants and Culture Trials

Stock plants were collected from two Alaska locations. Plants with large fruit size and upright growth habit were collected 440 km N of Anchorage, Alaska. Plants with small berries but large clusters (7 to 12 fruit per cluster) and upright growth habit were collected from MooseCreek Farm, 185 km N of Anchorage. These selections were transplanted to flats of peat and grown under fluorescent lights in the lab.

Trials were conducted using two culture media: Anderson's Rhododendron Medium (Economou et al., 1984) and Woody Plant Medium (Kyte et al., 1996) both with 20 mg l^{-1} sucrose and pH 4.8 for explant initiation. Trials also included media with and without 2.5 ml l^{-1} Plant Preservative Mixture™ biocide (Plant Cell Technology, Inc. Washington D.C.) as an experimental anti-fungal agent.

The growth regulator 2iP (isopentyladenine) has been shown to be very effective in initiating microshoots on lingonberries (Norton et al., 1985). Trials included 2, 6, 10, 15, and 20 mg l^{-1} 2iP growth regulator to identify optimum levels for microshoot formation. Trials also included comparison of 3.5, 3.65, 3.75, 4, 5, and 6 g l^{-1} agar. Baby food jars with magenta caps were used as vessels with 20 ml media per jar then sterilized in a pressure cooker. Explants were surface sterilized in 5 % bleach solution with 2 drops Tween 20 in 500 ml solution. Full strength Woody Plant Medium plus 20 ml l^{-1} Plant Preservative Mixture™ was used to soak the cleaned explants prior to transfer.

Explants, 4cm to 6cm in length, were trimmed to expose lateral buds, scrubbed in the bleach solution with a soft brush, rinsed three times in sterile water, then soaked for 72 hours in the 20 ml l^{-1} Plant Preservative Mixture™ biocide. Explants were positioned horizontally or vertically in the medium with six to eight explants per jar.

Culture jars were placed in a homemade controlled environment cabinet with average 22.5°C temperature and 55 % relative humidity. The first explants were placed beneath 40W cool white fluorescent bulbs (distance 33 cm) for a 16-hr photoperiod. Later, the light regime consisted of 30 min alternating light and dark for 16 hr, followed by 8 hr of continuous darkness. Explants were subcultured every four to five weeks. Because of space limitations, all projects were not true replicated experiments but a series of trials to identify optimum conditions for the farm laboratory.

Facilities

A storage area in the farmhouse (4.3 m x 3.7 m) was converted into a laboratory. Four tiers of shelves (46 cm x 1.3 m), were built to accommodate standard nursery flats, and were used for growing cultures and stock plants. All surfaces were painted to facilitate cleanliness. Work benches and shelves for lab supplies were added along with a large sink and dishwasher. A small portion of the lab (2.5 m x 1.8 m) was isolated as a transfer room. This room contained an enclave (45 cm x 90 cm) and a portable sterile hood originally used for mushroom culture.

One of the shelf units was enclosed with clear plastic sheeting as an environment cabinet because of problems with maintaining proper temperature and humidity for cultures. The cabinet was equipped with a circulating fan and heater to maintain the temperature at 21-24°C. Originally a humidifier was enclosed in the chamber but was replaced with a evaporative cooling unit. Other equipment included a gram scale, hotplate stirrer, pH meter, a refrigerator, dissecting microscope, large capacity pressure cooker for sterilizing equipment and cultures, and various glassware and tools.

Microshoots, 6 cm to 8 cm in length, were harvested for rooting. Rooting trials for best medium included perlite, vermiculite, coconut husk fibers, peat moss, and a 1:1:1 by volume combination of perlite, coconut husk and peat moss. The moistened medium was

applied in a layer (2.5 cm depth and 10 cm width) onto a strip of plastic film. The strips were rolled jellyroll fashion into 18 cm-diameter rounds. The rounds were enclosed in plastic bags and placed beneath the same light conditions as cultures. Rooted microshoots were transferred to other rolls for subsequent growth.

A cold room (2.5 m x 3.7 m) with shelves and heater was built to fulfill the chilling requirements for lingonberries. Once rooting had occurred, and terminal buds were visible, plants were transferred to the cold room for 720 hr of chilling at 10°C, then returned to the culture shelves for a second growth cycle. Stock plants were also transferred to the chilling room after setting a terminal bud and returned to the lab after 720 hr.

RESULTS

The Rhododendron Medium at all levels of 2iP was not successful for the development of microshoots. Only callus developed from explants. Woody Plant Medium with 15 mg l^{-1} 2iP and 3.75 g l^{-1} agar provided the best medium for microshoot production. Contamination was a problem in the home-made laboratory. Trials with high levels (10%) bleach as well as prolonged periods of scrubbing were unsuccessful. Contamination was unacceptably high or explants died due to the higher concentrations of bleach. Use of the Plant Preservative Mixture™ biocide, reduced contamination levels to less than 5% of the jars. It was also possible to salvage new explants that showed signs of contamination by re-cleaning and soaking in the Plant Preservative Mixture™.

