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Chilling requirement studies on flower buds in some male pistachio genotypes (*Pistacia vera* L.)

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Abstract

Effects of different chilling periods were evaluated on growth and development of floral buds of male seedling trees **(***Pistacia.vera* L.**)** for chilling requirements of male genotypes helpful in predicting overlapping of flowering with female trees and escape from spring cold damage. The chilling requirement and responses of male genotypes to chilling treatment were determined by applying eight levels of chilling to shoots (*i.e.* 600-1300 h) at 3±1 °C. Based on the effect of chilling hours on bud break on four male pistachio genotypes were grouped to early (P_1 and P_6) and late flowering (P_7 and P_{10}) types. Percentage and rate of bud break, duration of flowering, growth and development of bud (length and width) were evaluated. The results indicated that genotypes had different chilling requirement. Among the male pistachio genotypes, the adequate chilling hours (bud break >80%) for P_1, P_6, P_7 and P_{10} genotypes were 800, 700, 1100, and 1300 hours, respectively. P_1 and P_6 had low chilling requirement (700 hours) for 50% bud break compared to P_7 and P_{10} (900 and 800 hours). Increased chilling led to decreased heat unit requirements for sprouting, resulting in greater overall growth and development. Chilling was a determining factor in floral bud break for all the genotypes, increasing chilling also produced greater bud break percentages. All genotypes required fewer heat units for bud break as chilling increased. Increasing the chilling hours also increased the length and width of flower buds and reduced duration of flowering.

Key words: Dormancy, bud break, cold storage, chilling requirement, bud development

Introduction

Dormancy in plants has been described as a state in which visible growth is temporarily suspended (Samish, 1954), a phase in plant development allowing it to survive under winter conditions (Saure, 1985) and a state in which deciduous plants are without leaf or are lacking visible growth (Westwood, 1993). Endodormancy released from within plant parts, as controlled by chilling temperatures, is a major factor in determining a plant's performance in a given climate or hardiness zone (Westwood, 1993). Temperate zone plants must be exposed to a certain period of chilling temperatures above freezing (Westwood, 1993) or a minimum number of hours below 7°C (45°F) (Saure, 1985) for dormancy break. This exposure period is referred as the chilling requirement. Dormancy requirements of landscape trees are of particular interest to the arborist and urban forester. Trees noted to perform well in northern climates, such as flowering cherries, spruce, or beech, may perform poorly or flower not at all in southern climates. In other cases, trees noted to perform well in the south may leaf out too early in the north, resulting in cold and frost damage (Lechowicz, 1984).

Much work has been reported on fruit species with respect to dormancy and chilling requirements. In a study with peaches, once chilling requirement was satisfied, prolonged chilling induced enhanced leafing over blooming (Citadin *et al.,* 2001). There are also cultivar differences in heat requirement for bloom. In a study with several fruit tree species, once chilling requirement was satisfied, prolonged chilling led to a decreased need for heat units for bud break (Couvillon and Erez, 1985).

The chilling optimum of temperate latitude forest trees varies between 0 and 2,000 h of below 5°C (Jensen and Gatherum, 1965; Steinhhoff and Hoff 1972; Van den Driessche, 1975; Burr *et al.,* 1989). Differences in chilling optimal within species may be caused by genetic variability, perhaps related to the different elevations and geographic regions in which the seed source was found (Rehfeldt, 1990). This genetic variation could in turn lead to the differences in chilling requirements between and within species. Most pistachios have a chilling requirement of 600-1200 hours. Not all buds of a plant have equal chilling requirements. Generally flower buds require less chilling than lateral buds. This is because flower buds often appear several days earlier than vegetative buds. Similarly, terminal buds have a lower chilling requirement than lateral buds. Therefore, in moderate climates (without severe cold in winters), terminal buds can begin to grow soon enough to establish apical dominance over laterals. In areas of severe winters, by the time the growing season begins both the apical and axillary's buds may have all of the chilling requirements met. Therefore when spring finally arrives, the plant will begin to grow from both lateral and terminal buds simultaneously.

Samish and Lavee (1962) indicated about the lack of standardized method to evaluate the depth of dormancy. To properly evaluate the depth of dormancy in the entire plant, it should be exposed to temperature for growth as in a greenhouse, but this is difficult with large plants. In a series of experiments, Erez and Lavee (1979) used rooted cutting of peach to study the effect of alternating temperature in breaking bud dormancy. One might ask whether the presence of roots in close proximity to the buds might affect response; however, Couvillon and Erez (1985) had previously demonstrated that bud break on rooted cutting paralleled that of mature trees. Therefore, cuttings bearing many buds will be the better choice for researchers wishing to predict the field response. The larger cutting has better expected response. The source of cutting is also important, especially for theoretical studies; previous year's shoots are normally used, but their vigor could affect response. Therefore, selected shoots should be similar in length and taken from similar position on the plant (Dennis, 2003).

