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COLD-TOLERANCE OF SOME AUTOCHTHONOUS AND IMPORTED COMMERCIAL SEEDLESS GRAPEVINE (*Vitis Vinifera*) CULTIVARS IN IRAN

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ABSTRACT

Winter cold is one of the main environmental factors limiting the production of grapes in Iran. Grape cultivars belonging to the *Vinifera* species are very sensitive to winter cold and depending on the cultivar, they are usually affected by cold damage in the range between -15°C and -20°C. There is genetic diversity among cultivars and even clones of a single cultivar with regard to cold tolerance. In this research, the cold tolerance of several cultivars of foreign and domestic commercial grape cultivars was evaluated. One-year-old 8-buds canes of the studied genotypes were harvested from the vineyards of the Urmia Kahriz Horticultural Research Station after the fall of leaves and they were placed a freezer temperature below zero (-15°C, -18°C, -21°C, and -24°C and 4°C as control). After applying the treatments, the number of open buds in greenhouse conditions and the rate of primary and secondary buds death were determined. Moreover, the electrolyte leakage of the buds and canes was measured. With increasing cold intensity, damage to primary and secondary buds increased and there was a significant difference between the cultivars in cold tolerance. Domestic cultivars showed a higher tolerance than foreign cultivars. Among the foreign cultivars, two cultivars of Thomson Seedless and Superior were more tolerant to cold. The results of this research are valuable in terms of determining the new seedless cultivars with a higher tolerance for cultivation in relatively cold weather regions.

1. INTRODUCTION

Temperature is one of the major determinant factors in plant growth and determines the range of their cultivation on the planet. Low temperature or cold causes serious damage to the crop production, quality, and even life of the plant. Grapes can grow in almost all climates of the world. Grapes have been cultivated for a long time in very hot and very cold areas, and it has been revealed that some

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cultivars grow and produce fruit better in hot regions, while some others grow and produce fruit better in cold regions. In cold regions, the biggest challenge is extreme winter cold. Grape cultivars belonging to the Vinifera species, that all grape cultivars in Iran belong to this species, are all semi-sensitive to highly sensitive to cold. The maximum temperature tolerated by buds is -15°C, and in the temperature below it, the cane, arm, and even trunk will be seriously damaged. In the grapevines that are in decline, buds and phloem are the most sensitive plant tissues to cold damages. Grape roots, like other plants, are more sensitive to cold than airborne organs and they tolerate up to -7°C on average (Okamato, 2000). Although grape flowers appear late and they are not often exposed to late spring cold, the grapes blossom disappear at -1°C and newly-formed fruits disappear at -0.5°C in cold conditions. The intensity and duration of cold stress, the storage of carbohydrates in canes and the amount of free water in plant tissues are the factors affecting the occurrence or non-occurrence of cold damage. The primary buds of grapes are more sensitive to cold than secondary buds (Fennell, 2004).

Cold-tolerance genetic variation within the Vinifera species is about -15 to -20°C (Zhang et al., 2012). North American species such as V. labrusca and V. riparia are good sources for cold tolerance. The riparia species tolerate cold temperatures of -31°C and lower, and Asian species such as amurensis tolerate similar cold temperatures without damage. Many of these cold-tolerant species are not usually edible and they are often used as a base or a genotype for breeding cultivars to cold (Winkler 1974). One of the breeding systems in fruit trees is a transfer of germplasm, such as importing of tolerant and commercial cultivars with more crop and better quality as well as cultivars tolerating the pests and diseases from the ecological area to another location (Janick and James, 1996). The goal of importing foreign cultivars and species is to use them directly for the construction of new gardens and the replacement of old gardens (if they show superiority over the commercial cultivars of the region and having favorable conditions for exporting). There are three major problems in importing the genotypes. First, access to these genetic resources is not easy. Second, they need to be quarantined so that pests and hazardous diseases such as viral diseases or phylloxera cannot be spread in their destination country. Third, some important crop traits in some new regions do not have genetic stability and show signs of incompatibility. For proper use of these imported genotypes, their compatibility with environmental conditions should be tested in several regions in the destination country (Baneh and Marandi, 2014). Winter cold is one of the most important damaging factors in most vineyards in the world and in Iran. Most of the grape fields are located in cold regions of Iran and most of the planted commercial cultivars of grape belong to vinifera species that are sensitive to cold. In this situation, in some years, extreme winter cold reduces the crop yield and eliminates some of the permanent organs of the grapevine, such as the arm and even the trunk, which reduces the production potential and imposes an additional cost on the farmers Eshghi and Kiamarsi, 2016). Among the most important cultivars planted in Iran and are suitable for the production of raisins and freshly use of them inside the country include *Bidaneh Sefid, Bidaneh Qermez, Pikami, Shahani* and several other cultivars, which have the largest share of their sales in domestic markets and low levels of them are exported. In recent years, a number of commercial cultivars have imported to Iran and have been planted to examine their compatibility and to compare them with domestic cultivars in several regions of Iran. Due to frequent threats of cold in Iran, it is necessary to gain knowledge and information on the sensitivity of these imported cultivars to cold stress. Hence, the aim of this study was to investigate the reaction of foreign cultivars to artificial cold stress and compare it with some native cultivars.