Under the 16-hr continuous light and 8-hr continuous dark photoperiod, the microshoots became a rusty red color with very few green leaves. The reduced light levels of the alternating 30 min light/dark cycles eliminated the red coloration.

Microshoots were subcultured to a maximum of 300 jars. Generally, the lab was capable of supporting 150 jars of mature cultures and 150 jars of developing cultures. Approximately 3000 shoots were harvested per transfer from the 150 jars of mature cultures.

The rolled medium used for rooting allowed for handling of large quantities of shoots in a very small space. Rooting success has been inconsistent with this method because of difficulties maintaining high humidity within the bags and also avoiding disease. Mortality in some rolls reached 100 % inside the plastic bags. Rolls covered with plastic rather than inside a closed bag had up to 95 % successful rooting.

Rooting and plant establishment was satisfactory on all media except the shredded coconut husk fibers. Microshoots rooted well in coconut husk fiber, but subsequent growth was poor. Cuttings became rusty red and did not show vigorous above-ground growth. Rooted cuttings transferred from the coconut husk fibers to a peat base medium recovered fully and continued development.

The cold room used for chilling the lingonberry plants allowed for two season's growth in one winter on the newly rooted plants. During the long winter months, two growth cycles could be completed because of the low chilling requirements of Alaska plants. The cold room also provided two cycles of young, actively growing shoots for explants during the winter.

DISCUSSION

Through trial and error, a successful tissue culture facility was built at MooseCreek Farm. Major obstacles included difficulties with temperature and humidity control for stock plants, cultures and rooted microshoots. Prior to construction of the environment cabinet, maintenance of proper humidity and temperature was nearly impossible. The humidifier proved unsatisfactory, and heat from the lamps could not be controlled. A fan was installed that circulated cool air into the cabinet from beneath the laboratory floor, but contamination rates in the culture jars increased to unacceptable levels. The humidifier was replaced with an evaporative cooler that helped with temperature control, but not humidity. During summer months, temperatures often exceeded 27°C in the cabinet. The evaporative cooler operated for long periods, causing a

rise in humidity to a high of 86 %. This problem required frequent monitoring and manual adjustment of the doors of the cabinet. A compressor-type air conditioner with a humidifier probably would provide better control.

During construction of the environment cabinet, cultures were moved temporarily from beneath the lights. Some cultures received very low light levels for nearly one week after which they had significantly better green color than those in the 16-hr continuous light. The "normal" rusty red-colored leaves and stems had become green, and plants appeared healthier. This observation led to experiments with light levels, and adoption of the alternating 30 min light/dark cycles. Space and construction limitations precluded raising the lights or changing the fixtures to reduce total light levels.

The propagation roll method of rooting cuttings yielded an unacceptable level of cutting loss, especially when rolls were enclosed in plastic bags. The cause of cutting death remains unknown, but other methods of rooting thousands of cuttings in limited space without greenhouse facilities must be identified.

An unexpected addition to the tissue culture facilities was a cold room. On two occasions, the stock plants succumbed very quickly following about five months of continuous growth in the lab. This problem was eliminated by storing plants at chilling temperatures to fulfill rest period requirements. The added bonus of the cold room was the ability to add an additional growth cycle during winter months for rooted microshoots before transferring them to the field.

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Literature Cited

Economou, A.S. and Read, P.E. 1984. In vitro shoot proliferation of Minnesota deciduous azaleas. HortScience. 19(1):60-61.

Cross, J.E. 1984. Propagation tips on some less widely grown plants. Proc. International Plant Propagator's Soc. 34:565-570.

Hjalmarsson, I. 1993. Domesstication of wildgrowing lingonberries (Vaccinium vitis-idaea) in Sweden. Acta Hort. 346:305-309.

Holloway, P. 1984. Lingonberry Cultivation. Agroborealis. 16(2):15-20.

Holloway, P. 1985. Rooting of lingonberries, Vaccinium vitis-idaea, stem cuttings. The Plant Propagator. 31(4):7-9.

Holloway, P. 1995. Rhizome production in lingonberry, Vaccinium vitis-idaea following propagation by tissue culture and conventional stem cuttings. The Plant Propagator. 7(2):21-23.

Hosier, M.A., Flatebo, G., and Read, P.E. 1985. In vitro propagation of lingonberry. HortScience. 20(3):364-365.

Kyte, L. and Kleyn, J. 1996. Plants from test tubes. Timber Press, Portland, OR.

Labokas, J. and Budriuniene, D. 1989. Vegetative propagation of lingonberry. Acta Hort. 241:270-272.

Lehmushovi, A. 1976. Methods of propagating the cowberry. Ann. Agric. Fenn. 14:325-333.