



Cold tolerance of some Iranian wild grape (*V. vinifera* ssp *sylvestris*) genotypes

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ABSTRACT

All grape cultivars belong to *Vinifera* species are cold sensitive and (depend on cultivar) can be damaged by the temperature around -15 to -20°C. In this research, the cold tolerance of some Iranian wild grape (*Vitis vinifera* ssp *sylvestris*) genotypes was evaluated. After the leaf fall the 4 bud cuttings were prepared from the growing vines in Kahriz Horticultural Research Center in Urmia and then treated with freezing temperatures (-15, -18, -21, -23 and 4°C as a control) in a cold chamber. The percentage of burst buds in greenhouse condition and the percentage of died primary and secondary buds of each genotype in chilling temperature were measured. Electrical conductivity (EC) of buds and cane tissue was also measured. With decreasing temperature, the rate of primary and secondary bud injury increased and there was a significant difference in cold tolerance among tested genotypes. Approximately all Iranian wild grape genotypes were sensitive to cold stress above -15°C but among them, there were moderate cold tolerant genotypes too. H6 and H4 interspecies hybrids with *Labrusca* species showed higher cold tolerance compared to wild grape genotypes. These results will be valuable for selection of cold tolerance germplasm to use for cold tolerance breeding.

Key word: Chilling stress, Cold index, Electrical conductivity

Freeze injury is the greatest environmental threat to long-term successful grape growing in northern latitudes, where critically low winter temperatures occur and can dramatically reduce crop productivity and even be a limiting factor to growing grapevine belongs to *vinifera* species (Fennell 2004). Freeze injury can occur during the dormant period and certainly cause severe damage to buds, canes and also trunks in less hardy cultivars. The majority of commercial *vinifera* grape cultivars are cold-sensitive however, there are many cultivars that are less sensitive than others (Zhang *et al.* 2012).

Selection of cold tolerance cultivar is one of the important factors for sustainable fruit production in a cold area. This trait is genetically controlled and involves a combination of morphological, physiological and biochemical features which develop by natural selection over long periods. There are many cold tolerance resources in grape species such as *V. amurensis* and some American and Chinese species. One of the strategies adopted in overcoming cold stress is the use of tolerant genotypes

through the characterization of local genetic resources and the selection of potential tolerant genotypes (Fisarakis *et al.* 2001). Old cultivated grapevine (*Vitis vinifera* spp *sativa*) is thought to have been domesticated from a wild population of *Vitis vinifera* ssp *sylvestris* (Lacombe *et al.* 2003). Generally, they are predominantly forest climbers and prefer humid condition. Adaptation to diverse climates resulted in the development of some biotic and abiotic stress resistance or tolerant genes in these wild grape germplasm (Zhang *et al.* 2012). For these reasons, the evaluation and conservation of wild grape biodiversity are very important (Grassi *et al.* 2006). In Iran wild grapevine (*Vitis vinifera* ssp *sylvestris*) populations were found generally in riparian wood habitats on river margins located in Alborz and Zagros mountains in North and North-Western part of the country (Doulati Baneh *et al.* 2011). The availability of these genotypes provides an excellent opportunity to determine their tolerance to biotic and abiotic stress. So the aim of this study was to investigate the cold tolerance of some Iranian wild grapevine genotypes.

MATERIALS AND METHODS

Pencil-thick canes from 12 grape genotypes were sampled from the grape repository of Kahriz Horticultural Research Station (latitude 45°N, Longitude 72°W), Urmia, Iran (2015). These samples included 9 accessions of wild grape and 2 interspecific *Vitis* hybrids, H4 (*V. vinifera* cv.