Investigators often speak of the 'end of rest' when evaluating bud dormancy. This is usually defined as the time when 50% of the buds on excised shoots are capable of growth within a given period of time when held at an appropriate temperature with their bases in water. Greening of the bud scales is some times taken as evidence of bud break. The bases of cutting must be cut frequently to prevent vessel occlusion. Another problem is the danger of desiccation unless they are kept under high humidity, as in a mist bed. Growing cutting and/or bubs *in vitro* can prevent this (Dennis, 2003). Not much information is available about the chilling requirements of pistachio during the winter to ensure adequate bloom and pollination in the following spring (Crane and Iwakiri, 1981; Crane and Takeda, 1979).

The aim of this study was to evaluate the chilling requirement of male genotypes by exposing shoots to varying degree of chilling hours. The study will be helpful in the understaning of overlapped flowering with female trees and resistance to spring cold damage.

Materials and methods

The experiment was carried out during 2005-2006 using four male pistachio genotypes (P_1, P_6, P_7, and P_{10}) growing in Pistachio Research Institute at Rafsanjan, Iran. Their chilling requirements were calculated according to the chill unit. Temperatures between 0-7°C in winter of past year was 800-900 hours, latitude and longitude; 30° 25´N, 55° 45´E, respectively. To determine chilling requirement of mentioned genotypes, after leaf fall in the early November, 96 shoots of 30 -35 cm length from each genotype were picked up. After treating with Benomyl (2%) to protect from fungi, the shoots were warped in humid cloth and plastic then placed at temperature 3±1°C in referegerator.

The shoots were taken out from referegerator after chilled for desirable time (600 to 1300 h). These shoots were placed in buckets with half Hoagland medium. At 100-hours interval bud sticks were removed from the referegerator and placed in nutrient solution in the growth chamber of Horticulture Department of Tehran University. The growth chamber at the laboratory, programmed to simulate a typical day in mid April (9 hour night with 11°C and15 hour light with 19°C), these parameters were based on the average of the past seven years. Fresh cuts were made in the shoot bases and the water was changed every 4 days. The experiment had a factorial design with two factors including chilling hours at 8 levels and four genotypes (early flowering (P_1, P_6) and late flowering (P_7, P_{10}) in base of randomized complete block. The flower bud breaking percentage was determined in each treatment and the data were analyzed by SAS software.

Results and discussion

There were significant differences among genotypes for chilling requirements (Table 1). The adequate chilling hours for P_1, P_6, P_7, P_{10} genotypes were 800, 700, 1100, and 1300, respectively. P_1, P_6 and P_{10}, had minimum chilling hour requirement (700, 700, 800 hours) to initiate 50% bud break than P_7 (800 h). None of the genotypes responded to 600 hours treatment, except P7. (Table 1).

Compared to early flowering genotypes, late flowering types required less chilling hours. In all genotypes, increasing the level of chilling accelerated the rate of flower bud break (Table 1). Differences in the number of heat units required to reach bud break at every chilling level were determined for each genotype. The level of chilling exposure required for flower bud break was inversely related to heat unit accumulation. The higher chilling treatments also generally exhibited the highest mean percentage bud break over the course of the experiment (Fig. 2). These observations were similar to those recorded by Ashby *et al.*(1991) and Couvillon and Erez (1985).

Increasing the chill hours also increased the length and width of flower buds (Fig. 3) which is in accordance to the findings of Ferguson *et al.* (2003). Duration of flowering decreased from 26 days (in 700 hour treatments) to 11 days (in 1300 hour treatments) (Fig. 4).

Information from the present and future studies may be used to facilitate the development of models for regional planting recommendations based on the amount of chilling received

Table1. Effect of chilling on mean flower bud opening percentage and its rate in different genotypes

Chill hours	P_1		P_6		P_7		P_{10}	
	Flower bud opened (%)	Rate of flower bud opening	Flower bud opened (%)	Rate of flower bud opening	Flower bud opened (%)	Rate of flower bud opening	Flower bud opened (%)	Rate of flower bud opening
600	0.00	0.00	0.00	0.00	57.9cde	1.73i	0.00	0.00
700	69.33bcd	2.47hig	85.913ab	4.92d	56.44cde	2.25hig	87.05ab	2.375ghi
800	86.235ab	3.35efg	85.53ab	3.32efg	39.27e	3.03efgh	71.48bc	2.09ih
900	88.77ab	3.61ef	51.083de	3.01efgh	49.25e	1.77i	85.02ab	5.34d
1000	84.49ab	2.83fgh	76.3abc	3.05efgh	42.51e	2.78fgh	80.93ab	3.4e
1100	81.493ab	3.95e	89.76ab	5.282d	89.92de	5.01d	73.02bc	5.31d
1200	83.46ab	8.37b	88.65ab	3.65ef	74.1bc	2.982efgh	75.42bc	3.3efg
1300	80.2ab	8.05b	97.72a	9.77a	44.9e	3.94e	83.77ab	6.4c