2. METHODOLOGY

For an accurate evaluation of the tolerance of these genotypes to different intensities of artificial temperatures under zero, this study was conducted as a factorial experiment in a completely randomized design with four replications at the Kahriz Horticultural Research Station belonging to the Agricultural Research and Education Center of West Azarbayjan province. All of the vine grapes studied in this study have been trained as a single-wire bilateral cordon system. Among one-year-old completely-wooded canes without symptoms of mechanical damages of the vine grapes of P*erlette, Flame Seedless, Black seedless, Superior, Fiesta Thompson seedless, Rasha, Bidaneh sefid, Bidaneh Gomez* and *Vitis labrusca* cultivars, 8-bud cuttings at required number (50 cuttings of each cultivar) were prepared and placed at refrigerator at 4°C (as a control without being affected by cold) and in a freezer at temperatures of -15, -18, -21, -24°C. To apply the cold treatment, with gradually reducing of temperature by 3°C/hr, the temperature reached to considered level. Then, the samples were kept at the same final temperature for 24 hours. Then, they were removed from the freezer and kept at room temperature for 48 hours so that the browning process of damaged tissues occur. After applying the treatment, the cuttings were divided into two groups. The first group was cut as four-bud cuttings and planted in the greenhouse within the medium that one half of it included windy sand and another half of it included perlite. The number of open and non-open buds was counted in two intervals. The second group of cuttings was transferred to the laboratory.

Using a sharp razor and three stages of gradual cutting of the buds, the death or survival rates of the primary, secondary and tertiary buds were calculated by observing through eyes. In order to evaluate the rate of cold damage to the vascular tissue and the germinal layer, the rate of necrosis of cambium of cuttings was also measured. Transversal thin sections were prepared from internode of cuttings and the area of the browned region was recorded under binocular. The rate of cold damages was in one of the following groups based on the percentage of browned areas in the transversal section (He and Niu, 1989): 1) 0 to 3%, 2) 3.1 to 6%, 3) 6.1 to 12%, 4) 12.1 to 25%, 5) 25.1 to 50%, 6) 50.1 to 75%, 7) 75.1 to 88%, 8) 88.1 to 94% 9) 94.1 to 97% and 10) 97.1 to 100%. Based on the percentage of cold, the genotypes examined in different groups were classified into different groups of 1- fully tolerated (deaths less than 20% of buds), tolerated (death from 20 to 60% of buds), semi-tolerant to semi-sensitive (death from 60 to 80% of buds), sensitive (over 80% death) and completely sensitive (drying of all cuttings).

Moreover, the rate of ion leakage of buds and stems was measured separately. To measure the ion leakage of the buds, the test tubes were disinfected in an oven at 70°C and then thin transversal sections were prepared from single-node buds of grapes and half a gram of these sections of buds separated. To eliminate surface contamination, the samples were washed double-distilled water 3 times. Finally, they were washed in 10 ml of double-distilled water and placed in test tubes. These tubes containing bud samples were placed in a shaker device at room temperature (25°C) at 100 rpm for 4 hours. After 24 hours, electrical conductivity (EC1) of samples were measured by EC meter. Then, samples were placed in an autoclave at 121°C and at a pressure of 1.2 atmospheres for 20 minutes. After cooling, EC2 of samples were measured after cooling for a second time. The following equation was used to obtain ion leakage (Saltveil, 2002).

After applying cold treatments and to measure ion leakage in the canes, the epidermis tissue of the canes was first removed and the cuttings were washed with distilled water and dried with tissue paper. The canes were cut into 2 mm thin transversal sections and 3 g of this tissue were added to a 100 ml volumetric flask containing 50 ml of deionized distilled water and kept at room temperature for 15 hours Then, the EC1 of the extract was measured. In the next step, the flask was placed in a bin Mari bath at a temperature of 100°C for 45 minutes, so that the heat can destroy the tissue of the samples. After cooling and reaching room temperature, EC2 was measured over a period of 5 hours. Cold damages of the cane tissue are shown as the It_A index (Reynolds et al, 2014).

$$It_A = Ct_1 / Ct_2 \qquad (1)$$

The obtained data were analyzed by SAS® software. Finally, the means of data were compared using the Least Significant Difference (LSD) test.

3. RESULTS AND DISCUSSION

The results showed that with increasing cold intensity, the rate of damages and death of primary and secondary buds was increased. At -15°C, about 25% of the primary buds and 12% of the secondary buds were damaged. With the gradual increase of the cold, the rate of damage increased in both buds. The death of 50% of the primary buds occurred at temperature -18°C and -21°C (Figure 1). The highest rate of death of primary buds (80%) and secondary buds (55%) occurred at -24°C, although the severity of damage at this temperature and other temperatures in the secondary buds was lower than the primary bud (Charts 1 and 2).

