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Relationship Between Frost Injury and Ion Leakage as an Indicator of Cold Hardiness in 60 Almond Selections

[1]*A. Imani*, [2]*K. barzegar and* [3]*S. Piripireivatlou*

[1]Horticultural Department of Seed and Plant Improvement Institute (SPII), P.O. Box31585-4119 Karaj, Iran
[2]Horticultural Department of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[3]Department of Horticulture Science, Faculty of Agriculture, Abhar branch, Islamic Azad University, Abhar, Iran

Abstract: Frost damage to the flowers and early developing fruits is one of the most limiting factors in almond cultivation regions of the world. This study was undertaken to understand almond response to frost damage concerning ion leakage in order to develop a criterion for the selection of frost-resistant cultivars in field experiments. In this work, 60 almond cultivars and genotypes on the basis frosts damage and ion leakage was studied. Results showed that the severity of frost damage was influenced by genotype. Genotypes that had the more resistant to frost damage had less ion leakage. It is suggested that ion leakage may serve as indicator of frost tolerance in almond breeding material.

Key words: Spring frost % Frost damage % Flower hardiness % Ion leakage % Almond

INTRODUCTION

Freeze injury is one of the main limiting factors to production and distribution of almond (*Prunus amygdalus*, Batsch L.) worldwide. Almond is particularly sensitive to spring frosts and in most growing regions of almond, buds, flowers and developing fruits after dormancy is injured. Losses due to frosts during blooming stage are usually more important than those due to low winter temperatures (Kester and Gradziel, 1990 and Rodrigo, 2000). Consequently, the ecosystem is a main factor which determines if an almond tree can be commercially grown in a region or not. Low temperatures are very important, especially during winter and spring. Low winter temperatures can damage the trees and even kill them. But almond tree tolerance varies from one variety to another (Miranda *et al*., 2005 and Barranco and Natividad, 2005). For example some of almond varieties may suffer damage by temperatures below -20°C or -25°C in winter, but due to early blooming and frequently is damaging by spring frost. Hence, almond cultivation was restricted to regions with low risk of spring frosts (Kester and Gradziel, 1990). For this reason, selection of late-blooming cultivars is an important breeding objective (Kester and Gradziel, 1990 and Socias I Company, 1992). In almond, to know about the minimum threshold temperature causing damages in flowers and young fruits is very essential.

Cold hardiness is associated with multiple mechanisms which each one play a role in protection of plant from freezing injury (Lang *et al*., 1994 and Orvar *et al*., 2000). Frost affects cell membranes, which become less permeable and even break, giving rise to the leakage of solute from damaged cells. This electrolyte leakage can be measured in terms of the electrical conductivity (EC) of the medium (Linden, 2002). EC measurement is a simple, quick and effective way in selecting almond genotypes by cold hardiness. Several attempts have been made to evaluate the effects of frost injury on flower buds. This has been achieved mainly through the use of ion leakage as indicators of cold hardiness in fruit trees. Experiments on olive trees subjected to frost injury have shown that damage of flower buds is enhanced at low temperatures but ion leakage increased (Soleimani, 2003). Vervaeke *et al*. (2004) studied the effects of frost injury on *Aechmea* species and ion leakage in selecting resistance cultivars. Also, the effects of frost injury on rose (Ameglio *et al*., 2003) and walnut (Ameglio *et al*., 2005) in concerning ion leakage as indicator of cold hardiness has been emphasized.

Correspoding Author: A. Imani, Department of Seed and Plant Improvement, Horticultural Institute (SPII), P.O. Box31585-4119 Karaj, Iran.

There is often a good correlation between ion leakage and freezing tolerance (Levitt, 1980). Sugars may depress the freezing point of the tissue and act as a nutrient and energy reserve, alter phase properties of membranes in the dry state and act as cryoprotectants to preserve protein structure and function. Other compounds acting similarly are lipids, soluble proteins and free proline (Lindon, 2002). Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and sub cellular structures and protecting cellular functions by scavenging reactive oxygen species (Bates *et al*., 1973 and Vanrensburg *et al*., 1993).

After the break of endo-dormancy and at the flowering stage, when flower buds are open or go forward generally more susceptible to frost damage, some differences observed among almond selections (Kodad and Socias I company, 2004).

The aim of the study was to evaluate different degrees of sensitivity to low temperature at different genotypes and cultivars in relation to ion leakage in field conditions.

