

Estimation of Chilling and Heat Requirements of Some Persian Walnut Cultivars and Genotypes

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Abstract. The objective of this work was to determine the chilling and heat requirements of Persian walnut cultivars and genotypes using excised twigs. The experiment was carried out from Nov. 2006 and 2007 to Mar. 2007 and 2008. One-year-old twigs were prepared from four cultivars and four domestic genotypes of *Juglans regia* L. After leaf fall, the twigs were taken and placed in plastic bags and kept at 4 ± 1 °C to stimulate 400 to 1500 chilling hours. After chilling, the excised twigs were transferred to the greenhouse with a natural photoperiod and a temperature from 18 to 27 °C. The evaluation of budbreak was made three times a week and the number of accumulated growing degree hours (°C) was determined until the buds reached the balloon or green tip stage. The chilling requirements were lowest (400 h) for catkins and highest (1000 h) for lateral buds. The Serr cultivar and 'Z₃₀' genotype had the lowest chilling requirements (650 and 650 h). 'Lara', 'Z₆₃', 'Z₅₃', 'Pedro', and 'Z₆₇' showed intermediate chilling requirements with values of 900, 900, 800, 750, and 750 h, respectively. Finally, 'Hartley' completed its dormancy after an accumulation of 1000 h, being the walnut cultivar with the highest chilling requirement in our study. As the final result, the cultivars and genotypes were classified into three groups based on their heat requirements: low requirement ('Z₃₀' and 'Serr'), medium requirement ('Z₅₃', 'Z₆₇', 'Lara', and 'Pedro'), and high requirement ('Hartley' and 'Z₆₃').

Adaptation is a concept related to how plants can survive and reproduce in a specific environment (Hill et al., 1998), and it is reflected in the synchronization between the development stages and climate (Dietrichson, 1964). Chilling requirement is an aspect necessary to ensure that the plants are adapted to the appropriate area. Dormancy and freezing tolerance are the main mechanisms developed against very cold conditions, although they could be independent (Irving and Lamphear, 1967). Freezing tolerance cannot be developed adequately without growth cessation (Fuchigami et al., 1971), which marks the onset of dormancy. The interest in understanding the mechanism of dormancy set and release is mainly based on the necessity of manipulation of the dormant period to avoid spring frost damage (Faust

et al., 1997). Dormancy has a significant economic impact on the maintenance and production of herbaceous and woody plants (Fennell, 1999).

The study of the phenological behavior of crops, as part of a well-characterized environment, is important both to obtain satisfactory production and to determine the most suitable agronomic techniques (Valentini et al., 2001). In this way, if a cultivar is established in an area where its chilling requirements are not satisfied adequately, the vegetative and reproductive growth of the cultivar will be affected negatively (Black, 1952; Coville, 1920; Ruck, 1975; Samish, 1954; Weldon, 1934). On the contrary, in the case of a cultivar with low chilling requirements growing in cold winter areas, the blooming happens too early because the chilling requirement is quickly satisfied (Scorza and Okie, 1990). Furthermore, studies concerning chilling and heat requirements are thus of special interest in these species, being very important for the choice of parents in breeding programs looking for late-flowering cultivars (Spiegel-Roy and Alston, 1979). When the chilling requirement is satisfied, blooming will start. Early blooming increases the likelihood of damage by late winter or early spring frosts. The time of full bloom depends on two factors: the chilling requirements and growing degree hours Celsius (GDH °C) required after endodormancy for reaching full bloom (Raseira, 1986).

The risks related to the lack of knowledge for the heat requirements of walnut cultivars is less than the chilling requirement, but the knowledge will provide us with more possibilities for the management of this crop so that cultivars with low chilling requirements but high heat requirement could be cultivated in relatively cold areas (Citadin et al., 2001). Methods for determining the heat requirements of blooming have been developed (Richardson et al., 1974). These methods essentially consist of establishing the heat accumulation, above a threshold, to which a

Table 1. Chilling requirement of Persian walnut cultivars and genotypes (hours below 7 °C) to reach 50% of lateral and terminal buds and catkins to the balloon or green tip stage in 2 successive years.^z