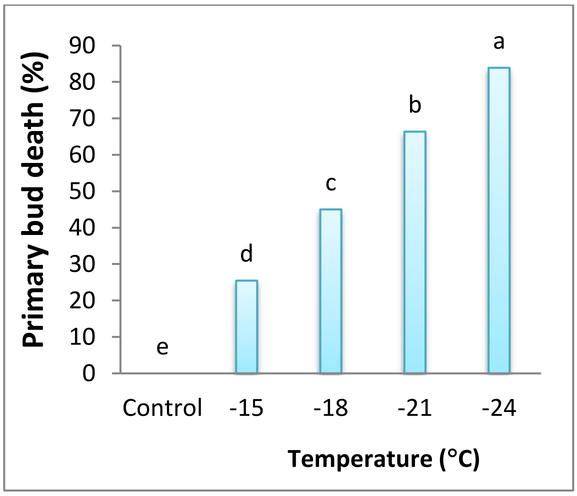

Chart 1: Comparison of the mean effect of temperature on the primary bud death

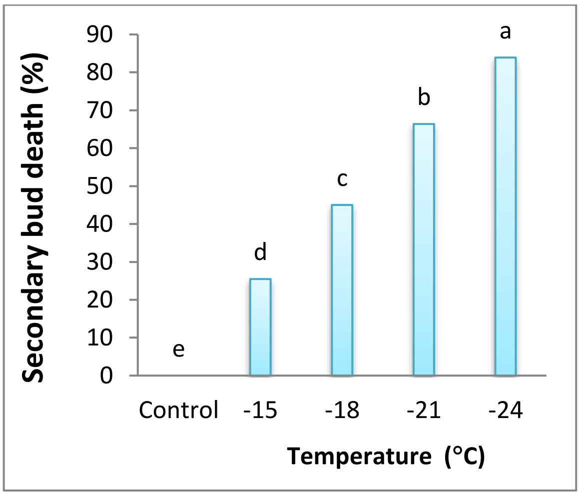

Chart 2: Comparison of the mean effect of temperature on the secondary bud death

The same letters indicate the lack of a significant difference at the level of 5% in the Duncan multi-range test.

In other words, primary buds are more sensitive to cold damage than secondary buds, and this result has been reported in several other studies. Rekika et al (2005) reported that there was a significant difference in the life of the primary buds of grapes among the cultivars. Moreover, the primary bud of grape is much more sensitive to cold than the secondary and tertiary buds. The results showed a higher sensitivity of primary buds to cold damage compared to secondary buds. The main reasons for the difference between grape buds in tolerating the cold are not clear. It has been reported that primary buds are less tolerant since larger organs and cells with more differentiation greatly reduce their ability in cooling or super-cooling and reduce their repair in response to cold damages. Less mature and less-differentiated cells (and compact arrangement) in the structure of secondary and tertiary buds increase the tolerability to the cold. Non-differentiation also makes it possible to improve the tissues in response to cold damage (Moyer, 2011).

Grape cultivars showed a significant difference in cold tolerance treatments. Grape cultivars belonging to the Labrusca species showed the highest cold tolerance (the lowest rate of death in primary and secondary buds) (Charts 3 and 4).

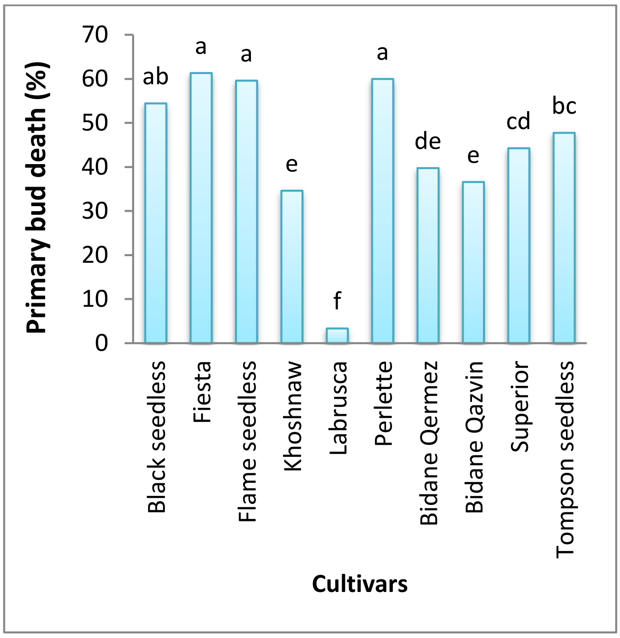

Chart 3: Comparison of the mean effect of cultivar on the primary bud death.

