Rhizome Production in Lingonberry, *Vaccinium vitis-idaea,* Following Propagation by Tissue Culture and Conventional Stem Cuttings

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Lingonberries from eight geographically-widespread selections were propagated by microshoots from tissue culture and conventional stem cuttings. Rooted plants were evaluated after two growth cycles to compare rhizome and daughter shoot production among selections and between propagation methods. Sixty percent or fewer of the plants from all selections produced rhizomes when propagated by conventional stem cuttings. Rhizome production among selections from microshoot propagation varied from 100 percent to zero. Overall, propagation by tissue culture produced the greatest number and biomass of rhizomes and daughter shoots. However, significant variation among selections highlights the importance of evaluating individual clones in breeding programs for the ability to produce rhizomes rapidly from tissue culture.

INTRODUCTION

Vaccinium vitis-idaea is a circumpolar plant composed of two races, subspecies *minus, and vitis-idaea* (Hulten 1949,1970). The only subspecies found in North America is *minus* which also occurs in the arctic-montane regions of Europe and Asia (Hulten 1970). Plants of subspecies *vitis-idaea* include the major European cultivars such as Koralle, Erntesegen, Emtedank and Erntekrone.

Both subspecies are being evaluated for field cultivation and breeding in Alaska. The native subspecies *minus is* the most reliably hardy race. This plant grows vegetatively by rhizomes located primarily within the top 10 cm of the soil surface. Field cultivation and propagation practices require rapid spread by rhizomes to form permanent wide-row plantings.

Stem cuttings of lingonberries root easily (Cross 1984, Dierking and Dierking 1993, Gorecka 1979, Holloway 1984, 1985, Labokas and Budriuniene 1988, and Lehmushovi 1976). However, in some instances, rhizome production is non-existent or very slow (Hjalmarsson 1993, Holloway 1984, 1985, Hosier *et al.* 1985 and Lehmushovi 1976). Vegetatively propagated 'Koralle' has been described as a non-runnering variety (Dierking and Dierking 1993).

Propagation of lingonberries by tissue culture is well documented, and in all instances, rooted microshoots produce rhizomes (Hosier *et al.* 1985, Norton and Norton 1985, Serres *et al.* 1993, 1994). Comparisons between tissue culture and conventional cuttings have had mixed results. Hosier *et al.* (1985) reported rhizome production from microshoots, but not from stem cuttings. In contrast, Serres *et al.* (1993) showed no difference in rhizome production between stem cuttings and microshoots for the cultivars, Koralle, Erntedank and Sussi. The purpose of this project was to compare lingonberry microshoots with conventional stem cutting propagation to determ~ne the optimum method of propagation for field establishment and to evaluate rhizome and daughter shoot production in eight geographically widespread selections from both subspecies *minus* and *vitis-idaea.*

MATERIALS AND METHODS

Eight wild and cultivated selections of lingonberries were grown in containers. at the University of Alaska Fairbanks (Table 1). They were identified according to subspecies by characteristics outlined in Hulten (1949). Parent material was seeds with the exception of the German cultivar, Erntesegen,obtained as rooted stem cuttings. Stem tip cuttings were randomly harvested from mature plants, treated with 0.8% powdered IBA (Hormodin #3), and rooted in a milled *Sphagnum sp.* peat medium in flats covered with clear plastic dome lids. Lighting consisted of high intensity sodium vapor lamps positioned lm above the flats which supplemented natural daylight for an 18-hour photoperiod. Minimum night temperature was 15C.

Twenty actively-growing shoot tip explants of each selection were propagated *in vitro* with 16 mg liter^{-1} isopentyladenine (2iP) following the protocol of Norton and Norton (1985). Microshoots were excised from the callus mass and recultured. Twelve weeks after proliferation, microshoots were excised and rooted in the greenhouse in the same manner as the stem cuttings.

Plants were grown in containers of milled *Sphagnum sp.* peat in the greenhouse then moved outdoors during the growing season. Fifty plants each from stem cuttings and microshoots from the eight selections were planted in peat-mulched silt loam soil for long-term evaluations of propagation techniques. The remaining plants were fall-acclimated

Table 1. Description and source of eight selections of lingonberries.

Plant	Subspecies	Source
Norway	*vitis-idaea*	Pasvik River Valley, collected from wild stands by C. Stushnoff
Finland	*vitis-idaea*	Lieto, southwest Finland, seeds collected by E. Stang
Germany	*vitis-idaea*	cuttings from A. Zillmer, Uchte, Germany
Japan	*minus*	seed from National Clonal Germplasm Repository, Corvallis (VAC 115)
Alaska I	*minus*	Fairbanks, Alaska collected from wild stands by P. Holloway
Switzerland	*vitis-idaea*	seed from National Clonal Germplasm Repository, Corvallis (VAC 348)
Lithuania	*vitis-idaea*	seed collected from cultivated field trials at Kaunas, Lithuania by J. Lubokas and D. Budriuniene, Lithuanian Scientific Research Institute of Forestry
Alaska II	*minus*	Talkeetna, Alaska collected from wild stands by P. Holloway

outdoors, stored for three months in a cold room at 3°C, then moved to the greenhouse. Plants were grown under the same temperature and light conditions used for rooting the cuttings. Plants were arranged both outdoors and in the greenhouse in four replicates of five plants for each selection and propagation method in a randomized complete block design.

