J. BIOL. ENVIRON. SCI.,
2011, 5(15), 153-157

Low Temperature Resistance of Developing Flower Buds of Pistachio (*Pistacia vera* L.) Cultivars

Zahra Pakkish[1*], Majid Rahemi[2] and Bahman Panahi[3]
[1]Horticultural Science, Department of Horticultural Science, Shahid Bahonar University, Kerman, IRAN
[2]Horticultural Science, Department of Horticultural Science, Shiraz University, Shiraz, IRAN
[3]Horticultural Science, Agricultural and Natural Resources Research Center, Kerman, IRAN

ABSTRACT

Percentage freeze damaged flower buds was estimated for four cultivars of pistachio (*Pistacia vera* L.) ʻKalle-Ghuchi', ʻOwhadi', ʻAhmad-Aghaei' and ʻAkbari' were determined. Samples were collected from bud swell stage to post bloom stage in 2008 and 2009. Four pistachio cultivars showed similar distribution of mortality during blossom bud development from bud swell to post bloom. During this period, the critical temperature for survival among the 4 cultivars in bud swell, green tip, tight cluster, full bloom and post bloom stage was -15°C, -12°C, -8°C, -4°C and -4°C respectively. Therefore, hardiness decreased as the stage of phenophase bud development increased. The tested pistachio cultivars exhibited less damage level in ʻOff' trees from ʻOn' trees.

Keywords: Bud flowers, Pistachio, Damage, Spring frost

INTRODUCTION

Low temperature is considered the most important factor determining the distribution of plant species on earth and can limit both the yield and the distribution of horticultural crops (Kalbrer et al. 2006), since many of them are either exotics or have arisen as a result of crosses of species or cultivars which are not indigenous to the region where they are cultivated (Nybom 1992). Horticulturists are constantly confronted with the problem of cold tolerance in relation to production of high quality fruit (Nybom 1992, Thomashow 1999). Yields may be changed faster by weather fluctuations than by any other factors. Spring frost hardiness in deciduous fruit trees is influenced primarily by genotype (Westwood 1993). Critical temperatures causing damage to reproductive organs are different among species and among cultivars. However, it is not easy to separate the effects of genotype from the effects of other factors. As a consequence it has been observed that critical temperatures not only vary with the phonological stage of flower buds but also can vary among species, cultivars, orchards and even within the trees (Westwood 1993). Although the variability of data shows that critical temperatures for species or cultivars are not biological constants, the results obtained can have an orientative value, and be useful to set the values to activate frost protection systems (Thomashow 1999 Westwood 1993, Xin 2000). In recent years freeze injury to pistachio flower buds has severely decreased yield in many commercial orchard in Iran. However, susceptibility to freeze damage appears to vary considerably between cultivars and should therefore be amenable to breeding efforts. Pistachio trees grow naturally where the summers and long, hot, and dry and the winters moderately cold (Crane and Iwakiri 1981).

The winter cold can limit their growth as can late spring frosts at time of blossoming, and lack of sufficient heat units at time of ripening in early fall limits good nut production (Rodrigo 2000). Pistachio trees in Iran, survive without injury at -7°C to -9°C in dormant season. In April 2006, pistachio trees were subjected to -2°C to -4°C that the most serious damages of buds were found in pistachio trees of ʻKalle-Ghuchi' grown in the Kerman, Iran.. However, pistachio blossom injured for cold and loss of crop.

This study was designed to determine critical temperature for frost injury of the pistachio cultivars flower buds during spring deacclimation.

MATERIALS AND METHODS

The experiment was conducted with four commercial pistachio cultivars: ʻKalle-Ghuchi' (early-bloom),ʻOwhadi'(middle-bloom),ʻAhmad-Aghaei'(middle bloom) and ʻAkbari' (late-bloom) a Pistachio Research Station, Iran, during 2008-2010 after dormant season. This station is located in the northeast of Kerman, Iran. The trees were 30 years old at the beginning of the study, and all trees received similar cultural practices such as irrigation and fertilization. The hardiness of pistachio cultivars was evaluated by subjecting excised 1-year old twigs to a controlled freezing stress, the samples were collected from March 2008 to April 2009 and from March 2009 to April 2010, with three week intervals, during dormant season. The single bud

* Corresponding author: zpakkish@yahoo.com

cuttings were placed in plastic bags and were weted. Then, single bud cutting sections were placed in ethylene-glycol (E-G) bath (Neslab, Model LT-50DD) at 1°C for 24h. Samples were placed in incubator and subjected to sequential freezing temperature, +5 (control), -4, -5, -10, and -15°C for bud swell stage, +5 (control), -2, -4, -8, and -12°C for tip green stage, +5 (control), -1, -2, -4, and -8°C for tight cluster stage and +5 (control), +1, 0, -1, -2 and -4°C for full bloom and post bloom stage. Sample temperatures were monitored using copper-constantant thermocouples inserted into the foil pouch and samples cooled at the rate of 5-7°C/h. The cuttings held for 24h at each temperature before being removed for evaluation. Individual buds were sectioned through the tip with razor blade and examined under a binocular microscope, checking for necrosis. The buds that appeared bright and green was considered alive and that appearing brown discoloration and straw coloured was considered dead (Rekika 2004, Stergio and Howell 1977).

