Screening Red Raspberry for Cold Hardiness in Vitro

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Abstract. Two selections and two cultivars of red raspberry (*Rubus idaeus* L.) were evaluated for cold hardiness in vitro. Tissue-cultured shoots were exposed to temperatures from 0 to -18°C and samples were removed at 2°C intervals. Injury was assessed by a visual rating of tissue browning after freezing. Only shoots subjected to step-wise acclimation at low temperatures before freezing revealed significant differences among the four types in the lowest shoot survival temperature. Acclimation treatments increased the lowest survival temperatures of in vitro shoots by a mean of 3.1°C. The hardiness obtained from this screening method agreed with that of winter survival in the field. Ranking, from the most to least cold hardy, was 'Boyne', Gu 72, Gu 63, and 'Comox'.

Plant cold hardiness is necessary for consistent perennial crop production in northern temperate latitudes and is an important consideration in many breeding programs. A rapid cold-hardiness screening method would be highly beneficial. Artificially freezing plant parts or seedlings has been used to assess cold hardiness rapidly and accurately without observing winter injury in the field during several seasons or at several locations (Quamme and Stushnoff, 1983). Tissue cultures may be even more efficient in a screening method because of space and time savings. In vitro shoots (Caswell et al., 1986), callus (Bannier and Steponkus, 1976), and cell suspensions (Chen and Gusta, 1983) have responded to the cold in a manner similar to that of intact plants; these results suggest that in vitro materials may be used successfully for cold-hardiness screening.

Differences among cultivars or species in cold hardiness have been reported for in vitro material, although this was not the primary objective of these studies. For example, Bannier and Steponkus (1976) found a greater ability for acclimation and thus greater cold hardiness in callus of outdoor chrysanthemum [*Dendranthema × grandiflorum* (Ramat.) Kitamura] cultivars than that of a greenhouse cultivar. A differential cold hardiness of callus and suspension cultures of various *Solanum* species was reported by Van Swaaij et al. (1987).

Although callus or cell suspension cultures may be appropriate for cold-hardiness screening on a cellular or tissue level, they may not

be the most suitable material to screen cultivars. Genetic changes in callus cultures may no longer reflect the cold hardiness of the source plant (Evans et al., 1984). Thus, a heterogeneous system may better represent a whole-plant situation. This is particularly true when assessing cold hardiness, because plant cultivars may differ in that respect due to the differential survival of their specific organs and tissues (Sakai and Larcher, 1987). Larger differences in cold hardiness suspected among genera or species maybe detected at the cellular level (Duda and Kacperska, 1983), but, to assess cultivar differences, using differentiated tissue may be more crucial. Thus, the objective of this study was to determine if cold-hardiness screening could be accomplished in vitro using cultured red raspberry shoots and to examine the effect of acclimation before freezing on this screening method.

Two raspberry selections from the Univ. of Guelph raspberry breeding program (Gu 63 and Gu 72) and two raspberry cultivars (Boyne and Comox) of known hardiness were used to assess cold hardiness in the laboratory. 'Boyne' was considered hardy (Ure and Morrison, 1962) and 'Comox' intermediate (Daubeney, 1987) in Ontario.

In vitro raspberry shoots were initiated by shoot-tip culture from stock plants grown in a greenhouse. The culture medium used was that recommended by Donnelly et al. (1980) for initiating and proliferating red raspberries. Shoot cultures were kept in a growth room at 24 ± 3°C and a 16-h photoperiod (light intensity from cool-white fluorescent lamps, 62 mol·m⁻²·s⁻¹).

The experiment was blocked over time. About 140 test tubes of shoots (2 to 3 cm in length) of each selection-cultivar for each block were subculture on fresh medium and grown in a growth room. After 2 weeks, half of the tubes of each selection-cultivar (≈70) was selected randomly for step-wise acclimation (Table 1). The acclimation regime was a modification of that used for shoot cultures by Caswell et al. (1986). Nonacclimated shoots remained in the growth room.

Acclimated and nonacclimated shoots in test tubes were selected randomly and divided evenly into nine groups (one control and eight temperature treatments) for exposure to various temperatures. Controls were set aside (20 ± 2°C) and the remaining shoots were placed in a freezer at 2 ± 1°C for 2 h. The freezer temperature was dropped at 2°C/h and a set of tubes was removed at -2, -4, -6, -8, -10, -12, -14, -16, and -18°C. The -2°C treatment was replaced with a -18°C treatment in blocks 3 and 4, as no injury occurred at -2°C. After being removed, samples were allowed to thaw at room temperature overnight and placed in the growth room. The degree of injury to each shoot was assessed 2 weeks after freezing by rating the portion of a shoot that was brown, where 1 = less than one-third, 2 = one-third to two-thirds, 3 = more than two-thirds to nearly all, and 4 = entirely. From the rating scores of each of the treatment temperatures, the temperatures of incipient (definitely appearing) injury and the lowest survival temperatures were derived using a method similar to that of Montano et al. (1987). Incipient injury was considered to occur at the lowest temperature at which the mean injury rating of the replicates was <2.5, at which ≈50% of the leaves was injured. The lowest survival temperature of the shoots was considered to be the lowest temperature at which the mean injury rating of the replicates was <4.