Cultivars and genotypes	Years	Lateral bud	Mean	Terminal bud	Mean	Catkin	Mean
Z ₃₀	2006	600 e ^y	650	500 d	550	400 d	400
	2007	700 d		600 e		400 d	
Z ₆₇	2006	800 c	750	800 b	700	600 b	600
	2007	700 d		600 e		600 b	
Z ₅₃	2006	800 c	800	800 b	750	700 a	650
	2007	800 c		700 d		600 b	
Z ₆₃	2006	900 b	900	700 c	750	700 a	650
	2007	900 b		800 c		600 b	
Serr	2006	700 d	650	700 c	650	400 d	450
	2007	600 e		600 e		500 c	
Hartley	2006	1,000 a	1,000	900 a	950	700 a	750
	2007	1,000 a		1,000 a		800 a	
Lara	2006	900 b	900	900 a	900	700 a	750
	2007	900 b		900 b		800 a	
Pedro	2006	800 c	750	500 d	600	500 c	500
	2007	700 d		700 d		500 c	
Difference between years	2006	a					
	2007						

^zEach value is the mean of three replications and eight cuttings per plot.

^yMeans in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.01$).

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Table 2. Range of chilling accumulation at which different Persian walnut cultivars and genotypes that exposed to various chilling temperatures from 400 to 1500 showed higher than 80% of the percentage of lateral, terminal, and catkins budbreak in 2 successive years.

Cultivars and genotypes	Years	Range of chilling accumulation
Z ₃₀	2006	900–1,500
	2007	800–1,500
Z ₆₇	2006	1,100–1,400
	2007	1,200–1,300
Z ₅₃	2006	1,000–1,500
	2007	900–1,500
Z ₆₃	2006	1,400–1,500
	2007	1,300–1,500
Serr	2006	1,100–1,400
	2007	1,000–1,500
Hartley	2006	1,400–1,500
	2007	1,300–1,500
Lara	2006	1,100–1,400
	2007	1,100–1,500
Pedro	2006	1,300–1,500
	2007	1,100–1,300

tree is exposed from breaking of dormancy until flowering date.

The chilling requirements for the termination of endodormancy (rest) were studied by many researchers (Chandler et al., 1937; Coville, 1920; Molisch, 1908). There are several methods for estimation of chilling requirements of plants such as using detached twigs (Citadin, 1999; Citadin et al., 1998; Herter et al., 2000), individual buds (Bianchi et al., 2000; Herter et al., 1992), and plants in containers (Camelatto et al., 2000; Citadin et al., 2001).

Each tree species has a specific chilling requirement that is related to the accumulated hours below a chilling temperature threshold or to cumulative chill unit, which are hours that are weighted for temperature effective-

ness for breaking dormancy (Erez et al., 1979; Weinberger, 1950). Although it has been mentioned that many walnut cultivars require ≈400 to 1500 h temperature below 7 °C to fulfill chilling requirements (Chandler et al., 1937), the available data for the chilling and heat requirements of walnut cultivars, especially for domestic walnut genotypes, are scarce. Therefore, the aim of this investigation, in 2 successive years, was focused on calculation of chilling and heat requirements to break bud dormancy of some cultivars and domestic walnut genotypes using 1-year-old twigs picked in the fall. Hence, the information obtained will provide better understanding of chill and heat requirements of walnut, which will be useful for best cultivation of the cultivars in the proper region.

Materials and Methods

Plant material collection. In Nov. 2006 and 2007, after leaf fall and before chilling accumulation, 1-year-old twigs were randomly collected from eight trees, 14 years old, of Serr, Pedro, Hartley, and Lara cultivars and 'Z₆₃', 'Z₅₃', 'Z₃₀', and 'Z₆₇' domestic promising genotypes that are being evaluated as potential cultivars in a breeding program from the experimental orchard of the Horticulture Department of the Seed and Plant Improvement Institute, Karaj, Iran.

Chilling treatment in the laboratory. The twigs were cut into 288 cuttings 20 cm long for each cultivar and genotypes (2304 twig totally) and transferred to the laboratory. Only one lateral and terminal bud and one catkin in their apical position were conserved, whereas the other buds were eliminated. After disinfection with Captan (Bayer Co.) 4000 ppm, a group of 20 cuttings were wrapped in moistened cheesecloth and

placed in a plastic bag to prevent dehydration during treatments and exposed to low temperature (4 ± 1 °C) to simulate 400 to 1500 h with 100-h intervals.

