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Screening of freezing tolerance in some dwarf selected mahaleb genotypes

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Abstract

Prunus mahaleb L. is the principal rootstocks used in Iran and world wide for sweet and sour cherries. This study was conducted with the main purpose of evaluation of freezing tolerance in eleven dwarf selected mahaleb (*Prunus mahaleb* L.) genotypes at Khorasan Razavi Agriculture and Natural Resources Research Center (Mashhad, Iran) during 2010- 2011. In two independ experiments, vegetative buds and annual shoots were placed in various temperature treatments (0, -5, -15, -25 and -25 °C with decreasing rate of 5 $^{o}ch^{-1}$) and percentage of ion leakage, soluble sugars and relative water content (RWC) in every stages were measured. Results showed that there was a significant difference in ion leakage and soluble sugars but there was not a significant difference in RWC between genotypes. Ion leakage and RWC percentage in buds and annual shoots increased and soluble sugars percentage decreased when the temperature was decreased. Ion leakage and RWC were the highest in 'DM129' (bud and shoot), but the 'DM265' (bud) and 'DM139' (shoot) had the lowest. The 'DM129' (bud and shoot) had the lowest and 'DM265' (bud) had the highest soluble sugars.

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Introduction

Mahaleb (*Prunus mahaleb* L.), a wild member of Rosaceae family, is an important rootstock for sweet and sour cherry cultivars (Ganji Moghadam, 2010). The mahaleb tree and its products have many uses. The mahaleb tree is robust and resistant to disease in nature; therefore, it has been used as the rootstock for the horticultural production of cultivar cherries in most Mediterranean countries (Gercekcioglu and Cekic,1999). This rootstock is compatible with light and loamy soils and rocky lands and also is compatible with cool and oceanic weather with which cherry is not compatible. Mahaleb roots are resistant to frost and freeze, so it can stand cold winters. One of the main problems in front of sweet cherry producer is late spring frost. Therefore, performing research projects with mahaleb breeding programs, especially to achieve to cold resistant is the most important mahaleb breeding objectives (Bonhomme *et al*., 2005).

Low temperature is a major abiotic stress factor affecting production in Iran and world wide. To survive this stress, plants need to acquire an increased freezing tolerance. During the acclimation of the plants, extensive changes are induced in gene expression and metabolic pathways (Iba 2002; Wang *et al.* 2003). Direct relationship has been founded between the increase resistances of woody and herbaceous plants to cold and carbohydrate content, generally assumed that the increase in the amount of carbohydrate in the cells, will lower the freezing point of cell sap (Barranco *et al*, 2005). Increasing concentrations of proline and carbohydrates and a decrease the amount of water in the leave of citrus generally has been associated with increased cold tolerance (Pakkish, 2009). Cold hardiness is associated with multiple mechanisms which each one play a role in protection of plant from freezing injury. Frost affects cell membranes, which become less permeable and even break, giving rise to the leakage of solute from damaged cells (Ameglio *et al*, 2005). Ion leakage increases with decreasing temperature, the temperature of –20°c than to temperature -15°c and -10°c had ion leakage highest (Nezami *et al*, 2011). Experiments on olive trees subjected to frost injury have shown that damage of flower buds is enhanced at low temperatures but ion leakage increased (Imani *et al*., 2011). (Vervaeke *et al*, 2004) studied the effects of frost injury on *Aechmea* species and ion leakage in selecting resistance cultivars. Cold resistant of different varieties of *Aechmea* to cool using measuring the ion leakage and observed that the amount of ion leakage in resistant cultivars was the result image. Also, the effects of frost injury walnut in concerning ion leakage as indicator of cold hardiness has been emphasized (Ameglio *et al*., 2005).

Some new techniques for the detection and classification have been identified for cold tolerant genotypes. Different ways of measuring the cold resistance in temperate zone trees there are, including measuring ion leakage and proline in the leaves (Jimenez *et al*, 2013; Mohammadzadeh *et al*, 2011).
Due to freezing the problem in Iran, is important to selection of freezing tolerance genotypes and there was not reported to evaluate the freezing tolerance of dwarf selected Mahaleb genotypes in Iran, so, the aim of this study was to evaluate different degrees of sensitivity to low temperature at different mahaleb genotypes.