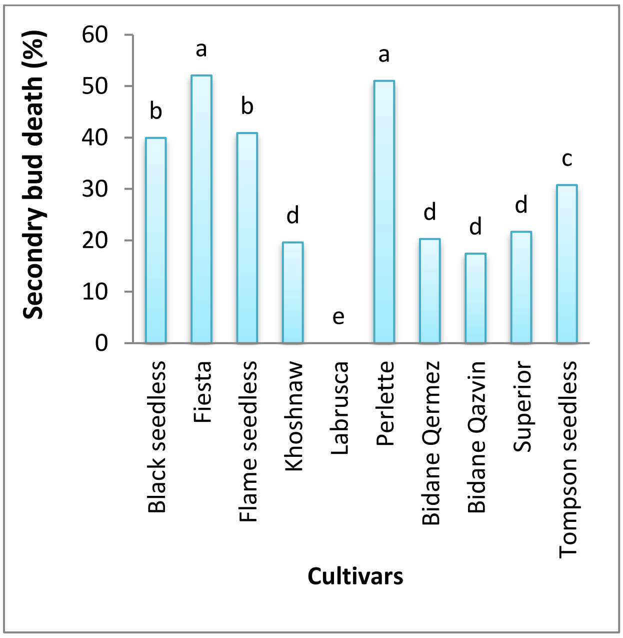

Chart 4: Comparison of the mean effect of genotype on secondary bud death.

The same letters indicate the lack of a significant difference at the level of 5% in the Duncan multi-range test.

The foreign seedless grape cultivars of Fiesta, Flame Seedless, Perlette, and Black Seedless had the highest rate of death in primary buds and they were very sensitive to cold treatments. Domestic cultivars showed the lowest rate of death in primary buds and showed the highest cold tolerance. Among the foreign seedless cultivars, Superior and Thomson Seedless cultivars showed greater tolerance, but this tolerance was lower than the Bidane Sefid, Bidane Qermez (Chart 3). Among the genotypes studied, there was a significant difference in secondary buds cold tolerance (Chart 4). Both primary and secondary buds of Labrusca cultivar showed a good cold tolerance, while the cultivars Fiesta, Flame Seedless, Black Seedless, and Perlette were sensitive to cold (while the sensitivity of secondary buds was less than that of primary buds).

Grape species and cultivars show a wide range of cold tolerance based on genetic traits, but the genetic basis for cold tolerance is in environmental conditions (Khanizadeh et al., 2005). The results of the comparisons of the mean of interaction effects of two factors of genotype and cold on the death rate of primary and secondary buds are presented in Table 1. At the temperature of -15°C, the highest death rate of the primary bud was seen in the Fiesta and Black Seedless cultivars, respectively, indicating the sensitivity of the buds of these cultivars to low temperatures. The highest tolerance of primary buds to the temperature of -15°C was observed in Rasha cultivar of Labrusca species (Table 1). With increasing cold intensity, the reaction of the cultivars to cold also changed, so that at this temperature, the highest rate of death of the primary bud was seen in foreign cultivars of Flame Seedless, Perlette, and Fiesta, respectively. At a temperature of -18°C, only the buds of Labrusca species grapes were not damaged, and among the cultivars, the lowest damage was seen in Rasha cultivar grapes. With increasing the temperature to -21°C, the rate of damage to the primary bud increased in all the cultivars examined. Among the foreign cultivars, Thomson Seedless showed better tolerance than other cultivars. At this cold level, the primary buds of the Labrusca species grapes showed no damage, indicating the inherent tolerance of the species to the cold. At a temperature of -24°C, many damages were imposed on primary buds, but there was a significant difference between the studied cultivars in the rate of death of the buds. The lowest damage was seen in the Labrusca species grapes, but in other cultivars, the rate of death of primary buds was more than 60% (Table 2). At a temperature of -24°C, no tolerance was observed in any of the cultivars belonging to the Vinifera species. A number of studies have reported that some cultivars of Vinifera such as the Riesling variety can tolerate a temperature of -22°C to -25°C (Lisek, 2009). Secondary buds also showed a reaction to cold similar to primary buds, so that as cold increased, the rate of death in buds increased. Secondary buds of grapes, in contrast to primary buds, have a higher tolerance to winter cold. Additionally, in some cultivars, the secondary buds grew after the damage of the early buds caused by cold and they produced an economic yield (Rekika et al., 2004). Therefore, it is necessary to examine the productivity of these buds in Iranian grape cultivars.

Table 1: Primary buds death index in the laboratory.

Cultivars	4		-15°C		-18°C		-21°C		-24°C	
Black seedless	0	O	45.833	LKJIM	42.1	LKJM	88.333	BAC	95.833	BA
Fiesta	0	O	62.5	FGIH	58.333	GJIH	85.7	BDAC	100	A
Flame seedless	0	O	33.333	NM	76.833	FDEC	87.733	BAC	100	A
Rasha	0	O	0	O	9.533	O	72.833	FGDEC	90.667	BAC
Labrusca	0	O	0	O	0	O	0	O	16.667	ON
Perlette	0	O	36.5	LKM	67.5	FGEH	95.833	BA	100	A
Bidane qermez	0	O	16.667	ON	47.833	LKJIM	54.167	KJIH	80	FBDEC
Bidane sefid	0	O	8.333	O	48.167	LKJIM	51.4	LKJIHM	75	FGDEC
Superior	0	O	34.833	LM	47.2	LKJIM	58.333	GJIH	80.833	BDEC
Thompson seedless	0	O	16.667	ON	52.667	LKJIH	69.333	FGDEH	100	A

Table 2: Secondary buds death index in the laboratory.