Budbreak in the greenhouse. After chilling, the excised twigs were placed with their basal ends in distilled water and forced to grow in the greenhouse with a natural photoperiod and varied temperature between 18 and 27 °C. The basal ends of the cuttings were cut three times a week (Citadin et al., 1998) and the water was replaced daily. Evaluation of budbreak was done three times a week for the number of buds reaching the balloon or green tip stage (Citadin et al., 2001). The accumulated GDH °C (Richardson et al., 1974) from the stage when the twigs were transferred to the greenhouse until 50% of buds reached the balloon or green tip stage was determined. One GDH °C is defined as 1 h at the temperature 1 °C above the base temperature of 4.5 °C. GDH °C was calculated from hourly temperature between 4.5 and 25 °C and all temperatures above 25 °C were considered equal to 25 °C.

Experimental design and statistical analysis. The experiment was conducted in a completely randomized design with three replications and eight cuttings per plot. Correlation coefficients between chilling and heating requirements of the cultivars and genotypes were determined using Pearson ranked-order correlation. Data were analyzed using SAS Software (SAS Institute, Inc., 2002). Means with significant differences were compared using Duncan's multiple range test at $P \leq 0.01$.

Results and Discussion

The calculated (or estimated) chilling requirements for walnut cultivars and genotypes in 2 consecutive years are shown in Table 1. The chilling requirements were considered to be satisfied when 50% of buds reached the balloon or green tip stage. The

Table 3. Heat requirement (GDH °C) of Persian walnut cultivars and genotypes to reach 50% of lateral and terminal buds and catkins to the balloon or green tip stage according to the Richardson et al. (1974) model in 2 successive years.²

Cultivars and genotypes	Years	Lateral bud	Mean	Terminal bud	Mean	Catkin	Mean
Z ₃₀	2006	10,656 ± 86 d ^b	10,512	11,355 ± 177 b	11,503	10,930 ± 87 a	8,567
	2007	10,368 ± 137 d		11,652 ± 216 de		10,795 ± 107 a	
Z ₆₇	2006	11,544 ± 233 cd	12,252	11,950 ± 95 b	12,743	9,715 ± 123 ab	8,680
	2007	12,960 ± 156 bc		13,536 ± 224 abc		10,460 ± 130 a	
Z ₅₃	2006	12,764 ± 224 bc	12,020	10,360 ± 114 b	11,064	7,930 ± 351 bc	8,232
	2007	11,276 ± 345 cd		11,768 ± 143 cde		8,534 ± 273 d	
Z ₆₃	2006	15,033 ± 89 a	14,636	15,267 ± 345 a	15,186	8,720 ± 176 bc	9,071
	2007	14,240 ± 128 ab		15,105 ± 278 a		9,423 ± 159 abc	
Serr	2006	10,850 ± 95 d	10,753	11,600 ± 380 b	10,934	7,075 ± 412 c	7,165
	2007	10,656 ± 122 d		10,268 ± 97 e		7,255 ± 235 d	
Hartley	2006	14,192 ± 431 ab	14,540	14,680 ± 447 a	14,322	9,525 ± 98 ab	9,647
	2007	14,888 ± 146 a		13,965 ± 198 ab		9,770 ± 185 ab	
Lara	2006	11,936 ± 167 cd	12,620	12,150 ± 265 b	12,957	7,480 ± 284 c	7,672
	2007	13,305 ± 247 ab		13,765 ± 364 ab		7,865 ± 341 cd	
Pedro	2006	13,065 ± 390 bc	13,142	14,396 ± 84 a	13,625	8,155 ± 105 bc	8,227
	2007	13,220 ± 107 ab		12,855 ± 231 bcd		8,300 b ± 216 cd	
Difference between years	2006	a					
	2007	b					

²Each value is the mean ± SE of three replications and eight cuttings per plot.

³Mean ± SE. Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.01$).

Table 4. Correlation coefficient of Persian walnut cultivars and genotypes between chilling treatments (hours below 7 °C) and heat requirements for reaching to the balloon or green tip stage in 2 successive years.