Statistical Analysis

The experimental design was a randomized complete with three replications. Analysis of variance using SPSS 8.0 windows was done for each year during the test. The data were statistically analysed and the means were compared by Dancans's Multiple Rang Test (DMRT). Differences between means at 5% ($p<0.05$) level were considered as significant.

RESULTS

The flower buds of all pistachio cultivars lost the capacity to frost injury as the buds advanced from bud swell to post bloom. There was no significant difference in the amount of injury to flower buds from the 4 pistachio cultivars in all temperature treatments. Date of developmental stage of flower bud in all pistachio showed T_{10}, T_{50} and T_{90}, -5°C, -10°C and -15°C respectively (Table 1). The results showed, in stage green tip, highest and lowest percentage of injury observed at -12°C and -4°C respectively (Table 2). During experiment, the flower buds in tight cluster stage were sensitive to frost, because, more than 90% buds were damaged at -8°C (Table 3). The full bloom stage and post bloom stage, flower buds injured at -4°C (Table 4, 5). However, between these four pistachio cultivars, 'Kalle-Ghuchi' was sensitive, 'Owhadi', 'Ahmad-Aghaei' were semi-sensitive and 'Akbari' was hardy, but this results were not significant in 5% Duncan's. The tested pistachio cultivars exhibited less damage level in 'Off' trees from 'On' trees.

Table 1. Percent dead flower buds of pistachio cultivars in bud swell stage.

Year	T(°C) / Cultivar	-15	-10	-5	-4	+5
2009(ON)	Kalle-Ghuchi	96.67a*	49.33c	14f	1.66h	0
	Owhadi	92.51ab	43.25d	8.33g	0	0
	Ahmad-Aghaei	90b	39.65de	9.32fg	0	0
	Akbari	89.30b	37.60e	9fg	0	0
2010(OFF)	Kalle-Ghuchi	96a	50b	12.61d	0.33e	0
	Owhadi	91.17a	45b	14d	0	0
	Ahmad-Aghaei	91a	47b	11d	0	0
	Akbari	89a	32c	8.33de	0	0

*Column means followed by the same letter are not significantly different (*P*=0.05)

Table 2. Percent dead flower buds of pistachio cultivars in green tip stage.

Year	T(°C) / Cultivar	-12	-8	-4	-2	+5
2009(ON)	Kalle-Ghuchi	94.77a*	52d	15.69g	1.29j	0
	Owhadi	91b	40e	10.22h	0	0
	Ahmad-Aghaei	90.12b	41.53e	7.5hi	0	0
	Akbari	85c	36f	6i	0	0
2010(OFF)	Kalle-Ghuchi	94a	48.69c	15f	0	0
	Owhadi	88.78b	38.50de	8gh	0	0
	Ahmad-Aghaei	91ab	41d	10g	0	0
	Akbari	86b	34e	4hi	0	0

*Column means followed by the same letter are not significantly different (*P*=0.05)

Table 3. Percent dead flower buds of pistachio cultivars in tight cluster stage.

Year	T(°C) / Cultivar	-8	-4	-2	-1	+5
2009(ON)	Kalle-Ghuchi	95.31a*	50.67c	16.49e	2.55g	0
	Owhadi	92.27ab	43.21d	12.32f	0.35g	0
	Ahmad-Aghaei	91.90b	48c	11.70f	0	0
	Akbari	88b	41.51d	3g	0	0
2010(OFF)	Kalle-Ghuchi	94a	48.69c	15f	0	0
	Owhadi	88.8b	38.50de	8gh	0	0
	Ahmad-Aghaei	90ab	41d	10g	0	0
	Akbari	86b	34e	4hi	0	0

*Column means followed by the same letter are not significantly different (P=0.05)

Table 4. Percent dead flower buds of pistachio cultivars in full bloom stage.

