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Twig pre-harvest temperature significantly influences effective cryopreservation of *Vaccinium* dormant buds

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ABSTRACT

Cryopreservation of temperate woody-plant material by dormant buds is less expensive than using shoot tips isolated from tissue cultured plants; however currently, dormant buds are used only for preservation of selected temperate tree and shrub species. Using dormant buds could be an efficient strategy for long-term preservation of blueberry (*Vaccinium* L.) genetic resources. In this study, viability of *V.* hybrid 'Northsky' (PI 554943) dormant buds was evaluated at 30 harvest dates over three consecutive fall/winter seasons to determine the optimal harvest time that promotes high post cryopreservation viability. Twigs with dormant buds were cut into 70 mm segments containing at least two nodes, desiccated, slowly cooled, stored in liquid nitrogen vapor and tested for post-cryopreservation regrowth. The highest regrowth of cryopreserved dormant buds was observed for buds harvested in mid-December and during the first half of January. Pearson's correlation coefficients were computed to evaluate the association between bud characteristics and viability at harvest date and logistic regression models were fit to test the ability of twig characteristics and temperatures to predict post cryopreservation bud viability. Post-cryopreservation viability was negatively correlated ($p < 0.05$) with average minimum, maximum and daily mean temperature preceding the bud harvest but was not correlated with the dormant bud initial and end moisture content, twig diameter, the number of dormant buds/cm of twig length and the number of days in desiccation. Regression tree analysis suggested post-cryopreservation viability to be between 52 and 80% for dormant buds harvested after a 10 day average maximum air temperature of <11.2 °C. Pre-harvest air temperature was a significant indicator of optimal dormant bud harvest time to produce adequate viability for long term preservation of blueberry genetic resources.

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1. Introduction

In cryopreservation, the use of dormant buds (DB) costs less, is simpler to accomplish and requires less time than using shoot tips because it does not involve aseptic cultures and the time consuming excision of shoots. The possibility of preserving plant dormant buds in liquid nitrogen has been known for years [17] and was reported in *Malus* [3,18,21,27], *Fraxinus* [29], *Ulmus* [4], *Morus* [8,9,10], *Pyrus* [23], *Pinus sylvestris* [6], *Betula pendula* [16], *Prunus cerasus* [25], and other tree species. Despite the successful reports, the dormant bud method is not widely practiced in plant germplasm preservation. Some possible reasons might be that the method is applicable only to shrubs and trees that undergo dormancy and dormant buds of these species may not always result in satisfactory post-cryopreservation viability.

In recent years, blueberry fruit consumption has increased due to its nutritional and potential medicinal value [1,19,20,22] and in many countries, blueberries are an everyday food. The United States contributes half of the world's commercial production [2]. Maintaining a diverse *Vaccinium* germplasm collection is vital for selecting and breeding new cultivars that will support domestic blueberry production. The U.S. Department of Agriculture-Agricultural Research Service, National Plant Germplasm System (USDA-ARS, NPGS) currently houses >1700 *Vaccinium* accessions (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?COR).

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Only a small fraction (<3%) of the blueberry genetic resources is backed up as cryopreserved shoot tips. The cryopreservation of *Vaccinium* by in vitro shoot tips was described by Reed [12,13] and Uchendu and Reed [24], and was readily applicable to >40 accessions at our laboratory. Niino et al. [7] reported experimental cryopreservation of blueberry dormant buds but their post-cryopreservation performance was tested only in vitro. Backing up the field maintained blueberry collection by dormant buds would increase the number of cryopreserved accessions in a shorter time period and with less cost than using shoot tips from in vitro cultures. However, effective cryopreservation requires the identification of protocols that ensure satisfactory post-cryopreservation viability that is measured by the ability of the stored material to grow a shoot or a plant.

Several *Vaccinium* L. species (Ericaceae, section *Cyanococcus*) make ideal candidates for cryopreservation by dormant buds. They are perennial shrubs and blueberry, cranberry and lingonberry exhibit a distinct dormancy phase; hence, they might be amenable to conservation via dormant buds. The objective of this study was to evaluate post-cryopreservation viability of blueberry dormant buds harvested at various dates during three fall-winter seasons and to determine the relationship of pre-harvest temperature to viability after storage in liquid nitrogen.