Cultivars and genotypes	Year	Correlation coefficient ²
Z ₃₀	2006	-0.64**
	2007	-0.86**
Z ₆₇	2006	-0.59**
	2007	-0.62**
Z ₅₃	2006	-0.87**
	2007	-0.95**
Z ₆₃	2006	-0.47**
	2007	-0.32**
Serr	2006	-0.65**
	2007	-0.63**
Hartley	2006	-0.45**
	2007	-0.31 NS
Lara	2006	-0.33**
	2007	-0.62**
Pedro	2006	-0.53**
	2007	-0.52**

²NS and ** = nonsignificant and significant at 1% statistical level, respectively ($P \leq 0.01$).

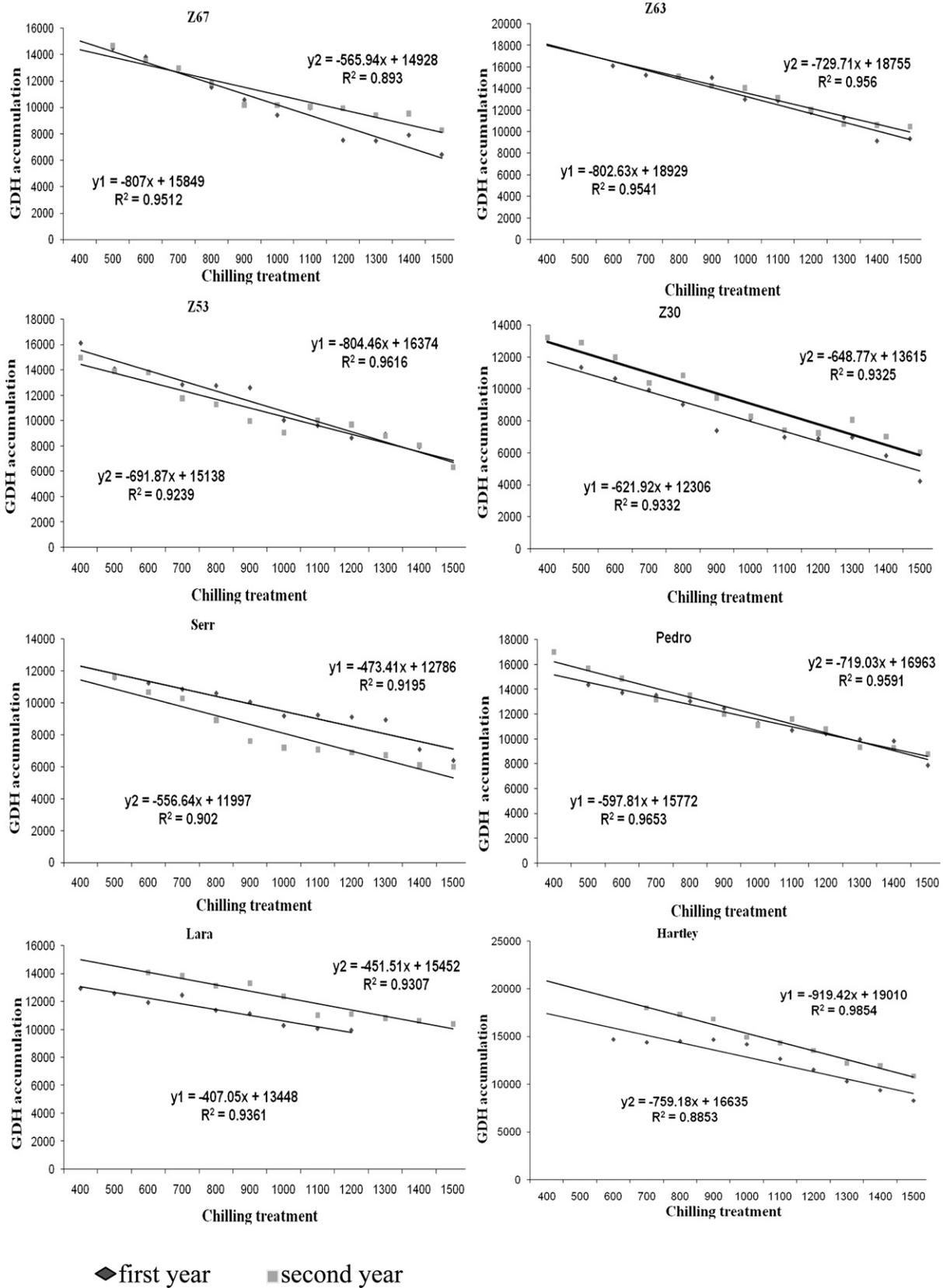


Fig. 1. The correlation between mean heat requirement and chilling treatments for all types of buds and catkin of Persian walnut cultivars and genotypes in 2 successive years along with the regression functions and coefficient of determination. Detached (or excised) twigs exposed to various chilling temperatures from 400 to 1500 and were forced to grow at 20 °C. The growing degree hours (GDH; °C) was calculated by the difference of 20 from 4.5 °C in 2 years.

chilling requirements of catkins were lower than lateral and terminal buds. The chilling requirements were lowest for catkins, except in 'Serr' and 'Lara', and highest for lateral

buds. This exception for these cultivars refers to the genetic characteristics. The chilling requirements ranged from 400 to 750 h for catkins, 550 to 950 h for terminal buds, and

650 to 1000 h for lateral buds. These results are in accordance with the hypothesis that terminal buds required less chilling temperatures than the lateral buds (Scalabrelli and

Couvillon, 1986). 'Serr' and 'Z₃₀' had the lowest chilling requirements (650 and 650 h, respectively). 'Z₆₇', 'Pedro', 'Z₅₃', 'Z₆₃', and 'Lara', with 750, 750, 800, 900, and 900 h, respectively, showed intermediate chilling requirements. Finally, 'Hartley' with 1000-h chilling requirement of terminal, lateral, and catkin bud dormancy, was the walnut cultivar with the highest chilling requirement in this study. The range of chilling requirements (for breaking buds and catkins dormancy) in the studied cultivars and genotypes varied from 650 to 1000 h. These results are in accordance with the suggestion of Chandler et al. (1937), who estimated the chilling requirement of walnut cultivars between 400 and 1500 h below 7 °C.