Year	T(°C) / Cultivar	-4	-2	-1	0	+1	+5
2009(ON)	Kalle-Ghuchi	95.28a*	58c	14f	1g	0.33g	0
	Owhadi	92ab	51.30d	11.65f	0.71g	0.65g	0
	Ahmad-Aghaei	94a	51.62d	12f	0.83g	0.67g	0
	Akbari	90a	43.29e	2.31g	0.21g	0	0
2010(OFF)	Kalle-Ghuchi	93a	53.70c	12.67f	2.3hi	2hi	0
	Owhadi	88.90	47.30d	9g	1hi	0	0
	Ahmad-Aghaei	92.28a	49d	10g	0	0	0
	Akbari	88.26b	43.77e	4h	0	0	0

*Column means followed by the same letter are not significantly different (P=0.05)

Table 5. Percent dead flower buds of pistachio cultivars in post bloom stage.

Year	T(°C) / Cultivar	-4	-2	-1	0	+1	+5
2009(ON)	Kalle-Ghuchi	95.68a*	54.30d	15.67g	2j	0.35j	0
	Owhadi	91b	50e	11hi	1j	0	0
	Ahmad-Aghaei	93a	48e	12h	0.83j	0	0
	Akbari	89c	37.39f	8i	0.2j	0	0
2010(OFF)	Kalle-Ghuchi	96a	55c	18f	1h	0	0
	Owhadi	92.20ab	50.30d	12g	0	0	0
	Ahmad-Aghaei	91.38b	48.70d	13g	1h	0	0
	Akbari	85c	45e	9.66g	0	0	0

*Column means followed by the same letter are not significantly different (P=0.05)

DISCUSSION

The results indicated that Kalleh-Ghuchi is a early blooming pistachio cultivars (with 750-800h chilling requirement) and it is prone to suffereing spring frost in the regions where frost occure frequently (Rahemi and Pakkish 2009). Therefore, the present study indicated that flower buds of all pistachio cultivars, during growth and development and after dormant season, if subjected to freezing and low temperature, will injury, and hardiness decreased as the stage of phenophase bud development increased and this finding was in agreement with the previous reports (Ashworth, 1990, Byers et al. 1994, Callan 1990, Rodrigo 2000, Westwood 1993). Survival of plants at freezing temperatures is dependent on their ability to cold acclimate in response to environmental stimuli such as low temperature (Anderson and Selcley 1993, Rodrigo 2000). Plant species in cold climates have evolved adaptations such as dormancy, rapid acclimation, and maintainence of high cold hardiness throughout winter singly or in combination. Although researchers have intensively studied various aspects of acclimation, the processes of cold deacclimation and reacclimation remain less understood. However, deacclimation occurs more rapidly (days to weeks) than acclimation (weeks to months) in both natural and controlled environmental (Ashworth, 1990, Callan 1990, Westwood 1993).

Frost damage is highly dependent on the stage of development of the flower buds (Simons and Doll, 1976; Westwood, 1993). From dormancy to fruit set, the flower bud undergoes a number of developmental stages that are associated with a progressive increasing vulnerability of the pistil to low temperatures

(Khanizadeh 1989, Lu and Rieger, 1993, Niobium, 1992). Thus, the early developing fruit is the most vulnerable stage. Anderws et al (1983) suggest four periods during spring deacclimation of *Prunus* flower buds: the dormant period with deep supercooling of the flower buds, a transition period when buds begin to swell and approach budbreak in which deep supercooling is progressively lost, a third frost tolerant period before petal tip emergence and a final frost sensitive period following petal tip emergence, in which flowers and early fruits are very sensitive to frosts.

Temperature fluctuation before and during bloom can also influence flower tolerance to low temperatures since warm conditions may increase flower vulnerability and cool temperatures may decrease it (Howe et al. 2003, Warren 1998). Likewise, temperature affects frost survival indirectly by delaying or advancing bloom (Kalberer et al. 2006), but it remains unclear whether intrinsic changes in flower freezing tolerance are produced. Yet did not increase the internal damage as the buds were still protected by bud scales in their early stage of development.The freezing temperatures were sufficiently low to cause uniform damage from all exposures (Arora et al. 2004, Webster et al. 2005). The phonological stage of flower buds did have an effect on the amount of damage. The stages observed , in order of development, were bud swell, green tip, tight cluster, full bloom and post bloom. These stages or phenophases are similar to those observed in apples (Byers et al. 1994, Fuller and Wishiewski 1998).

The amount of damage could have been increased by the moist condition of the bud. It has been suggested that additional free water can raise the critical temperature required for frost damage by increasing ice crystallization and propagation in tender growing tissues (Arora et al. 2004, Westwood 1993).

This study showed variability in frost resistance and phenophase development, both among genotypes and environmental effects (Anderson and Selcley 1993, Niobium 1992, Rodrigo 2000).

These 3 parameters associated with spring freeze injury avoidance (high flower bud set, long bloom period, and late bloom) can be selected in all years because they are not dependent on environmentally imposed selection pressure (i. e., freeze during bloom) (Callan 1990, Byers et al. 1994, Fuller and Wishiewski 1998). A potentially very important factor about which we have very little information is the difference in resistance between location, other than differences related to stage of development. A laboratory test may predict accurately the results for the orchard from which the sample was collected, but fail to represent the general situation because of differences in resistance related to unknown cultural factors.