The experiment was a randomized complete-block design with four blocks occurring over time. Five to eight replications were used within a block for each treatment and selection-cultivar combination. The analysis was conducted on the mean value for each treatment, selection-cultivar, and block on the incipient injury temperature and the lowest survival temperature. An analysis of variance was conducted on these two sets of temperatures using the Statistical Analysis System (SAS) program package (SAS, 1985). Treatment and selection-cultivar means were separated using SAS's least square mean option.

Shoot injury progressed from the bottom oldest leaves upward. The apical meristems were the last tissues to be injured, as leaf primordia and young leaves are the most cold hardy in herbaceous plants (Sakai and Larcher, 1987). Thus, the lowest survival temperature was the lowest temperature at which the apical meristems survived.

Acclimation lowered the incipient injury temperature from -8.0 to -10.5°C (significant at $P = 0.05$) and decreased the lowest shoot survival temperature by a mean of 3.1°C; however, the latter ranged from a decrease of only 1°C for 'Comox' to a decrease of 5°C for 'Boyne'.

Table 1. Step-wise acclimation treatments for in vitro raspberry shoots.

Temp (°C)	Duration	Light intensity ² (μmol·m ⁻² ·s ⁻¹)
15 ± 2	2 weeks	10.1
2 ± 1	2 weeks	26.5
-2 ± 1	24 ± 1 h	26.5
20 ± 2 (rewarming)	48 ± 1 h	26.5

²Eight-hour photoperiod.

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Although acclimation resulted in greater cold hardiness, the lowest survival temperature of acclimated 'Comox' shoots did not differ significantly from those of its nonacclimated control (Table 2). This lack of significant increase in low-temperature survival following acclimation may be attributed to a slower acclimation rate of 'Comox' or to a lower capacity to acclimate. Previous researchers also found an increase in cold hardiness after in vitro material underwent acclimation treatments at low temperatures (Bannier and Steponkus, 1976; Sakai and Sugawara, 1973; Tumanov et al., 1968) or a combination of a short photoperiod and low temperatures (Caswell et al., 1986; Chen et al., 1979; Hellegrren, 1983).

The lowest survival temperature of acclimated in vitro shoots differed among selections and cultivars, but selections and cultivars did not differ in cold hardiness when their shoots were not acclimated. Selection-cultivar differences averaged over treatment were not significant; however, a significant selection-cultivar $\times$ treatment interaction occurred. Acclimated 'Boyne', Gu 63, and Gu 72 shoots survived to lower temperatures than those of 'Comox' (Table 2). Acclimated 'Boyne' shoots were 3.5 and 2°C harder than those of 'Comox' and Gu 63, respectively. Selection-cultivar rankings of relative cold hardiness obtained in this study agreed with results obtained from a field assessment (Zatylny, 1992).

An assessment of incipient injury did not reveal any selection-cultivar differences among the acclimated (range -9.5 to -1.5°C) or nonacclimated (range -7.5 to -8.5°C) shoots. Injury progressed from the lower leaves upward in this study, damaging the leaves on the lower half of the plant at incipient injury temperatures. However, the apical meristem usually remained uninjured at the lowest survival temperature. Thus, apical meristem survival was the focus for plantlet survival. The cold hardiness of different plant organs and tissues within the same plant can differ greatly (Sakai and Larcher, 1987). Therefore, the differential ability of the leaves of these red raspberries to survive low temperatures may not reflect their cold hardiness accurately and may differ from the relative survival of their apical meristems, as was evident in this study.

In previous studies, acclimation treatments were not always necessary to reveal cultivar or species differences in cold hardiness. Van Swaaij et al. (1987) found differential low-temperature survival of callus and cell suspension cultures of various *Solanum* species without initially acclimating the tissue. Popular (*Populus nigra* L.) callus was harder than that

Table 2. Mean lowest survival temperatures, as determined by an injury rating of <4², of acclimated and nonacclimated in vitro raspberry shoots after low-temperature exposure.

Selection-cultivar	Lowest survival temp (°C)	
	Acclimated	Nonacclimated
Boyne	16.0 a ²	-11.0 d
Gu 72	-15.0 ab	-11.5 d
Gu 63	-14.0 bc	-11.0 d
Comox	-12.5 cd	-11.5 d
Treatment mean	-14.4 a	-11.3 b

²Tissue browning rated as 1 = less than one-third injury to 4 = entire injury.

³Mean separation within columns and across rows for selection-cultivar and treatment combinations and within rows for treatment means by least square mean option, $P = 0.05$.

of rape (*Brassica napus* L.) without prior acclimation (Duda and Kacperska, 1983). However, differences in cold hardiness may be greater from species to species than among cultivars of the same species, regardless of acclimation. Nevertheless, for cultivar screening, acclimated tissue was also necessary for citrus seedlings (Young, 1969). Acclimated material logically would be necessary for cultivar screening, as it is often the rate and onset of acclimation and deacclimation rather than the intensity of hardiness that influences winter survival of raspberries (Brierley and Landon, 1946; Jennings et al., 1972; Pacholak, 1978) and other crops (Stushnoff, 1972).

This study indicates that artificially freezing acclimated in vitro shoots successfully screened four red raspberry types for cold hardiness. Differences in cold hardiness did not exist among the types in their nonacclimated state, or the differences were too small to be detected using these methods. Thus, these red raspberry types probably differed in their ability to acclimate or the maximum level of cold hardiness they were capable of achieving. Since acclimated tissue was crucial in this study for successful screening, the type and duration of acclimation may have influenced the results obtained. Thus, various acclimation treatments should be examined to optimize this method of screening raspberries for cold hardiness.

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