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Evaluation the Resistance of Almond to Frost in Controlled and Field Conditions

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Abstract: Frost damage to the flowers and early developing fruits is one of the most limiting factors in the most almond cultivation regions of the world. This study was undertaken to help understand almond response to frost spring at same phenological stage, in order to develop criteria for the selection of cultivars with improved resistance to frost on the basis of field and laboratory experiments. In general, in this study, K-12-6, Ferragnes, Tuono and K-16-25 were negatively affected by frost at same popcorn and flowering stages in field test and laboratory constitutions. Experiment laboratory test showed, the four cultivar and selection suffering a greater frost damage rate at flowering at- 3.2° C (100%, 100%, 58 and 45% for K-12-6 , Ferragnes, Tuono and K-16-25 respectively) compared with the balloon stage at- 6.4^{0C} (100%,100%,85% and 58% for K-12-6 , Ferragnes, Tuono and K-16-25 respectively). Up to -3.1° C there was no damage at the balloon stage, where as in anthesis there was strict damage in four late flowering almond cultivars and selections at -3.2° C. Also, Results showed that the severity of frost damage was influenced by genotypes of almond. Genotypes that had the more resistant to frost damage had higher amount proline content. It is suggested that the content proline may serve as indicator of frost tolerance in almond breeding material.

Keyword: Frost, Almond, Flowers hardness

INTRODUCTION

Seasonality changes are one of the most common of climatic aspects due to various stresses. These stresses are affecting on vegetative and crop achievement [9]. Among these stresses, frost spring is one of the most factors affecting in plant productivity. Among of fruit crops, almond due to early flowering, most have been damaged with late spring frost [13]. It has been reported that almond cultivation in Iran and also in the most regions of the world is characterized by high degree risk from adverse climatic factors such as drought, soil salinity and spring frost [12 & 18]. Although, the almond is resistant to low temperature in the winter, but certain degree and duration of low temperature in the spring frost is lethal to the most reproductive organs at the blooming period. In some years even the resistant cultivars to cold can be damaged by low temperature [3]. The periods of major frost risk are from in the beginning of bloom and towards to active (shoot or fruit growing) growth [17 & 6]. The minimum temperature in which almond cultivars can resist in various phenological stages, may define its adaptation to specific agro-ecological zones. The temperature at which flower buds are injured depends primarily on their stage of development. Buds are most hard during the winter when they are fully dormant. As they begin to swell and expand into blossoms, they become less resistant to freeze injury [11 & 16]. Therefore in temperate climates, losses due to frosts during bloom are more important than those due to low winter temperatures.

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Cold resistance in flower buds possibly is the results of several factors including; structural, physiological and morphological features [5]. Factors such as genotype, stage of development, formation of ice, moisture content and nutritive status of pistil, have been reported that related to sensitive or tolerance of flower to spring frosts [18]. Resistance to frost among buds of different cultivars of the same species also is different. As critical temperature indices for almond have been obtained for the different stages of phenological development [12, 15 & 20]. Freezing temperatures can seriously damage to the plant tissues, the effects of spring frost on reproductive organs of almond are highly variable and depends on the characters of both the freezing stress and the plant material status [8, 14 &21]. The different responses to frosts observed among plant genotypes, tissues of the same plant and different seasons have led to study the mechanisms of injury [3 & 22]. In some cases, the accumulation of organic Osmolite like proline, glycine, betaine can be considered, that their amounts increase in stress conditions [9].Proline seems to have various roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species [7 & 23].

Cultivation techniques such as irrigation, reduction in nitrogen fertilizer rates, soil maintain systems and heater have been often used the commercial orchard to limit frost damage. However, the most effective way to combat damage, apart from simply avoiding frost prone areas, is to use frost spring resistant cultivars such as late flowering almond cultivars Selection of the less susceptible varieties among varieties with same phenological stages is the most effective indirect method of avoiding frost damage. The objective of the present research was to determine the damage caused to some late flowering almond cultivars and selections under the field and laboratory conditions in relationship with Proline.