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Jighjigha × Riparia Gloire), H6 (*V. vinifera* cv. Ghara Ozum × Kober 5BB) and a *Labrusca* species (Table 1 and Table 2). Cold treatments, 4°C (control), -15°C, -18°C, -21°C, -24°C, were performed according to the method of He and Niu (1989). To adapt the canes to cold conditions, the refrigerator was programmed to decrease the temperature by 3°C/h until the test temperature was reached (Khanizadeh 1991). Half of cuttings were placed in pots with half of sand and half of perlite medium and then transferred to a greenhouse. The second group was held for 48 h at room temperature. To evaluate the bud mortality, primary and secondary buds were sectioned through the tip of the buds with a razor blade and examined under a binocular microscope, checking for necrosis of the primary bud. The primary bud that appeared bright and green was considered alive, and that appearing dull, straw-colored or black/brown was considered dead.

Cold index: Twenty, 1-mm thick sections were cut from the middle of an internode and the area of browning of the

xylem was recorded with a light microscope and scored on a ten-point scale based on the area (%) of browning in the whole cross-section: (1 = 0-3.0 %, 2 = 3.1-6.0 %, 3 = 6.1-12.0 %, 4 = 12.1-25.0 %, 5 = 25.1-50.0 %, 6 = 50.1-75.0 %, 7 = 75.1-88.0 %, 8 = 88.1-94.0 %, 9 = 94.1-97.0 % and 10 = 97.1-100 % (He and Niu 1989). Cold index % (CI) is the standard of cold injury severity and can be calculated (Zhang *et al.* 2012):

$$\text{Cold Index (\%)} = \frac{\sum_{i=1}^{10} \frac{10}{i} ni \times i}{10 \sum ni} \times 100 \times 100$$

where i, the cold injury level (1, 2...10); ni, the number of shoot whose cold injury level was i.

Relative conductivity: Ion leakage rate of buds and stems were measured separately. The epidermis of the canes was first removed and then washed in distilled water. After drying with filter paper, shoots were cut into 2–5 mm

Table 1 Effects of genotype and freezing temperature on primary and secondary bud mortality

Genotype	4°C		-15°C		-18°C		-18°C		-24°C	
	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)
H4	0 l	0 p	0 l	0 p	0 l	0 p	0 l	0 p	33.3 d-i	16.7 nop
H6	0 l	0 p	0 l	0 p	0 l	0 p	0 l	0 p	0 l	0 p
Labrusca	0 l	0 p	0 l	0 p	0 l	0 p	0 l	0 p	0 l	16.7 nop
PR1B11	0 l	0 p	25 g-k	41.7 jkl	29.2 e-j	65.7 e-h	29.2 e-j	65.7 e-h	60 b	98.7 a
PR1B12	0 l	0 p	27.8 f-k-q	46.6 jkl	39.5 c-g	68.2 d-g	39.5 c-g	68.2 d-g	60 b	100 a
PR1B5	0 l	0 p	47.5 n-q	41.1 jkl	26.7 f-k	37.2 j-m	26.7 f-k	37.2 j-m	50.2 bcd	88 ab
PR1B8	0 l	0 p	23.2 b-e	59.2 f-i	35.8 c-h	69.6 c-e	35.8 c-h	69.6 c-e	93.3 a	100 a
R1B1	0 l	0 p	25 g-k	33.3 k-n	18.7 h-l	47.5 ijk	18.7 h-l	47.5 ijk	53.3 bc	80.8 b-e
R1B16	0 l	0 p	0 l	8.9 op	8.3 kl	25.6 lmn	8.3 kl	25.6 lmn	50 bcd	100 a
R1B2	0 l	0 p	0 l	0 p	12.8 jkl	25 l-o	12.8 jkl	25 l-o	42.8 b-g	81.7 b-e
R1B7	0 l	0 p	8.3 kl	25 l-o	13.1 jkl	29.2 lmn	13.1 jkl	29.2 lmn	50 bcd	86.2 abc
R2B2	0 l	0 p	0 l	0 p	17 h-k	40.7 jkl	17 h-k	40.7 jkl	53.3 bc	74.9 b-f

Table 2 Relative conductivity of grape genotypes under different low temperature treatments