Materials and methods

This study was carried out in two independ experiments at Khorasan Razavi Agriculture and Natural Resources Research Center (Mashhad, Iran). Eleven dwarf mahaleb genotypes (DM139, DM120, DM129, DM247, DM143, DM188, DM249, DM187, DM165, DM265, local) were used for the experiment. Four shoots of each mahaleb genotypes were collected with equal length (5 cm) and weight (5g) in time of bud swelling. Trees of the region with ages around 10 years and horticultural operations, such as irrigation, fertilization, pruning, etc carried out for them. The sampling after transfer to the laboratory were placed in cold chamber. In first and second experiment were evaluation respectively vegetative buds and annual shoot at 5 temperature levels (0,-5, -15, -25,-35°c). After 2h staying at each of temperature, samples brought outside to evaluate the freezing tolerance and following factors were measured.

Electrolyte Leakage

Buds and annual shoots sample were cut to 1- 2 cm pieces and placed in test tubes with 20 mL deionised distilled water (0.5-0.8 g fresh buds and annual shoots sample). After vortexing the samples for 3 s, the initial electrical conductivity (EC_0) of each sample was measured. The samples were stored at 4°c for 24 h, and conductivity (EC_1) was measured again. Samples were then autoclaved for 15 min, cooled to room temperature, and conductivity (EC_2) was measured for a third time. The relative permeability of cell membranes was calculated using a slight modification of the method of (Ameglio *et al*, 2005):

EC_0 (%)- Electrolyte conductivity

$$ECO = \frac{(EC1)}{(EC2)} \times 100$$

EC_1- Primaly Electrolyte conductivity

EC_2- Electrolyte conductivity after autoclaved

Relative water content (%) = RWC

In order to calculate RWC, fresh weight samples were weighted, then were submerged in distilled water and finally were direct at 60°C for 48 h and were weighed again. RWC was calculated according to (Maraghni *et al*, 2011):

RWC = (FW-DW/TW-DW) ×100

FW = fresh weight

DW = dry weight

TW = turger weight of samples

Soluble Sugar

To determine the total rate of soluble sugar solution 100 micro liters of leakage solution taken and 3 ml Known Antron freshly prepared (150 mg of net Antron + 100 ml of sulfuric acid 72 percent) was added. Then put it in boiling water bath for 10 minutes and after cooling of samples, their absorption in wavelength 625 nm was read with a spectrophotometer. Pure glucose was used for drawing standard curve. Concentrations of 0, 20, 40, 60, 80, 100 and 120 mg l^{-1} (ppm) were prepared. Like main samples, possible operation was performed on them (Xavier Morin, 2007; Annette *et al*, 2010).

Resistance or tolerance to frost the observational method

After the samples were removed from the system placed at room temperature (21±1 ° c) for 24h. The plant material for each treatment was separated by a knife were investigated by Microscope. Tissue green and healthy are living tissues and brown or black tissues were injury tissue (Afshari *et al*, 2013)

Statistical analysis

Experiment was carried out in a completely randomized design with three replications. Data analysis was performed in two trials using MSTATC statistical software and comparison of means using the Duncan multiple range test in level of 5 percent. The data obtained from the relative water content, soluble carbohydrates and ion leakage was calculated as percentage.

Results and discussion

Ion leakage

Results showed that highly significant differences between different temperature and genotypes based on ion leakage in the relationship with frost damage. The mean comparison showed a reduction temperature in bud and annual shoots significantly increased the percentage of ion leakage. The lowest ion leakage at 0°c in bud and annual shoots respectively were 61.33 and 34.88 and the most ion leakage at -35°C respectively were 79.12 and 44.70 (Table1 and 2). The genotype had a significant effect on the percentage of ion leakage, on the other hand, there was relation between frost damage and ion leakage in mahaleb genotypes that had the more resistant to frost damage had less ion leakage. The highest percentage ion leakage in genotype 'DM129' (bud and annual shoots) to the respectively 88.88 and 56.37 the lowest ion leakage in genotypes 'DM265' (bud) 49.91 and annual shoots 'DM139' were allocated to 32.30 (Table 1and 2). Genotypes 'DM129', 'DM120', 'DM143' were no significantly different with controls in the percentage of ion leakage.