MATERIAL AND METHODS

In this study, 60 genotypes and cultivars after occurring late frost spring naturally with temperature fall -3.2°C in 25 March 2010 were evaluated. Flowering in almond was started from 18 February in the earliest flowers (Sefied) and on 25 March 2010 in the latest flowers (Sh16 abbreviate Shahroud 16) cultivars. In this evaluation procedure, 24h before occurring frost spring in field conditions, flower samples of the genotypes and cultivars were collected and studied in relation to ion leakage as without stress set (Suleiman, 2003). 24h after frost spring, again samples of flower of genotypes and cultivars were studied using microscope (Barranco *et al*., 2005). Flowers were considered frost damaged when their pistils were brownish (Rodrigo, 2000). Since pistil is the effective organ for developing into nut. Also, ion leakage of flower samples determined as stress set.

Electrolyte Leakage (EL) Analysis: EL was measured according to Barranco *et al*. (2005). 0.5 g fresh weight of the flowers excised and washed in deionized water. Afterward samples were placed in an Erlenmeyer flask containing 15 mL of deionized water. The flasks were then shaken for 24h using a conductance meter (Consortmodel C831, Turnhout, Belgium) at 120 rpm in light conditions and temperature of 20 to 22°C. The initial electrolytic conductivity of each solution (initial EC, in µS.cmG^{1}) was measured, to obtain an indirect indication of the amount of ion released at each freezing temperature. Sample tubes were then autoclaved (1h, 120° C, 1 atm) to kill the tissues completely. After 2 h Shaking at 200 rpm in light conditions, electrical conductivity was measured again (autoclave EC), to obtain a reference value for total ions. Relative EC at each temperature (T) was calculated as ECr = (initial EC × autoclave EC) × 100.

The statistical analysis was performed using Statistical Analysis System (SAS Institute Inc, 1990) and means compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Results of this experiment showed highly significant differences between cultivars based on ion leakage in the relationship with frost damage (Table 1, 2 and 3).

As it is seen in Table 1, there are significant differences between 60 almond cultivars and genotypes. Also similar position between cultivars and genotypes according to ion leakage after frost stress has been showed in Table 2.

It was cleared that before frost occurring, there was no frost damage for each cultivar or genotype. All cultivars and genotypes were identical upon frost damage

Table 1: Analysis of variance of frost damage concerning 60almond cultivars and genotypes

SOV	DF	MS
Treatment	59	229.16770**
Error	119	10.03051
Total	179	

CV=3.353807

** Means are significantly different at $p<0.01$.

Table 2: Analysis of variance of post-frost ion leakage concerning 60almond cultivars and genotypes

SOV	DF	MS
Treatment	59	150.618892**
Error	119	0.781540
Total	179	

CV=0.969708

** Means are significantly different at $p<0.01$.

Table 3: Analysis of variance of Pre-frost ion leakage concerning 60 almond cultivars and genotypes

SOV	DF	MS
Treatment	59	142.680017**
Error	119	0.838644
Total	179	

CV=3.418665

** Means are significantly different at $p<0.01$.

Table 4: Relationship between ion leakage and frost damage in almond cultivars and genotypes in different Phenological stages