The higher chilling requirements in the cultivars indicated the risk of losses in yield or having low production growing these cultivars in the warmer regions (Erez, 2000). Based on the results, 'Serr' and 'Z₃₀' seem to be more suitable to cultivate in warmer winter climates. Alternatively, 'Hartley' might be the appropriate cultivar for cultivation in colder regions and especially with the risk of late spring frosts. Among the Iranian-evaluated genotypes, 'Z₆₃' seems to be more adapted to produce good yield in colder winter climates too. Although late-flowering cultivars had usually higher heat requirements, the flowering time is mainly determined by the chilling requirements. Therefore, when late-leaving walnut cultivars and genotypes are used in breeding programs for cultivation in cold climates with early spring or late winter frost, the progenies with high chilling requirements from parents like 'Hartley' and 'Z₆₃' could be crossed with them.

According to results, the cultivars and genotypes with similar chilling requirements showed different responses to chilling. For example, 'Z₃₀' and 'Serr' or 'Z₆₃' and 'Lara' had similar chilling requirements, but 'Z₃₀' and 'Lara' showed a higher percentage (80% or greater) of budbreak in a wide range of chilling accumulation treatments compared with 'Z₆₇' and 'Z₆₃', respectively (Table 2). On the other hand, these cultivars and genotypes needed more chilling to reach a higher percentage of budbreak compared with their chilling requirement.

The heat requirement for lateral and terminal buds and catkins was calculated by Richardson et al. (1974). The cultivars and genotypes were classified into three groups in accordance with their heat requirements: low ('Z₃₀' and 'Serr'), medium ('Z₅₃', 'Z₆₇', 'Lara', and 'Pedro'), and high heat requirement ('Hartley' and 'Z₆₃') (Table 3). Our results indicated that, except for 'Z₃₀', the heat requirements for catkins were lower than lateral and terminal buds and, except in 'Hartley' and 'Z₅₃', GDH °C accumulation for terminal buds was higher than lateral buds (Table 3). This observation was also observed in other experiments (Citadin et al., 2001; Gariglio et al., 2006). 'Z₆₃' and 'Lara' had similar chilling requirements, but their heat requirements were different; however, in

other cultivars and genotypes, there was a positive correlation between chilling and heat requirements. The results derived from this work as well as obtained by others (Brown, 1957; Spiegel-Roy and Alston, 1979; Swartz and Powell, 1981) indicated that this situation could be the result of residual effects of dormancy.