Generally with decreasing temperature the percentage of ion leakage increased in annual shoots and buds (Table 1 and 2). Results showed that the severity of frost damage was influenced by genotype. It is suggested that ion leakage may serve indicator of frost tolerance in almond breeding material (Imani *et al*, 2011).The researchers conducted to cold resistant apricot varieties, investigate in temperature treatments -1 To -2 0°c and reported the low temperature had highest the percentage of ion leakage. The lowest ion leakage was observed in temperature 1 to 5°c and the most percentage of ion leakage temperature to -20 °c (Asl Moshtaghi *et al*, 2009). According to investigations of (Imani, 2011), the level of cold tolerance among cultivare of species and the amount of ion leakage in response to stress had been the different. They also concluded that ion leakage, a public property for all species is not sensitive to freeze. In this present study, it was cleared that the electrolyte leakage of mahaleb genotypes in response to freeze stress increased. So this criterion may be used to evaluate sensitivity or resistance genotypes to freezeing damage.

Table 1. Effect of temperature on bud ion leakage 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	85.55 a*	87.09 a	62.88 a	90.27 a	92.91 a	88.88 A
DM120	83.01 a	85.95 a	88.26 a	89.30 a	90.04 a	87.26 A
DM143	76.99 ab	87.34 ab	82.27 ab	88.66 a	89.11 a	84.89 A
control	60.84 c	71.09 abc	81.68 ab	84.98 a	80.75 abc	75.86 A
DM188	60.69 bc	68.22 abcd	74.08 abc	81.84 ab	85.03 abc	73.97 A
DM165	59.55 c	60.09 abcd	70.68 bcd	77.65 abc	84.85 abc	70.56 A
DM249	53.49 c	56.47 cde	61.32 cde	71.54 bcd	80.75 abc	64.71 B
DM139	53.29 c	56.26 cde	58.22 de	71.21 bcd	78.57 abc	63.53 B
DM247	48.67 c	50.19 cde	58.13 de	68.51 cd	69.20 bcd	58.94 B
DM187	46.75 c	47.88 de	53.28 e	66.11 cd	68.24 cd	56.45 C
DM265	45.85 c	43.33 e	48.80 e	60.63 d	50.97 d	49.91 C
Mean	61.33 A	64.87 AB	69.59 BC	77.33 C	79.12 D	

*In each column, row, mean that at least one letter in common are significantly different according to Duncans test at 5% level.

Soluble sugars

With reduction temperature in bud and annual shoots significantly increased the percentage soluble sugars. The results in relation with the effect of different temperatures on the percentage soluble sugars showed that the highest percentage was observed at 0°c in bud and annual shoots respectively to the 38.18 and 65.5 and in temperature -35°c in bud and annual shoots respectively to the 34.71 and 54.67 (Table 3 and 4), because the percentage soluble sugars was significantly more than other temperatures. The most percentage soluble sugars in genotype 'DM265' bud and 'DM139' annual shoot were respectively, 38.57 and 69.76 (Table 3 and 4).

Result showed that sugar, starch, proline and relative water content during to cold resistance of in some apricot cultivars on vegetative and reproductive buds reviewed. They reported in between cultivars of percentage soluble sugars in different vegetative organs (branch and buds) was different significantly (Abedi *et al*, 2010). Correlation between carbohydrate rate and cold resistance has been reported in some woody species. They had also reported that carbohydrate rate increased in some organ of plants in the winter and carbohydrate rate in root was higher than leaves, stems and buds (Afshari and Parvaneh, 2013). The results from the idea that high levels of soluble carbohydrates or osmotic activation can not be considered as cold resistance mechanisms, but rather it is suitable because of increased metabolic activities at the time of approaching to hot growth season (Annette *et al*, 2010). Some researchers have indicated direct relationship between increased sugar content and cold tolerance in woody and herbaceous plants. It is commonly assumed that increasing in sugar content of cell, lowered the freezing point of the cell (Rohaninia *et al*, 2008), these results confirmed with our findings in this study.