MATERIALS AND METHODS

In this study, responses of four late flower almond cultivars and selections with index morphology characteristics (Ferragnes: late bloom, longed flower bud; Tuono: late bloom, round –longed flower bud; K-12-6: late bloom, Round –longed bud, early leafing; K-16-25: late bloom, Round –longed flower bud, late leafing) were investigated. Establishment of experimental orchard was in 2005. Experimental trees were planted 5×5 m apart. This experiment was based on randomly complete design with three replications. Each plat consisted of three trees.

Field test

All trees were planted in 2005, in the Kamal-Shahr Collection Orchard, Seed and Plant Improvement Institute (SPII), 50 km west of Tehran, Iran. Kamal- Abad station enjoys specific geographical conditions such as benefiting from approximately 250 sunny days per year, minimum temperature, maximum temperature and altitude (above sea level),-15,38 and 1258 M respectively. Most 150 almond varieties are available in the kamal-abad collection orchards. Flowers (full bloom stage) of the selective almond cultivars and selections in orchard were affected by frost spring, falling – 2.9° C, 2006. Frost damage to parts of flower of almond cultivars and selections were evaluated after 24h and then detached from the spur, placed for dissection into a Petri dish and observed for frost damages on asteroo microscope. The pistil is the most frost sensitive of organ in the flower. In almond, the first tissue affected can be the pistils, the petals, or both. For this reason, flowers were considered frost damaged when pistils in them were brownish, because of pistil is the effective organ for developing into nut.

Laboratory test

In 2005 spurs with equal length and diameter were harvest from four late flower almond cultivars and selections in early morning in two phenological stages (popcorn and flowering) were placed in an insulated (container and carried quickly to the laboratory. Spurs were taken are represented of all positions on the trees. For frost treatment, spurs were spread in to a chamber(432 L;ASLAparatos Cientificos,Madrid Spain).This programmable chamber model is equipped with a heat – cold unit working in the – 20° C to 30 ° C range – 0.3° C precision. Five thermopar probes connected to a datalogger (LI-100; LI-COR, Inc., Lincoln, Neb) were placed near the samples.

Air temperature in the chamber was maintained at 7° C for 50 min. and then programmed to decline by 3°C. h^{-1} until the desired frost temperature was reached. The frost temperature was maintained for 30 min. and was then increased up to 7°C by 3°C. h^{-1}. Frost was applied in the popcorn stage three temperature (-3.1, -4.9 and -6.4°C) and also in the flower stage three temperature (-1.2, -2.1 and -3.2°C). Frost damages rate was evaluated 24 h after Frost treatment. Flower buds were regarded as frost damage when they showed brownish.

To determining the proline

For determining the proline rate, the plant material was crushed in a mortar with 10 ml sulfosalicylic acid and centrifuged at 4000 rpm for 20 min. The 2ml of the supernatant was mixed with 2ml ninhydrin and 2ml acetic acid, and incubated for 1 h at 100°C. The 4ml toluene was added, and absorbance was determined by a spectrophotometer at 520nm.Proline content was derived from a standard curve obtained with pure proline (Merck KGaA,Darmstadt, Germany), according to Bates et al. (1973). The statistical analysis was performed using Microsoft Excel (2007) and SAS software [19] and means were compared using Duncan’s Multiple Range Test (DMRT).

RESULTS

Field test

Results of field fest showed that the severity of frost damage was influenced by variety; frost damage rate was significantly affected by morphological and phenological stage of flower buds and leafing time. Also, Proline of almond cultivars/selections concern to variety type was different (Table 1).

Table 1. rost damage and proline content in four late flowering almond cultivars/selections in same anthesis stage, exposed to -2.9 in orchard conditions

Almond cultivar/selection	Frost damage in anthesis stage (%)	Proline (mol gμ^{-1})
Ferragnes	96.41a*	2.09 a
K-6-12	98.42a	2.21 a
Tuono	85.14b	1.45 b
K-16-25	59.68c	1.01 c

*Means with the same letter are not significantly different at p=0.05using Duncan’s multiple range test.