Genotype	4°C		-15°C		-18°C		-18°C		-24°C	
	Cane	Bud	Cane	Bud	Cane	Bud	Cane	Bud	Cane	Bud
H4	26 r-u	30.6 wxy	31 p-r	34.8 u-x	36.5 l-n	38.6 r-v	49.1 j	50.9 m-o	64 d-f	70.8 e-h
H6	23 u-w	26 y	29 q-s	30.9 w-y	37.8 lm	49.4 m-p	39.2 k-m	59.2 kl	55.3 g-i	72 e-g
Labrusca	18.2 w	28.6 y	29q-s	32.7 v-x	30.9 p-r	42q-t	51.5 ij	59.5 kl	56 g-i	71.7 e-g
PR1B11	19 w	37.8 s-v	21 u-w	67.2 g-j	49.3 j	69.3 e-i	59.6 fg	74c-g	78.9 ab	82.2 ab
PR1B12	27.7 q-t	39 r-v	31.8 n-q	39.3 r-v	37 lm	45 o-r	39.8 k-m	61.4 jk	66.7 de	74.9 c-f
PR1B5	21.5 u-w	46.5 n-q	31.6 n-q	53.6 lm	42.8 k	74.3 c-f	53.3 h-j	79 a-d	78.4 ab	85.1 a
PR1B8	19.8 vw	40 q-u	23.2 u-w	43.5 p-s	24.7 s-u	69.4 e-i	39.8 k-m	72 e-g	73.7 c	80.1 a-c
R1B1	21.7 u-w	38 r-v	28.2 q-s	42 q-t	42.8 k	73.2 d-g	62.2 ef	75 c-f	67.9 d	75.5 c-f
R1B16	22.7 u-w	36.2 t-w	28.5 q-s	35.8 u-w	36.9 lm	42.3 q-t	56.7 gh	52.7 mn	75.5 bc	84.9 a
R1B2	21.7 u-w	33.7 u-x	30 qr	36.7 s-w	35.2 m-p	42.2 q-t	63.9 d-f	53.2 lm	62.8 ef	76.3 b-e
	31.3 o-q	41.8 q-t	36 l-o	44.9 o-r	40.6 kl	64.8 h-k	49.9 j	62.9 i-k	63 d-f	82.8 ab
	21.5 u-w	46.5 n-q	31.6 n-q	53.6 lm	42.8 k	68.9 f-i	53.3 h-j	75.2 c-f	81.7 a	85.1 a

thick slices (He and Niu 1989). Next, 1 g samples of each material were placed in graduate test tubes with 20 ml of deionized water. After resting at room temperature for 25 min, the tubes were then evacuated for 15 min to remove air from the tissues. After the vacuum had been released the samples rested for a further 15 min at room temperature and then conductivity (C1) was measured using a SENSDirect Conductivity Meter (Germany). After boiling for 30 min and resting at room temperature for 5 h, conductivity (C2) were measured again.

To measure the ionic leakage of the buds, the test tubes were disinfected in an oven at 70°C, and then 0.5 gr of thin slices from the single buds was prepared and washed 3 times with double distilled water and placed in test tubes. These tubes shacked for 4 h at room temperature (25°C) at 100 rpm. After 24 h, electrical conductivity (EC1) was measured by EC meter. The samples were then placed in an autoclave at 121°C and 1/2 atmospheres for 20 minutes. After cooling, EC2 samples were measured again. The following equation was used to obtain relative conductivity of cane and buds samples:

Relative conductivity (RC) % was calculated as: $RC = C1/C2 \times 100$

Data analysis: The arc-sin transformed data for the survival of the primary bud were used to perform the analysis of variance by SAS (SAS 1989). The Least Significant Difference (LSD) test with $\alpha=0.05$ used to separate the means. Also the descriptive statistics, the correlation between traits, factor analysis, and the cluster was performed by using SPSS 16 software. Cluster analysis and grouping the genotypes was used using Ward's Method based on the Euclidean distance as a criterion for the standard interval.