*Means followed by different letters are significantly different by Duncan's Multiple Range Test at P=0.01

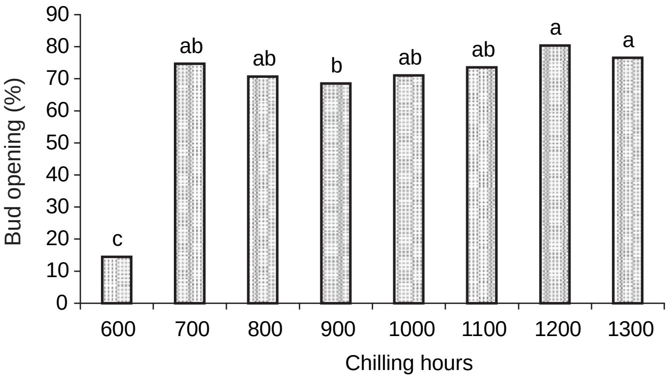

Fig.1. Influence of chilling hours on bud opening

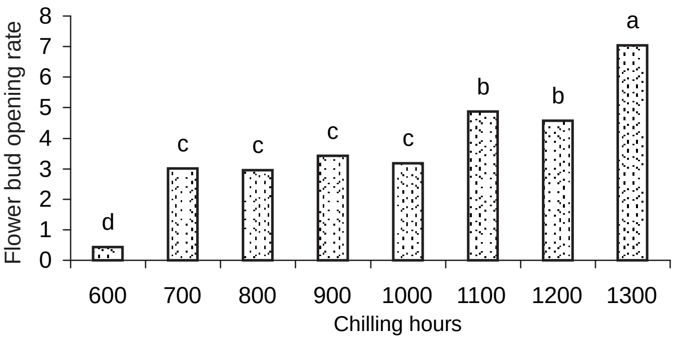

Fig. 2. Effect of chilling hours on the flower bud opening rate

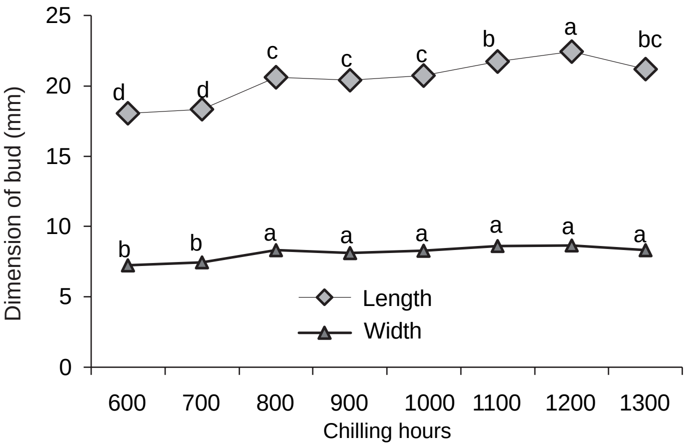

Fig. 3. Chilling effect on dimension of flowering buds of pistachio male genotypes in growth chamber.

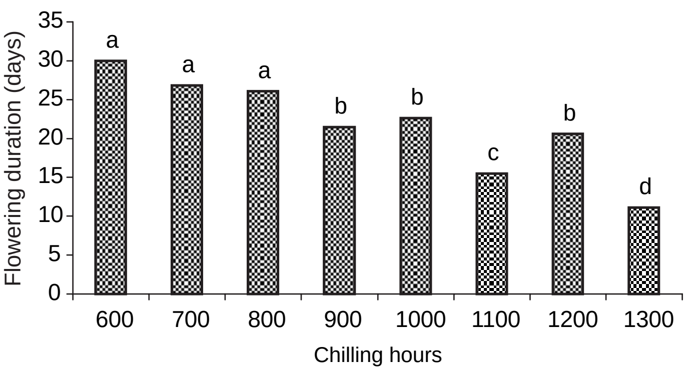

Fig. 4. Effect of chilling hours on the flowering duration

Fig. 5. Shoot bud showing anthesis under growth chamber conditions

at a given location. More research will be needed to develop regional planting models for adequate pollination. The processes that lead to dormancy and bud break within a plant consist of many interacting factors (temperature, light, physiological and chronological age of plant, apical dominance, provenance, hormonal balances, environmental conditions, drought, fertility, etc.). These factors are related to chilling and heat unit accumulation and must be studied to present a accurate picture of specific chilling requirements in individual cultivars. Finally, one of the most critical concerns yet to be addressed is a determination of the optimal temperatures for break dormancy.

The present study assumed ambient temperatures below 7°C and constant at 3°C as adequate to accomplish chilling requirement and maintaining the greenhouse environment above 22°C was ideal for flushing. Perhaps lower or higher temperatures could be considered more effective for breaking dormancy. Also, differences between constant versus fluctuating temperatures in a natural or simulated environment are the areas of additional study.

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