Cultivars	4		-15°C		-18°C		-21°C		-24°C	
Black seedless	0	P	18	ONMP	38.333	IJHLK	60	EDF	83.333	BAC
Fiesta	0	P	50	IGHF	45.833	IJGHF	74.233	BDC	90.4	BA
Flame seedless	0	P	20.833	ONML	29.167	JNMLK	68.567	EDC	85.833	BAC
Rasha	0	P	0	P	0	P	39.667	IJGHLK	58.333	EGDF
Labrusca	0	P	0	P	0	P	0	P	0	P
Perlette	0	P	20.333	ONML	42.333	IJGHFK	95.833	A	96.667	A
Bidane qermez	0	P	4.167	OP	12	ONP	33.333	IJMLK	51.833	IEGHF
Bidane sefid	0	P	0	P	16.933	ONMP	20.833	ONML	49.333	IGHF
Superior	0	P	0	P	25	NMLK	29.167	JNMLK	54.167	EGHF
Thompson seedless	0	P	4.167	OP	22.167	ONML	38.333	IJHLK	89.167	BA

4. GREENHOUSE STUDIES

The results showed that the rate of opening of primary buds in treated cuttings decreased with increasing cold intensity. In cuttings treated at temperatures of -21- and -24°C, the burst of the primary buds in greenhouse conditions decreased sharply and 50% of the buds were not opened at -18°C. The highest burst rate was recorded in control treatment (without cold) (Chart 5).

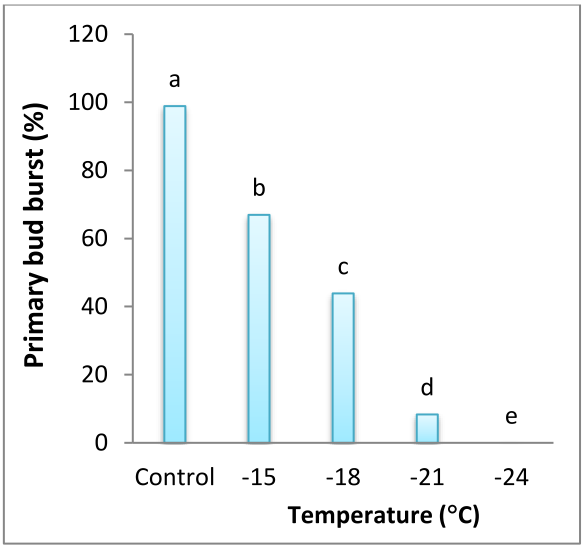

Chart 5: Comparison of the mean effect of temperature on the burst of the primary buds

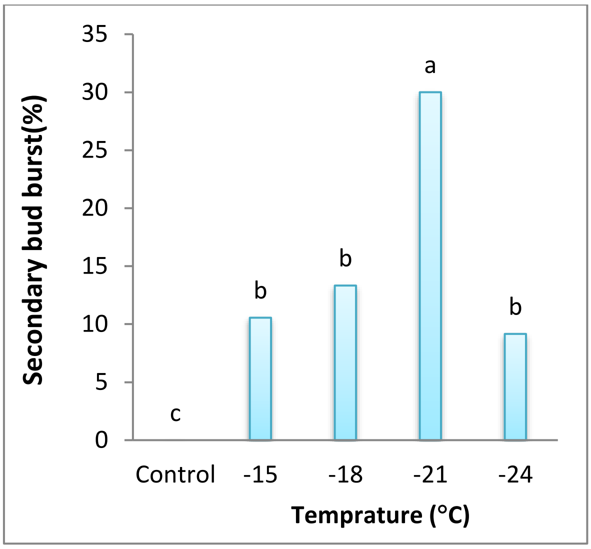

Chart 6: Comparison of the mean effect of temperature on the burst of the secondary buds

The same letters indicate a lack of significant difference at 5% level in the Duncan multi-range test.

The results of mean comparisons showed that, unlike primary buds, with increasing cold intensity to -21°C, the burst of secondary buds increased and burst decreased in the cuttings treated at a temperature of -24°C. In general, the rate of opening of these buds was very low in greenhouse conditions in non-cold cuttings or control cuttings (Chart 6). The low rate of a burst of the secondary buds in the control is due to the physiological effect that occurs in the composite bud of the grape so that at burst time, the primary buds inside the main bud of the grape first grow. In this case, the secondary bud does not usually grow. However, in the case of damage or death of the primary bud (for example, due to extreme cold), the opportunity for burst and growth of the secondary bud will be provided (Dolati Baneh, 2016). This condition is well seen in cuttings treated with the applied colds (Chart 6). In these cuttings, the primary buds disappeared by extreme cold and, as a result, the conditions for the growth of secondary buds, which are more tolerant to cold, were provided.