Table 2. Effect of temperature on annual shoots ion leakage of 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	49.20 a	54.16 a	58.16 a	59.84 a	60.23 a	56.37 C
DM120	39.75 b	44.14 b	47.14 b	52.95 b	62.27 a	49.27 C
DM143	31.95 bc	23.30 cd	36.56 cde	40.42 cd	45.78 b	37 60 B
control	36.63 bc	38.01 c	39.14 c	43.28 c	43.32 bc	40.07 C
DM188	36.59 bc	37.53 c	38.13 cd	42.57 c	41.59 cd	39.28 C
DM165	31.34 bc	32.45 d	35.76 cde	37.78 de	56.39 cde	35 .40 C
DM249	30.89 bc	32.14 d	34.97 cde	26.10 ef	40.25 cd	34 .87 B
DM139	28.32 c	32.00 d	32.85 e	23.29 f	35.04 e	32 .30 B
DM247	38.13 bc	34.61 cd	38.41 cd	40.98 cd	46.07 cde	39 .64 C
DM187	29.89 bc	32.16 d	33.82 de	34.65 ef	39.98 cde	34.10 B
DM265	30.52 bc	32.02 d	34.29 cde	35.76 ef	37.51 de	34.02 B
Mean	34.88 D	36.59 C	39.04 BC	41.61 AB	44.70 A	

*In each column, row, mean that at least one letter in common are significantly different according to Duncans test at 5% level.

Relative water content (RWC)

Genotype had a significant effect on the percentage of RWC ($p<0.05$). Bud and annual shoot of 'DM129' had the highest values of 31.83, 45.16, respectively. 'DM265' (bud) and 'DM139' (annual shoot) had the lowest RWC (Table 5 and 6).

The rate of RWC in plant with high cold resistance is the lowest than others. Plant having higher suloble sugare under cold stress should have low RWC. These results are correlated with the findings of other researchers (Abedi *et al*, 2010; Mellisho *et al.*, 2011). So, based on results, mentioned genotypes which are classified as high and medium suloble sugare genotypes in condition of cold stress, should be of low-content RWC.

Table 3. Effect of temperature on bud suloble sugare of 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	35.04 e*	34.54 d	23.87 f	33.41 d	33.04 d	33.98 A
DM120	35.16 e	34.83 d	34.29 ef	34.08 cd	33.83 bcd	34.43 A
DM143	36.29 de	35.29 d	34.62 def	34.25 cd	33.75 cd	34.84 A
control	37.08 cd	35.58 d	35.37 cde	35.04 bcd	34.54 abcd	35.52 A
DM188	37.96 c	35.71 d	35.50 cde	35.08 bcd	34.58 abcd	35.76 A
DM165	38.29 bc	35.79 d	35.62 cde	35.41 bc	34.95 abc	36.01 A
DM249	39.79 ab	37.12 c	35.83 cd	35.75 bc	35.00 abc	36.69 A
DM139	40.04 a	37.25 c	34.41 bc	35.79 bc	35.20 abc	36.88 A
DM247	40.21 a	38.00 bc	37.37 ab	36.78 ab	35.46 abc	37.54 A
DM187	40.41 a	38.87 ab	38.62 a	36.91 ab	35.67 ab	38.05 A
DM265	38.18 a	39.58 a	36.01 bc	38.45 a	35.83 a	38.75 A
Mean	38.18 A	36.59 AB	36.01 BC	35.54 C	34.71 D	

*In each column, row, mean that at least one letter in common are significantly different according to Duncans test at 5% level.