Cultivar/ Genotype	Phenological stage (%)	Cultivar/ Genotype	Pre-frost ion leakage (%)	Cultivar/ Genotype	Post-frost ion leakage (%)	Cultivar/ Genotype	Frost damage (%)
K68	100%	K1625	35.44a*	K5132	99.27a	K121	99.83a
K46	100%	K1016	34.96ab	k34	99.16a	Sh21	99.70ab
K527	Petal fall	Fragiulio	34.95ab	K147	99.02a	K144	99.74 ab
K234	100%	K717	34.93ab	Bala 9	98.73ab	K46	99.56 ab
K414	100%	K155	34.39abc	K1011	98.50ab	K1110	99.56 ab
K121	100%- Petal fall	Sh21	34.21abcd	K155	98.43abc	K527	99.48 ab
K132	100% - Petal fall	Shah15	34.11abcd	K414	97.98abcd	Mamaie	99.40 ab
D101	100% - Petal fall	Bala 9	33.84abcde	K97	97.36bcd	Sh15	99.39 ab
K1625	100% - Petal fall	Azar	33.70abcde	Sefied	97.32bcd	K155	99.30 ab
K84	Petal fall	Falsa Barese	33.53bcde	K717	97.25bcd	K414	99.30 ab
K131	100%	Sefied	33.26bcdefg	Mamaie	97.25bcd	K5132	99.13 ab
K168	100%	Supernova	33.11cdefgh	Supernova	97.197bcd	K147	99.11 ab
K155	100%- Petal fall	Genco	33.10cdefgh	Sh21	97.12bcde	K234	99.06 ab
K147	100% - Petal fall	Flippo Ceo	32.90cdefghi	K1016	96.73cde	K717	98.83 ab
K8	100%	K1630	32.88cdefghi	K1625	96.56def	K1625	98.83 ab
K1322	100%	K84	32.55 defghi	K1014	96.44def	Bala 9	98.80 ab
K1623	100%	Tuono	32.52 defghi	K121	96.33efg	K1016	98.65 ab
K119	100%	K937	32.44defghi	Sh13	95.50efg	Sefied	98.60 ab
K1340	100%	K1110	32.16 efghij	Genco	95.43fgh	K937	98.36 ab
K97	100%- Petal fall	Rabi	32.04 efghij	Sh12	95.22fgh	K97	98.36 ab
Sh13	100%	K47	31.85fghij	K47	95.01fghi	K118	98.30 ab
K144	100%- Petal fall	K97	31.64ghijk	Marcona	94.93ghij	K1014	98.16 ab
K1110	100% - Petal fall	Mamaie	31.41hijk	K118	94.407ghij	K920	98.10 ab
K1630	100% - Petal fall	K936	31.41hijk	K1110	94.3ghij	D101	98.06 ab
Sh16	10%	K121	31.38hijk	Tuono	94.07ghij	Genco	98.02 ab
Sh21	100% - Petal fall	K101	31.36hijk	Sh15	94.00hijk	Fragiulio	97.90 ab
K102	100%	K118	31.23ijk	K84	93.50hijk	Rabi	97.70 ab
Bala9	100% - Petal fall	K920	31.18ijk	D101	93.43ijk	K47	97.50 ab
k834	100%	K1011	31.15ijk	K920	93.28ijk	K1623	96.61 ab
Sh18	100%	Marcona	30.56jk	K527	93.26ijk	K1322	96.40 abc
K92	100%	K5132	29.85k	K937	93.20jkl	K1630	96.39 abc
K924	100%	K168	28.20l	Fragiulio	93.02klm	K1011	96.30abcd
Sh15	100% - Petal fall	K132	28.14l	Rabi	92.01klm	Falsa Barese	96.30 abcd
K920	100% - Petal fall	K144	27.440m	K936	91.96lmn	k34	96.13 abcd
K118	100% - Petal fall	K34	26.700mn	K1630	91.50mno	K936	96.06 abcd
K1014	100%	K147	26.14mn	K234	90.50mno	K92	96.00 abcd
K1016	100% - Petal fall	K131	25.38mn	K132	90.43nop	K84	95.94 abcd
K936	100%- Petal fall	K527	23.53o	K1340	90.11nop	K132	95.63 abcd
Sh12	100%	K1340	22.50op	K144	90.06opq	A200	95.40 abcde
K937	100% - Petal fall	K102	22.10opq	Nonpareil	88.67pqrs	K102	95.37 abcde
K717	100% - Petal fall	K119	21.68pq	Flippo Ceo	88.58qrst	K8	95.22 abcde
K1011	100% - Petal fall	K414	21.63pq	K168	88.36qrst	Marcona	95.22 abcde
Flipe Ceo	100% - Petal fall	K1014	21.61pq	Azar	88.09qrst	K168	95.17 abcdef
K5132	100% - Petal fall	Sh12	21.53pq	Falsa Barese	87.44qrstu	K1340	94.67 abcdefg
K47	100%	Sh13	21.18pqr	A230	87.37rstuv	Sh12	94.02 abcdefg
Falsa Barese	100% - Petal fall	A200	20.51qrs	K8	87.37rstuv	Shekofeh	93.83 abcdefg
Genco	100% - Petal fall	K46	20.41qrs	K46	87.24rstuv	K119	93.80 bcdefgh
Fragiulio	100% - Petal fall	Nonpareil	19.79rst	Sahand	87.21rstuv	K131	93.10 cdefghi
Tuono	100% - Petal fall	K92	19.55rstu	K1623	87.16stuv	FlipeCeo	90.10defghi
Marcona	100% - Petal fall	K234	18.84stuv	K131	86.93tuv	Sh18	89.93efghi
Supernovaa	100% - Petal fall	K1623	18.82stuv	Sh18	86.84uvw	Tuono	89.91fghi
Mamaie	Fruitlet	K1322	18.37tuv	K924	86.20vw	K924	89.17ghi
Rabie	Fruitlet	Sh18	18.00uv	K102	84.96x	Azar	89.13hi
Sefied	Fruitlet	A230	17.25v	K92	83.35x	Supernova	88.56hi
Azar	Petal fall	Sahand	15.66w	K119	83.33x	Sahand	87.96i
Nonpareil	Petal fall	K8	15.32w	A200	81.81xy	Nonparei	87.50ij
Sahand	100%	K68	13.92y	K1322	81.43y	A230	87.40ij
A230	100%	Shekofeh	13.69y	K68	81.38y	K68	86.46j
Shekofeh	100%	K924	13.65y	Shekofeh	80.12yz	Sh13	84.06jk
A200	100%	Sh16	15.32y	Sh16	54.23z	Sh16	36.56k