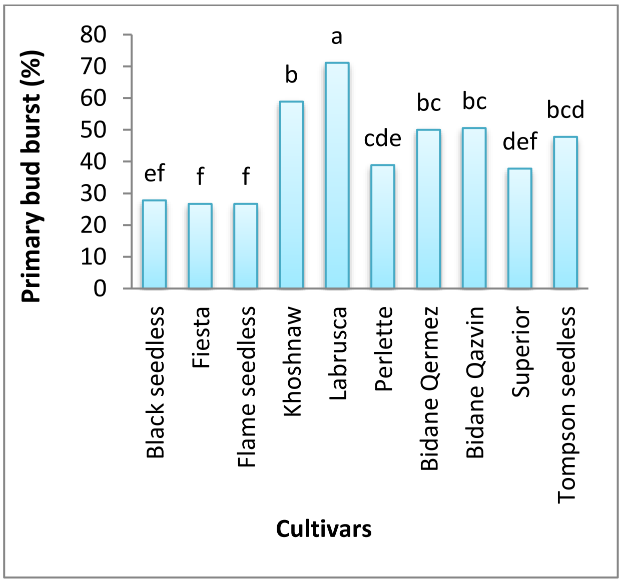

Chart 7: Comparison of the mean effect of cultivar on the burst of the primary buds

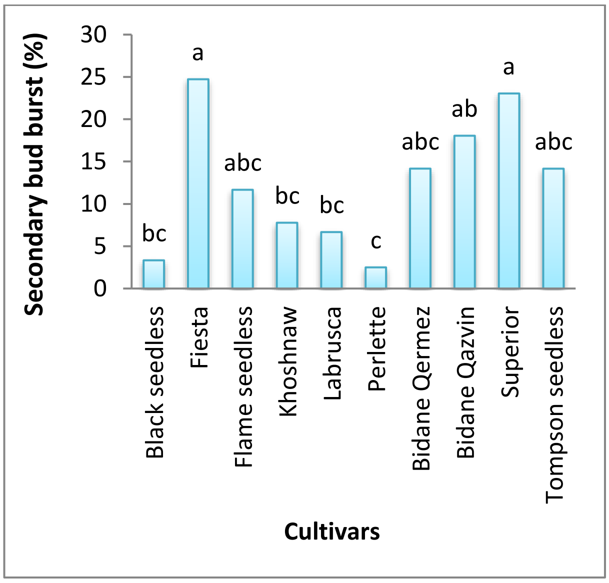

Chart 8: Comparison of the mean effect of cultivar on the burst of the secondary buds

The same letters indicate the lack of a significant difference at the 5% level in the Duncan multi-range test.

Based on the results obtained from the rate of opening of buds in greenhouse conditions, it was found that the highest number of germinated primary buds belonged to Rasha and Labrusa species. Black Seedless, Fiesta, and Flame Seedless had the lowest burst (Chart 7).

The results obtained from burst rate of secondary buds after planting of cuttings in sand and perlite in greenhouse conditions showed that there are significant differences among the cultivars in terms of rate of burst and growth of secondary buds, so that in the Black Seedless grapes, both primary buds and secondary buds had lower burst, while in the Fiesta cultivar, high number of secondary buds were opened compared to primary buds (Charts 7 and 8).

5. THE EFFECTS OF COLD ON CANES AND CAMBIUM

The results of cold effects on xylem showed that this part of the one-year cane of grape had a good cold tolerance so that there was no significant difference with the control up to the temperature of-21°C. in fact, no damage was seen in it. However, at a temperature of -24°C, signs of damage caused by cold were seen (Chart 9). Studies have shown that xylem of cane is more tolerant to cold compared to primary buds (Winkler, 1974). Among the cultivars studied, the cultivars Flame, Perlite, and Black Seedless were more resistant to cold than other genotypes and the Labrusca species showed the lowest damage (Chart 10). The highest damages to cambium tissue were recorded at -24°C. Although there were damages at -24°C, this effect had no significant difference with the temperature of -18°and -15° (Chart 11). In general, there was a significant difference between the studied genotypes in terms of damage in the cambium layer. Flame Seedless and Fiesta cultivars showed the highest damages to the cambium compared to other cultivars (Chart 12). The sensitivity of the cambium layer to cold was more in comparison to xylem and these results are consistent with the results obtained in several studies (Howell, 2000).

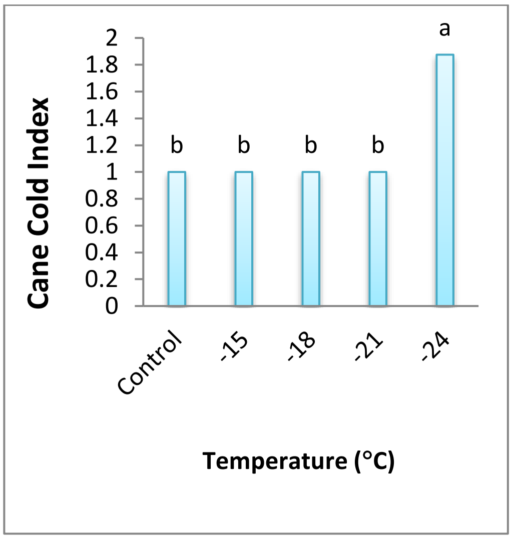

Chart 9: Comparison of the mean effect of temperature on cane cold index.