RESULTS AND DISCUSSION

With the increase of cold intensity, death percent of primary and secondary buds were increased. At 15°C, about 21% of the primary and 11% of the secondary buds were damaged. The death of 50% of primary buds occurred at temperatures between -18°C and -21°C (Table 1) so the critical temperature was -18°C, in which, there was marked primary bud mortality in many of wild accessions. The highest death rate of the primary (70%) and the secondary buds occurred at -24°C, although the damage severity in secondary buds at all temperatures was lower than the primary buds (Table 1). This result has been reported in several studies (Rekika *et al.* 2005, Eshghi and Quamarsi 2016). The primary bud is less cold hardy because the larger organs and more differentiated (specialized) cells reduce its ability to super-cool and heal in response to damage. The less mature, less specialized cells (and denser arrangement) in the secondary and tertiary buds afford more extensive cold hardiness. Lack of differentiation also allows a more rapid healing response to damage (Moyer 2011). Secondary buds also showed a reaction similar to primary buds in cold treatments. In some grapevine varieties, the secondary buds are fruitful and after the cold damage of primary buds, they will grow and can bear an economical fruit (Rekika *et al.*

2004). Many of wild grape genotypes have fruitful secondary buds which incrementally harder than the primary buds so it is a way to use them for improvement of cultivars that be able to produce fruit in the region with cold winter or late spring frost when primary buds may be injured.

Greenhouse study: The primary buds burst of treated cuttings was diminished in greenhouse conditions, although among the genotypes there was discrepancy in each level of cold treatment. 50% of the buds did not open at -18°C and from -21 to -24°C primary bud burst decreased sharply. Unlike the primary buds, with increasing cold intensity to -21°C, the secondary buds burst increased and at -24°C it decreased. The low percent of secondary buds burst in the control is due to physiological effects that occur in the grape component buds. At the time of bud opening, the primary bud first begins to grow and in this case, the secondary bud does not usually grow, but in the event of injury and death of the primary buds (for example, due to extreme cold), the opportunity for growth of the secondary bud will be provided (Doulati Baneh *et al.* 2011). There are significant differences in the percentage of secondary buds burst among genotypes in greenhouse conditions. The highest percentage of bud burst recorded in wild genotype R1B1. High levels of primary buds burst indicate a low degree of cold damage. In extremely cold temperatures (-21°C and -24°C) the highest tolerance was recorded in the H6 with the highest percentage of primary buds burst but in many wild genotypes, the primary buds completely destroyed.

Cold index: The results of cold damage to xylem showed that this part of the cane had a good tolerance to low temperature, so there was no significant difference with the control temperature up to -21°C and no harm was actually happening. Studies have shown that the cold tolerance of xylem was more than the primary buds (Howell 2001). Frost damage symptoms were observed at -24°C. Among the examined genotypes, wild genotypes showed more susceptibility to cold in comparison to H6, H4 and *Labrusca* species.

Electrical conductivity: Cold treatments had a significant impact on the Electrical conductivity of buds and canes tissues as with decreasing temperature the EC was increased. The lowest EC in the control and the highest was recorded at -24°C in all genotypes. The electrical conductivity of buds was approximately higher than the EC of canes. From -18°C to -24°C the EC of some genotypes was significantly increased. The lowest EC of bud tissue at -24°C was measured in H4, H6, and *Labrusca* and then in three wild grape genotype (Table 2). It was reported that the increase in EC reflects the damage to the cell membrane (Saltveit 2002). This result is in agreement with studies involving 25 wild *Vitis* species (Zhang *et al.* 2012) and 19 grape rootstock and cultivars (Reynolds *et al.* 2016). Also it has been reported that in some plants, the degree of EC increases with the application of cold stress, but if the intensity of stress and time of application is low and the plant is in better condition, the EC rate will decrease and accordingly EC cannot be a good initial index for