Observation method

Observation showed that there was significantly different between genotypes and temperatures in the annual shoots and buds 'DM129' (annual shoots and buds) and 'DM120' respectively fig (1. a) and fig (2. a), freezing percent dicereced with lowest temperature, whereas genotypes 'DM139' and DM'265' respectively fig (1. b) and fig (2.b), had the highest injuries in the lower temperature (fig 1and 2). Symptoms of freezing in the genotypes 'DM129' and 'DM120' (bud and annual shoots) was observed in the temperature -15°C,while in genotypes 'DM139' and 'DM265' was in temperature -5°C.

Table 4. Effect of temperature on annual shoots suloble sugare of 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	56.62 g	53.12 i	50.46 h	47.41 h	47.70 fg	51.06 A
DM120	59.41 ef	54.74 h	52.16 gh	48.21 gh	47.08 g	52.33 A
DM143	61.45 e	59.50 ef	56.83 e	55.08 e	49.54 ef	56.48 A
control	58.68 fg	55.57 h	52.87 g	48.91 g	50.87 e	53.40 A
DM188	58.83 fg	57.23 g	53.71 fg	51.19 f	54.79 d	55.17 A
DM165	65.75 d	50.60 e	59.50 d	58.71 d	60.50 b	60.99 A
DM249	70.83 c	62.79 d	62.25 c	61.66 c	57.41 c	62.98 A
DM139	77.83 a	71.41 a	70.54 a	65.17 a	64.00 a	69.76 A
DM247	59.00 efg	58.25 fg	54.57 f	51.95 f	48.33 fg	54.45 A
DM187	74.87 b	69.62 b	68.66 b	64.83 ab	59.62 bc	67 .52 A
DM265	72.37 c	67.75 c	67.16 b	63.66 b	61.54 b	66.49 A
Mean	65.05 D	60.97 AB	58.99 BC	56.07 C	54.67 A	

Table 5. Effect of temperature on bud RWC of 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	20.87 a	25.80 a	32.25 a	38.25 a	41.75 a	31.83 A
DM120	19.78 ab	25.62 ab	30.81 a	35.23 ab	40.78 a	30.44 A
DM143	18.04 abc	24.40 ab	29.51 a	34.45 ab	38.54 a	28.98 A
control	17.40 abd	24.07 ab	28.93 a	34.19 ab	37.47 a	28.41 A
DM188	17.10 abc	22.06 ab	27.87 ab	33.46 ab	38.97 a	27.49 A
DM165	17.27 abc	20.43 ab	26.34 ab	33.01 ab	35.04 a	26.41 A
DM249	17.64 abc	18.30 ab	25.56 ab	32.88 ab	34.71 a	25.81 A
DM139	14.14 abc	18.17 ab	25.35 ab	32.61 ab	34.43 a	24.94 A
DM247	11.70 bc	16.57 ab	23.09 ab	31.77 ab	34.10 a	23.44 A
DM187	11.71 bc	13.17 ab	22.89 ab	28.52 ab	33.95 a	22.04 A
DM265	10.89 c	12.61 b	15.19 b	27.53 b	29.47 a	21.13 A
Mean	16.04 B	20.10 AB	26.98 AB	32.92 A	36.11 A	

*In each column, row, mean that at least one letter in common are significantly different according to Duncans test at 5% level.

Table 6. Effect of temperature on annual shoot RWC of 11 selected mahaleb genotypes.

	Temperature (°C)					
Genotype	0	-5	-15	-25	-35	Mean
DM129	37.86 a	34.83 a	46.78 a	50.57 a	55.77 a	45.16 B
DM120	28.56 ab	42.18 a	44.71 a	49.42 a	57.76 a	44.53 B
DM143	28.01 ab	30.83 ab	36.47 a	45.26 a	51.38 a	38.39 B
control	30.31 ab	34.12 ab	42.90 a	49.08 a	49.00 a	41.08 B
DM188	28.87 ab	32.11 ab	40.23 a	48.29 a	48.92 a	39.70 B
DM165	25.09 b	30.06 ab	34.21 a	41.11 a	43.86 a	34.86 B
DM249	24.32 b	26.98 b	34.08 a	37.50 a	46.46 a	28.43 B
DM139	19.17 b	23.54 b	28.94 a	33.49 a	37.02 a	26.26 B
DM247	27.12 ab	32 34 ab	38.47 a	46.10 a	55.30 a	39.86 B
DM187	21.67 b	26 93 b	32.54 a	35.74 a	44.0 a	32 .21 B
DM265	22.42 b	26.97 b	32.82 a	36.77 a	43.24 a	32.44 B
Mean	26.67 A	30.99 A	37.46AB	43.03 AB	48.54 B	