100%=100% opened flowers; Fruitlet= small fruit within jacket; 10%=10% opened flowers; 100%- Petal fall =Initiation of fall petal

* Means with similar letters there are no significant difference by Duncan test($P<0.05$)

in this stage. Thus, cultivars and genotypes from this view point, no analyzed statistically. Ofcourse, cultivars and genotypes in this phase had the lowest ion leakage, too. However, among cultivars and genotypes there was significant differences based on this physiological index (Table 3).

On the other hand, it has been showed in Table 4, there was relation between frost damage and ion leakage in almond cultivars and genotypes that had the more resistant to frost damage had less ion leakage. It can be mentioned, in this process, phenological stage of flower bud development is very important. For example, Sh16 as the late blooming had the maximum resistant to frost with at least ion leakage. But Sefied cultivar was very sensitive to this late spring frost stress due to very early blooming. If both cultivars were at same phenological stage of flower bud development (for example both cultivars at anthesis stage), It may be equal resistant to frost damage will be observed, or may possibly early blooming cultivar such as Sefied more resistant than late blooming Sh16. Therefore, frost resistance in almond especially late spring frost directly depended on flower bud development stage (Levitt, 1980 and Miranda *et al*., 2005). So that freezing damage in cultivar K121 with 100% opened flowers to petal fall in the temperature -3.5°C was%99.83 while cultivar Sh 16 with 10% opened flowers in same the temperature,%36.56 damaged (Table 4).

In general, Phenological stage seems to be important regarding the degree of frost damage, as trees were more affected at full bloom than at the popcorn (balloon) stage. Although flower buds are the most frost-sensitive parts of the trees in the cold season, but this sensitivity not only in relation to their phenological and histological stage but also for a protective effect, plants activate mechanisms involving many enzymes and antioxidative compounds (Kang, *et al.,* 2002). Miranda *et al*. (2005) concluded that *prunus* species, such as almond, resist to frost without major damage before the bloom phase, but is susceptible to frost during and after full blooming.

Results of frost test and percentage of ion leakage, in almond cultivars in different of phenological stages have been showed in Table 4. The frost resistance of almond has been recognized for many years, but tolerance and avoidance mechanisms of freezing resistance have not been investigated previously. Results of present showed that a position correlation was observed between the percentage ion leakage and frost damage for all the cultivars under study in bud swell stage. A200 cultivar (late blooming) with freezing damage 87.5% in -3.5°C with 81.81% ion leakage, while Nonparei cultivar (medium blooming) 87.50% damage in same temperature with 88.67% ion leakage (Table 2). According to investigations of Murata and Tatsumi (1979), Hardwick and Anderews (1980) and Lindon (2002), the level of cold tolerance among cultivars of species and the amount of ion leakage in response to stress had been the different. They also concluded that electrolyte leakage, a public property for all species is not sensitive to freeze. In this present study, it was cleared that the electrolyte leakage of almond cultivars flowers in response to freeze stress increased. So this criterion may be used to evaluate sensitivity or resistance cultivars to freeze.

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