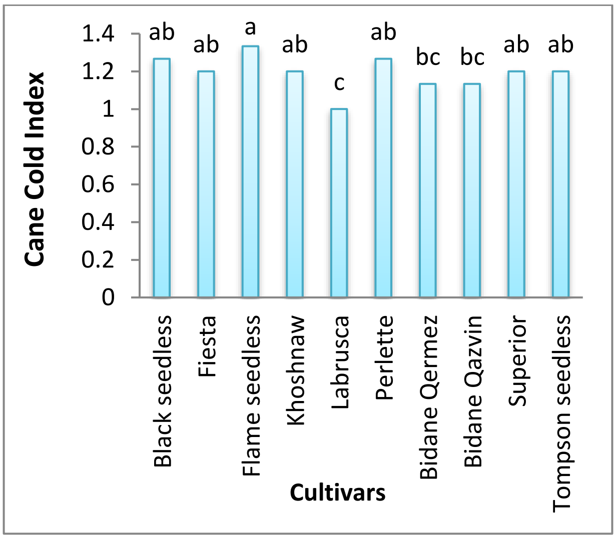

Chart 10: Comparison of the mean effect of cultivar on cane cold index.

The same letters indicate the lack of a significant difference at a 5% level with Duncan multiple -range test.

6. COLD EFFECTS ON ELECTROLYTE LEAKAGE OF BUD AND CANE

The results showed that by decreasing the temperature below zero, the electrolyte leakage of buds and canes increased (Charts 13 and 14) so that the lowest ion leakage was found in the control and the highest electrolyte leakage was found at -24°C in all cultivars. The amount of electrolyte leakage in the buds of the Perletee and Fiesta cultivars was more that of than other genotypes, while this index was lower in the Bidane Sefid and Labrusca species (Chart 15). Electrolyte leakage was also different in the cane of the grape cultivars studied, so that electrolyte leakage was the lowest in the Labrusca species, while in Thomson Seedless and Black Seedless cultivars; the electrolyte leakage was higher than that in other samples.

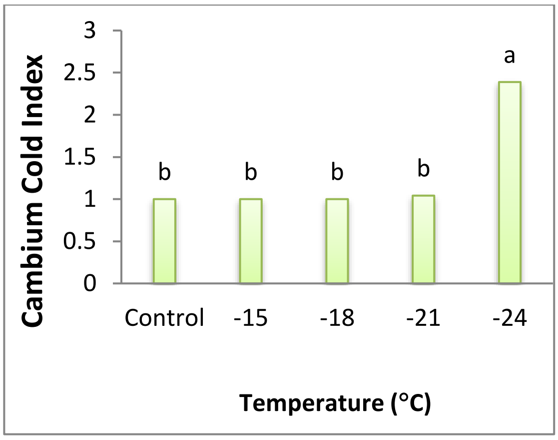

Chart 11: Comparison of the mean effect of temperature on cambium cold index

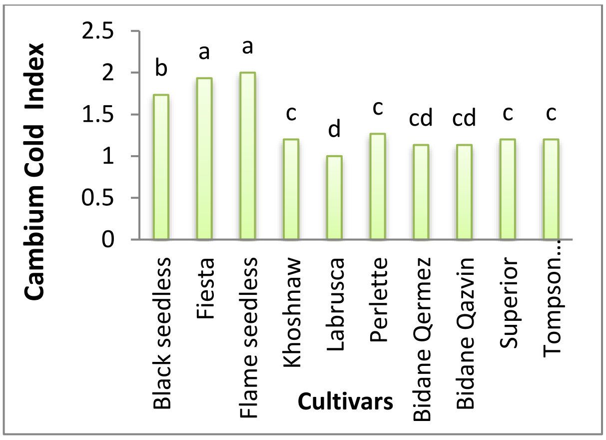

Chart 12: Comparison of the mean effect of cultivar on cane cold index.

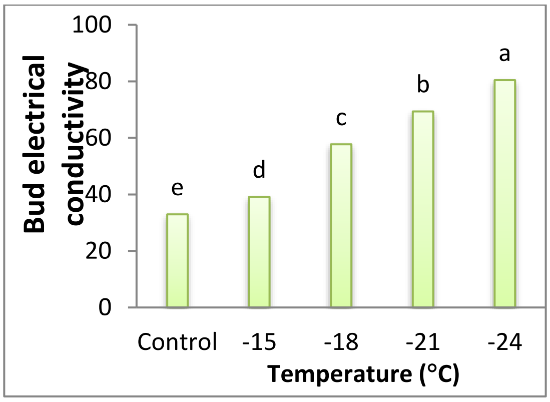

Chart 13: Comparison of the mean effect of temperature on the bud electrolyte leakage

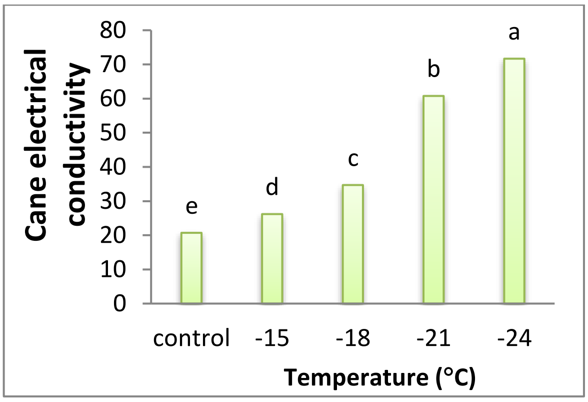

Chart 14: Comparison of the mean effect of temperature on the cane electrolyte leakage