In general the K-16-25, Ferragnes, Tuono and K-12-6 were negatively affected by frost respectively. The comparison of means with the reaction of different cultivars on proline rate indicated that most proline rate related to variety K-12-6 (2.21 mol gμ^{-1}.), and the lowest rate was allocated to K-16-25 (1.01 mol gμ^{-1})(Table 2). Results of laboratory test showed, the four cultivars and selections suffering a greater frost damage rate at flowering at- 3.2°C (100%, 100%, 58 and 45% for K-12-6 ,Ferragnes, Tuono and K-16-25 respectively) (Table 2) in compared with the balloon stage at- 6.4°C (100%,100%,85% and58% for K-12-6 ,Ferragnes, Tuono and K-16-25 respectively)(Table 3).).Also the results in relation to effect of temperature on *f*rost damage (%) in four late flowering almond cultivars and selections in same anthesis stage indicated that by temperature decreasing, rate of proline and *f*rost damage increased (Table.3)**.** Up to-3.1*°C* there was no damage at the balloon stage, where as in anthesis there was strict damage in four late flowering almond cultivars and selections at -3.2*°C*.On the hand, at the same lower temperature, there was little damage at the popcorn stage, but, at the anthesis severe frost damage of almond cultivars and selections was observed. In this study, sternness of frost damage among almond cultivars and selections was different base on morphological features and leafing time.

Table.2 Frost damage and proline content in four late flowering almond cultivars and selection in same popcorn stage

Temperature (0C)	Ferragnes		Tuono		K-6-12		K-16-25	
	Frost damage in popcorn stage (%)	Proline (mol gμ^{-1})	Frost damage in popcorn stage (%)	Proline	Frost damage in popcorn stage (%)	Proline (mol gμ^{-1})	Frost damage in popcorn stage (%)	Proline (mol gμ^{-1})
-3.1	0.00f†	0.00 c	0.00f	0.00 c	0.00f	0.00 c	0.00f	0.00 c
-4.9	87.33b	7.33 b	23.33d	5.56 b	100.00a	10.87 b	8.00e	2.33 b
-6.4	100.00a	21.12a	85.00b	17.21a	100.00a	25.62a	58.66c	21.12a

†Mean with the same letter are not significantly different at p=0.05using Duncan's multiple range test.

Table.3 Frost damage and proline content in four late flowering almond cultivars and selections in same anthesis stage

Temperature (0C)	Frost damage in anthesis stage (%)							
	Ferragnes	Proline (mol gμ^{-1})	Tuono	Proline (mol gμ^{-1})	K-6-12	Proline (mol gμ^{-1})	K-16-25	Proline (mol gμ^{-1})
-1.2	0.00c†	0.00 c	0.00g	0.00 c	0.00g	0.00 c	0.00g	0.00 c
-2.1	50.33b	0.640 b	10.33f	0.07 c	35.00e	1.04 b	3.33g	0.01 c
-3.2	100.00a	1.89 a	58.33b	1.17 a	100.00a	2.54 a	4500d	1.21 a

Table.4 Morphological and phenological characteristics of almond cultivars/selections