The time of bloom depends on the heat requirement of the cultivars (GDH °C accumulation) during endodormancy (Arnold, 1959; Citadin, 1999). Furthermore, among promising genotypes, 'Z₆₃' seems to be appropriate for cultivation in the regions with late winter or early spring frosts. Among the cultivars, 'Serr' with the lowest heat requirement is unsuitable for such a climate, but 'Hartley' was the best for the region with late winter or early spring frosts.

According to the earlier researches, dormancy and subsequently chilling requirements are affected by atmospheric and soil condition such as temperature (Welling, 2003), nutrition (Almond and Young, 1990), light, rainfall (Buchanan et al., 1977), and water stress and could change in different years. We also observed 100- to 200-h differences in the chilling and heat requirements in 2 successive years in the same cultivars, which indicates the environmental factors affected the chilling and heat requirements in 2 successive years (Tables 1 and 3).

There was a negative correlation between chilling treatments for breaking dormancy and heat requirements in walnut cultivars and genotypes (Table 4). These coefficients were negative and high except in 'Hartley', 'Lara', and 'Z₆₃' that imply the prolonged exposure to chilling temperatures, when dormancy is overcome, could reduce the heat requirement for budbreak. These results confirm that resting or partially chilled trees require much more heat accumulation in comparison with the trees in which their chilling was satisfied before they were able to bloom (Richardson et al., 1975; Samish, 1954; Swartz and Powell, 1981). In contrast, extra chilling given after completion of rest reduces the needed heat unit accumulation for blooming (Citadin et al., 2001; Couvillon and Erez, 1985; Couvillon and Hendershott, 1974; Felker and Robitaille, 1985; Scalabrelli and Couvillon, 1986). Monet and Bastard (1971) demonstrated that peach flower buds develop slowly under low temperature but are physiologically and biochemically active. Rapid growth resumes when temperatures rise. Thus, prolonged exposure to low temperature prolongs the slow growth phase, thereby reducing the rapid growth phase. Among the cultivars and genotypes, 'Serr' and 'Z₅₃' had the highest correlation coefficients, respectively. The results also indicated that except for 'Z₆₃', correlation coefficient of domestic walnut-promising genotypes were higher than cultivars. Meanwhile, heat requirements of promising genotypes were more affected by increasing chilling treatments.

The correlation between mean heat requirement and chilling treatments for all

types of buds and catkins in 2 successive years along with the regression functions for 2 years (y_1 and y_2) and coefficient of determination are shown in Figure 1 for each cultivar and genotype. Based on the linear function with excising chilling time, the synchronism of budbreak will increase and the heat requirements of cultivars and genotypes will modify by prolonged chilling. These results are in agreement with the findings of Couvillon and Erez (1985), who found that when cultivars with low chilling requirements cultivated together with cultivars with high chilling requirements in the same field condition, they must show lower heat requirements too. Because the excessive chill causes 90% of the heat requirement variations, in consequence, the cultivar shows no specific heat requirements. Several other studies have shown that the heat requirements of cultivars and genotypes can be modified by a continuous chilling accumulation after the breaking of dormancy (Couvillon and Hendershott, 1974; Spiegel-Roy and Alston, 1979; Swartz and Powell, 1981).

In conclusion, chilling treatment increases the percentage of budbreak and decreases the heat requirement in the cultivars and genotypes, but the responses of cultivars and genotypes and different buds to the chilling treatments are not similar. Figure 1 is a more appropriate model to study the heat requirement during increasing a chilling period in various cultivars and genotypes. Also, the estimated chilling and heat requirements (Tables 1 and 3) are good predictors for time of budbreak, but should be used in appropriate areas. The domestic Iranian genotypes are also suitable for a range of different climates from warm climates ('Z₃₀') to cold areas ('Z₆₃').

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