*In each column, row, mean that at least one letter in common are significantly different according to Duncans test at 5% level.

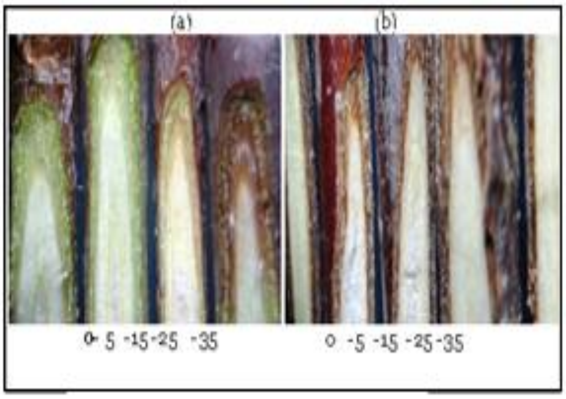

Fig. 1. The effect of freezing injuries on annual shoots. (a-Genotype DM129 b- Genotype DM139).

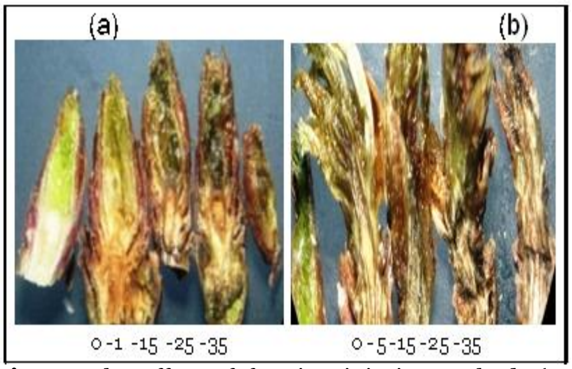

Fig. 2. The effect of freezing injuries on bud. (a-Genotype DM120 b- Genotype DM265).

Can be expressed temperature 0°c had lowest RWC and ion leakage and the highest sugar content. While the most ion leakage and RWC, and lower sugar content showed in temperature -35°c. Genotype 'DM129' (annual shoots and buds) had the highest RWC and ion leakage, whereas lowest RWC and ion leakage was reported in the 'DM126' (bud) and 'DM139' (annual shoot). The highest percentage of sugar in the 'DM139'(annual shoot) and lowest the percentage of sugar was reported in the 'DM129' (bud and annual shoots). Considering with decrease temperature in the bud and annual shoots cause to increase ion leakage and decrease the percentage of soluble sugar, and it seems that this genotype is susceptible to cold. Observation in studies have indicated that the most injury of 'DM129' was at -35°c and low injury showed in 0°c, whereas low injury in genotype 'DM265' (bud) and 'DM139'(annual shoot) were found. Injury rate is influenced not only by temperature but also by developmental stage that means the buds had the highest cold resistance in dormancy stage which is related to increase growth inhibitors in buds, reduce water of buds, and form scale on the buds at this time. Damaged organs seem brown or yellow-brown which is distinctive morphological sign of chilling injury. Damage percentage is not only influenced by temperature but is also by phenological stage too. Studies reported that survival at low temperature of some cultivars is related to cell membrane structure and higher amount of unsaturated fatty acids. Some cultivars have supercooling ability which prevents cell damage at lower temperature. Ice formation prevents the trees from supercooling. Other cultivars such as *Prunus padus* don't have supercooling ability but do to raceme inflorescence some flower survive (Rouhani Nia *et al.*, 2011).

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