2. Material and methods

Terminal twigs (last year growth) of blueberry cv. Northsky (*Vaccinium* hybr. PI 554943) were collected during three fall/winter seasons in 2011–2012 (12 harvest dates), 2012–2013 (14 harvest dates) and 2013–2014 (4 harvest dates; a total of 30 harvests; Table 1) at the National Clonal Germplasm Repository, Corvallis, OR. In the 2013/2014 season, the selection of the four harvest dates was based on the collection dates of the previous two seasons that resulted in the highest post storage viability. At the day of harvest,

Table 1
Selected twig characteristics and viability (%) of dormant buds of 'Northsky' blueberry (*Vaccinium* hybr.) cryopreserved in three fall/winter seasons (2011/2012, 2012/2013 and 2013/2014).

Harvest season	Harvest date	Initial MC (%)	End MC (%)	No. of days in desiccation to reach 25–30% of MC	DB fresh weight (mg)	Twig top diameter (mm)	Twig bottom diameter (mm)	No. of DB/cm of twig length	Average viability of control DB (%)	Average viability of cryopreserved DB (%)
2011/2012	11/14/11	48.5	28.4	16	31.5	1.8	2.7		100	57
	11/21/11	44.6	25.2	20	77.7	2.0	2.8		100	70
	11/28/11	44.7	27.3	20	31.0	1.6	2.0		100	50
	12/5/11	49.0	28.8	36	111.4	1.4	2.1		100	67
	12/12/11	46.7	26.1	20	107.4	1.3	1.9		100	59
	12/19/11	45.3	29.0	28	59.9	1.4	3.6		100	75
	1/3/12	32.9	27.6	23	128.2	1.6	2.6		100	45
	1/9/12	48.6	30.6	24	155.7	2.1	2.5		94	100
	1/16/12	49.5	26.9	20	126.1	1.6	2.2		100	100
	1/23/12	49.8	27.5	14	124.8	1.7	2.5		100	70
	1/30/12	47.9	29.8	23	56.0	2.1	2.5		100	50
	2/13/12	48.1	29.6	16	181.6	1.7	2.8		100	0
2012/2013	10/1/12	48.8	29.2	36	165.7	1.6	2.9	0.6	57	0
	10/15/12	49.8	29.2	23	93.1	1.6	2.3	0.6	80	0
	10/29/12	43.0	29.4	19	163.8	1.5	2.3	0.6	25	0
	11/12/12	48.4	29.7	20	141.7	1.6	2.7	0.6	67	0
	11/26/12	48.3	30.3	20	107.6	1.8	2.7	0.6	67	25
	12/3/12	48.3	30.7	17	142.4	1.6	2.5	0.6	65	30
	12/10/12	46.6	30.4	24	61.4	1.7	3.3	0.6	100	90
	12/17/12	46.4	29.8	17	88.4	2.1	2.8	0.5	100	88
	12/31/12	46.5	28.1	36	75.7	1.7	2.8	0.7	100	45
	1/7/13	48.6	29.4	29	85.7	1.7	2.3	0.7	100	84
	1/14/13	46.4	30.2	22	115.4	1.6	2.2	0.7	92	67
	1/21/13	49.3	30.6	25	87.0	1.5	2.0	0.8	100	79
	1/28/13	47.2	30.7	24	40.2	1.5	2.3	0.7	92	75
	2/4/13	51.4	30.4	23	86.2	1.6	2.2	0.7	100	58
2013/2014	12/16/13	47.0	30.2	29	26.2	2.7	3.2	0.5	100	35
	1/6/14	46.4	29.8	29	27.0	1.9	2.6	0.5	100	85
	1/21/14	48.4	30.6	28	43.2	1.8	2.3	0.4	95	45
	2/3/14	47.7	28.9	38	28.8	1.8	2.9	0.4	100	65
	Mean value	47.1	29.1	24	92.4	1.7	2.6	0.6	94	54
	SE±	0.41	0.21	0.9	8.4	0.03	0.02	0.02	2.3	4.4
	n	60	60	60	60	180	180	108	60	60

Hybr.-hybrid; No.-number; MC-moisture content; DB-dormant bud; %-percent; g-gram; mm-millimeter.