After four months, plants were harvested and separated into stems, leaves and roots, rhizomes and daughter shoots. Rhizomes were identified by their horizontal growth habit, whitish growing tip, and occasional lateral, reddish, negatively-geotropic branches (daughter shoots) that terminated in leaves. Occasionally, shoots developed from buds on the buried, rooted portion of the original shoot that were reddish-green and strongly negatively-geotropic. Because these shoots did not exhibit the growth habit of a true rhizome, these shoots were counted as stems. Each component was dried in a forced-draft oven at 65°C for 24 hr then weighed for individual and total biomass. Data were analyzed using analysis of variance. Percent data were subject to arcin transformation prior to analysis.

RESULTS AND DISCUSSION

All of the lingonberry selections rooted easily by both conventional stem cuttings and microshoots. Rooting percentages were 95 percent or higher for all treatments. Seven of the eight selections differed significantly in the production of rhizomes from rooted cuttings following two growth cycles. Alaska II had no rhizome production from either cuttings or microshoots, and the Norway selection produced rhizomes from microshoots but not cuttings (Table 2). All other selections produced rhizomes from both microshoots and cuttings. With the exception of Alaska II and Japan, all selections showed a higher percentage of plants with rhizomes from microshoots than from cuttings.

In the seven selections with rhizomes, production differed significantly among selections and propagation methods. Analyzed separately, all selections except Lithuania had significantly greater numbers and biomass of rhizomes from microshoots than from cuttings. In the case of Alaska I, plants from microshoots produced nearly 10 times the number of rhizomes per plant than from cuttings.

In five of the selections, the trend in leafy daughter shoot production from rhizomes paralleled the trend in rhizome production: greater number and biomass of daughter shoots per plant from microshoot propagation than from conventional cuttings. Selections from Japan, Switzerland and Germany produced few daughter shoots (average fewer than four per plant) from either propagation method.

The only selection that showed no rhizome production, Alaska II, grew poorly frommicroshoots. The total plant biomass after two growing seasons was significantly lower from microshoots than from cuttings.

The ability to produce rhizomes and daughter shoots is not related to subspecies. Both subspecies showed significant variation in rhizome production with the greatest differences between the two Alaska selections of subspecies *minus*. All selections produced rhizomes on fewer than 60 percent of the plants when propagated by conventional stem cuttings. Labokas and Budriuniene (1989) reported 41-52 percent of plants from stem cuttings produced rhizomes (1-3 per plant) following two growing seasons. These authors were the source of seed for the Lithuania selection used in the present study (Table 1).

Tissue culture propagation of young shoot tips provides the best method for rapid asexual propagation and future field establishment of lingonberries. Methods for micropropagation have been well established (Serres *et al.* 1994, Norton and Norton 1985, and Hosier *et al.* 1985). Careful evaluation of individual clones is necessary to ensure optimum rhizome production by microshoots.

Table 2. Rhizome and daughter shoot production in eight selections of lingonberry when propagated by rooted microshoots and conventional stem cuttings.

Plant Selection	Propagation Method	Plants with Rhizomes	Numbers per plant		Biomass (g)		
			Rhizomes (%)	Daughter shoots	Rhizomes	Daughter shoots	Total
Norway	Microshoots	76**	1.8**	3.8**	0.12**	1.10*	8.36
	Cuttings	0	0	0	0	0	7.32
Finland	Microshoots	80**	13.0**	12.4**	0.36**	0.34**	4.92
	Cuttings	48	1.2	0.6	0.04	0.04	5.44
Germany 'Erntesegen'	Microshoots	100**	6.8*	3.6*	0.24*	0.28	5.74
	Cuttings	40	1.8	1.0	0.06	0.08	5.64
Japan	Microshoots	44	8.2*	1.2	0.16	0.06	4.45
	Cuttings	40	3.4	2.6	0.16	0.18	6.26
Alaska I	Microshoots	100**	93.4**	40.6**	0.44**	0.70**	4.10
	Cuttings	36	9.8	5.6	0.16	0.20	5.40
Switzerland	Microshoots	100**	6.0**	2.0	1.14**	0.12	5.88*
	Cuttings	44	1.6	1.1	0.18	0.14	7.48
Lithuania	Microshoots	100**	8.0	13.4**	0.4	2.02**	5.86
	Cuttings	60	6.0	0.8	0.3	0.12	8.12
Alaska II	Microshoots	0	0	0	0	0	0.78**
	Cuttings	0	0	0	0	0	4.96

*,** mean pairs in each column for indivdual plant selections differ significantly at P< .05 and .01, respectively.

Where micropropagation is not feasible, field establishment by seedlings may be necessary since rhizome production from cuttings is reduced or at least delayed. Long-term field studies will elucidate whether the differences in rhizome and daughter shoot production between cuttings and microshoots changes with time. Future breeding programs will necessitate screening individual selections for the ability to produce rhizomes.

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