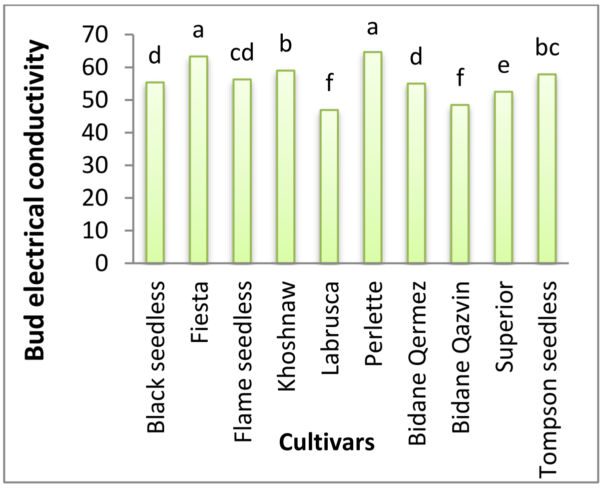

Chart 15: Comparison of the mean effect of cultivar on the bud electrolyte leakage

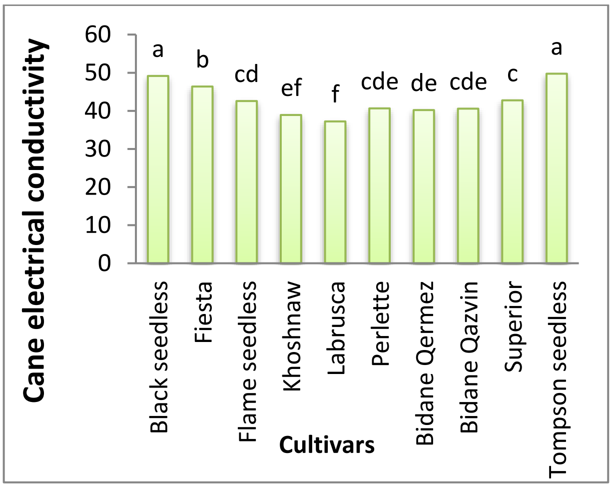

Chart 16: Comparison of the mean effect of cultivar on the cane electrolyte leakage

The same letters indicate the lack of a significant difference at the 5% level with Duncan multiple -range test.

The results of the comparison of the mean of interaction effects of genotype and cold on electrolyte leakage showed that at all levels of temperature, electrolyte leakage of buds and canes was different in grape genotypes. The lowest electrolyte leakage of the bud was measured at the coldest temperature (-24°C) in the Labrusca species and the highest was found in the Flame Seedless and Fiesta cultivars. Studies have shown that the increase in ion leakage and penetration reflects the damage to the cell membrane and as ion leakage is lower in the plant under stress, it indicates that the sensitivity of the cell membrane to stress is lower and vice versa (Saltveit, 2002). The results of this study showed that with increasing cold intensity, the electrolyte leakage increased, indicating the damage of the membrane due to cold. The grape of Labrusca cultivar showed the lowest ion leakage and the lowest damage in the bud and wood. The relationship between ionic leakage and the rate of cold damage in the tissues studied in two Flame and Fiesta cultivars were also proven. It has been reported that with applying the cold stress, the level of ion leakage increases in some plants, but if the intensity of stress and time of application is low and the plant is in better condition, the ion leakage rate will decrease. Accordingly, it is argued that ion leakage is not an appropriate initial index for determining the rate of stress damages, but at high-stress levels, it can be a useful index for the indirect assessment of stress damages (Tripathi et al, 2006).

7. CONCLUSION

Grape cultivars and species can tolerate the cold based on their genetic capabilities. However, genetic tolerance of cold is a function of environmental and crop conditions. Poor management operations in vineyards including inappropriate nutrition and irrigation, high productivity rate, inappropriate pruning, and inadequate control of pests and diseases during the growing season are the main factor preventing the process of proper storage of food in the organs of the vine and reducing cold tolerance. In contrast, storage and proper management increase cold tolerance. Accordingly, planting the cold tolerant cultivars in areas with a temperature below zero in winter and proper management operations in vineyards can reduce the damages caused by winter cold. An increase in the level of cold tolerance can reduce the rate of damages caused by winter cold. In general, Iranian cultivars showed higher cold tolerance in comparison with foreign cultivars. Among the foreign cultivars, Thomson and Superior cultivars showed lower sensitivity to cold in comparison with other foreign cultivars. The grape of Labrusca species showed high cold tolerance in laboratory and field tests and it can be currently introduced as an important source of cold tolerance in grape breeding work in the country.

8. DATA AVAILABILITY STATEMENT

The used or generated data and the result of this study are available upon request to the corresponding author.

9. ACKNOWLEDGMENT

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