Cultivar	**Source**	**Tree traits**	***Phenology***	**Fruit traits**	***Kernel traits***	**Other characteristics**
TUONO	unknown	Growth habit: spreading Vigor: very high Productivity: high Bearing habit: spur	Flowering: late, on the small bundle Pollinizers: Ferragnes, Ferrastar, Genco, Filippo Ceo, Texas Harvest date: early-intermediate	Shape: globular – almond-shaped Colour: light brown Size (h,w,t) (mm): 35, 24, 18 Weight (g): 3.8	Size (h,w,t) (mm): 24, 14, 8 Weight (g): 1.6 Kernel/almond ratio: 41%. Double seeds: 25-35% Colour: light brown Shape: flat Blanching rate of kernel: good	Cultivar interesting for the late Flowering but is devalue to the high number of double seeds
FERRAGNES	Cristomorto x Ai	Growth habit: upright Vigor: very high Productivity: high Bearing habit: spur	Flowering: late, on the small bundle Pollinizers: AÏ, Cristomorto, Ferraduel, Ferrastar, Filippo Ceo, Primorski, Tardy Texas, Yaltinski, Tuono Harvest date: intermediate	Shape: globular - almond-shaped Colour: light Size: larg Weight (g): 3.7	Size: larg Weight (g): 1.5 Kernel/almond ratio: 41%. Colour: brown Shape: flat Blanching rate of kernel: optimal	Cultivar interesting for the high yield, late flowering and almond characteristics. The only problem is the self-incompatible
K-6-12	locality	Growth habit: upright Vigor: very high Productivity: high Bearing habit: spur	Flowering: late, on the small bundle Pollinizers: Ferragnes, Ferrastar, Genco, Filippo Ceo, Texas Harvest date: early-intermediate	Shape: globular - almond-shaped Colour: light Size: larg Weight (g): 3.7	Size: larg Weight (g): 1.2 Kernel/almond ratio: 35%. Colour: light brown Shape: flat good	New cultivar , interesting for the high yield and , late flowering
K-16-25	locality	Growth habit: upright Vigor: very high Productivity: high Bearing habit: spur	Flowering: late, on the small bundle Pollinizers: Ferragnes K-6-12, Tuono Harvest date: intermediate	Shape: globular - almond-shaped Colour: light Size: larg Weight (g): 4.4	Size: larg Weight (g): 1.6 Kernel/almond ratio: 37%. Double seeds: 40-45% Colour: light brown Shape: flat Blanching rate of kernel: good	New cultivar , interesting for the high yield and , late flowering

DISCUSSION

The risk of frost injury of the reproductive organs may increase with phenological development stage and growth and low temperature. At the popcorn stage the four almond cultivars/selections were damaged only when exposed to –4.9°C.

Phenological stage seems to be important regarding the degree of frost damage, as trees were more affected at full bloom and anthesis than at the pubcorn stage. Almond has demonstrated hardiness at pre bloom and less hardly at post bloom [15]. Miranda et al (2005) concluded that *prunus* species such as almond resists to frost without major damage before the pre bloom phase, but is susceptible to frost during and after anthesis. The results presented here have confirmed that almond is most susceptible to frost from the first swell bud stage on ward, and much less susceptible in fully dormant.

The variation in spring tolerance between the Ferragnes, Tuono, K-12-6, and K-16-25 almonds at same phenological stages could be due to difference in the several factors including structural, physiological phenological and morphological features (Table 4). In this study, high variability between different cultivars/selections to frost spring tolerance at same phenological stage was observed. An explanation for these results is the many factors still unknown or difficult to control which influence bud or flower resistance. So, Saunier (1960) and Proebstin and Milk (1978) determined that frost resistance varies within the tree itself in the same extent varies within orchard, cultivars, flowers of cultivars in the same penological stage. As well, in a similar position on the tree often present differences in frost resistance [5, 11, 21 & 22].

Frost was more detrimental during anthesis forward than the bud swell to backward stage. On the hand frost at the same phenological stage with index morphology characteristics is vary risk to the crop production [21].

Proline production increases in higher plants under stress conditions take place using two Glutamate (nitrogen deficiency) and Urnetin (nitrogen is high in the cell) cycles.

In sensitive plants to cold, cell proline increase is not sufficient to cause cold resistance increase, unless high amounts of proline are added before the stress. Of course cell proline increase always doesn't cause an increase in cold resistance [9]. In this regard proline rate of samples was measured after cold treatment, it is observed the highest proline rate was in anthesis stage and the lowest rate was in pobcorn stage of sprout. The results are similar with Rodrigo (2000) reports. His emphasizes the important attention on structural, phenological, morphological and physiological feature of almond cultivars when selection plant material for new varieties, as this is a mechanism of frost escape.

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