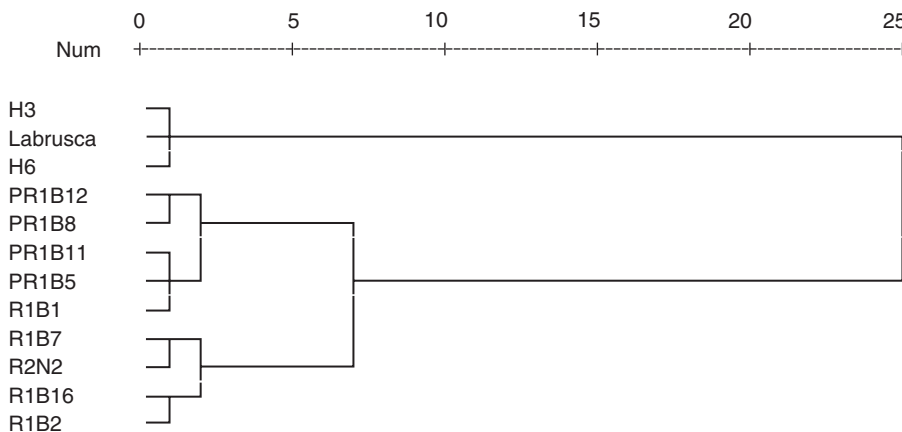


Fig 1 Dendrogram of grape genotype using Ward Method.

determining the amount of stress damage, but at high-stress levels it can be a useful indicator for the indirect assessment of tension stress (Tripathi *et al.* 2006).

Cluster analysis: In the grouping of the genotypes, the grapes were evaluated based on the extent of the damage to the primary bud of 3 different groups. In the first group H4, H6 and *Labrusca* were the most tolerant to cold. Second group included five wild genotypes that very sensitive to frost damage. In the third group, four wild genotypes with moderate cold tolerance were located (Fig 1). Primary bud death correlated significantly and positively with secondary bud death, cold index, secondary bud burst and bud electrical conductivity correlated negatively with primary bud burst. Significant positive correlations were also found between secondary bud death and cold index, secondary bud burst, and bud electrical conductivity. A significant negative correlation was observed between secondary bud death and primary bud burst, too. In this study, there was good agreement between greenhouse tests of bud burst and laboratory evaluation of bud mortality.

Grape genotypes can tolerate cold stress based on their genetic ability but their genetic tolerance to frost is also a function of environmental and agronomic conditions (Foodlad *et al.* 2003). Poor management practices in the vineyard including inappropriate nutrition and irrigation, high fertilization rate, inappropriate pruning and lack of proper control of pests and diseases during the growing season are noticed as the main factors in preventing the process of hardening and proper carbohydrate preservation and storage in the vine organs. Accordingly, with cultivation of cold tolerant cultivars in cold winter areas, it is necessary to apply proper management operations in the vineyards in order to reduce winter frost damage. There are different strategies to protect the grapevine from winter cold stress, but breeding to produce genetically cold hardy cultivars is very important. Although absolute hardiness is critical in cultivar selection in cold viticulture districts, but many factors like cultural practices and geographical aspects plays large role, especially to acclimate prior to potential cold events (Zhang *et al.* 2007). H6, H4 interspecies hybrids with *Labrusca* species showed higher tolerance to colds and

as a genetically resource for cold hardiness, H6, H4 genotypes can be apply as donor gene in breeding programmes, but due to genetically distance between them to return the quality traits in offspring the pseudo-backcross have to be done for several generations. *Labrusca* species as a cold hardiness source is suitable for transmission of tolerant genes. Today there are many hybrids between *labrusca* and *vinifera* species that can tolerate cold winter, but the foxy flavors of berries are not acceptable for Iranian clients and marketing. Wild

Grape germplasm resources in Iran can be found almost in temperate climates with cold winter. It was assumed that in this situation several cold hardy genotypes have been developed by natural selection. In this study, approximately all Iranian wild grape genotypes were sensitive to cold stress, but among them there was moderate cold tolerance as well, but this tolerance were not more tolerable than to the local cold cultivars. It was reported that altitude and latitude have a significant effect on cold tolerance of accessions of *V. amurensis* and *V. adstricta* (Zhang *et al.* 2007). In future studies, it will be better to conclude both latitude and altitude factors in evolution of cold tolerance of wide accessions of Iranian wild grape genotypes.

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