ACKNOWLEDGEMENT

The authors thank M.S Hadi Asghari for help and useful comments during the experiment.

REFERENCES

Anderson JL, Secley S D (1993). Bloom delay in deciduous Fruits. Hort Rev 15: 97-144.

Andrews Pk, Proebsting EL, Gross DC (1983). Differential thermal analysis and freezing injury of deacclimating peach and sweet cherry reproductive organs. J Am Soc Hort Sci 108: 755 – 759.

Arora R, Rowland L J, Ogden E L, Dhanaraj A L, Marian C O, Ehlenfeldt M. K, and Vinyar B (2004). Denardening kinetics, bud development, and dehydrin metabolism in blue berry cultivars during deacclimation at constant, warm temperatures. J Am Soc Hort Sci 129: 667- 674.

Arpaci S, Atli H S, Tekin H, and Burak M (2006). Studies on spring frost resistance of some pistachio (*Pistacia vera* L.) cultivars. 725: 391- 396.

Ashworth E N (1990). The formation and distribution of ice within forsythia flower buds. Plant physiol 92: 718 – 725.

Byers R E, Marini R P (1994). Influence of blossom and fruit thinning on peach flower bud tolerance to an early spring freeze. Hortscience 29: 146 – 148.

Callan N (1990). Dormancy effects on supercooling in deacclimated 'Meteor' tart cherry flower buds. J Am Soc Hort Sci 115: 982- 986.

Crane JC, Iwakiri BT (1981). M orphology and reproduction of pistachio. Hort Rev, 3: 376-393.

Fuller M P, Wisniewski M (1998). The use of in frared thermal imaging in the study of ice nucleation and freezing of plants. J Therm Biol 23: 81- 89.

Howe G T, Aitken S N, Neale D B, Jermstad K D, Wheeler NC, and Chen T H H (2003). From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81: 1247- 1266.

Kalberer S R, Wisniewki M, and Arora R (2006). Deacclimation and reacclimation of cold hardy plants: Current understanding and emerging concepts. Plant Sci 171: 3- 16.

Nybom H (1992). Freeze damage to flower buds of some apple cultivars. J Hort Sci 67: 171- 177.

Kalberer S R, Leyva-Estrada N, Krebs S L, and Arora R (2006). Frost dehardening and rehardening of floral buds of deciduous azaleas are influenced by genotypic biogeography. Env Exp Bot 1016.

Khanizadeh S, Buszard D, Fanous M A, and Zarkadas C G (1989). Effect of crop load on seasonal variation in chemical composition and spring frost hardiness of apple flower buds. Can J Plant Sci 69: 1277 – 1284.

Lu S, Rieger M (1993). Effect of temperature preconditioning on ovary Freezing tolerance of Fully opened peach flowers. J Hort Sci 68: 343- 347.

Niobium H(1992). Freeze damage to Flower buds of some apple cultivars. J Hort Sci 67: 171-177.

Rahemi M, Pakkish z (2009). Determination of chilling and heat requirements of pistachio (*Pistacia vera* L.) cultivars. 8: 803-807.

Rekika, D, Cousineau J, Levasseur A, Richer C, Fisher H, and Khanizadeh S (2004). The use of a bud freezing technique to determine the hardiness of 20 grape genotypes. Acta Hort 640: 207-212.

Rodrigo J (2002). Spring frosts in deciduous fruit trees morphological damage and flower hardness. Scientia Hort 85: 155-173.

Simons RK, Doll CC (1976). Morphological and anatomical response of apple to a late spring frost in relation to stage of fruit development. J Am Soc Hort Sci 101:315-320.

Stergio B G, Howell G S (1977). Effect of site on cold acclimation and deacclimation of Concord grapevines. Am J Enol Viticult 28: 43-48.

Thomashow M F (1999). Plant cold acclimation: freezing tolerance gens and regulatory mechanisms. Ann Rev Plant Physiol Plant Mol Biol 50: 571- 599.

Warren G J (1998). Cold stress: Manipulating freezing tolerance in plants. Curr Biol 8: R514 – R516.

Webster D E, Ebdon JS (2005). Effects of nitrogen and potassium fertilization on perennial rye grass cold tolerance during deacclimation in late winter and early spring. Hortscience 40: 842-849.

Westwood MN(1993)Temperate-Zone Pomology: Physiology and Culture. Timbwer Press,Portland.

Xin Z, Browes J (2000). Cold comfort farm: The acclimation of plants to freezing temperatures. Plant cell Env 23: 89 3 – 902.