

Persian Walnut Phenology: Effect of Chilling and Heat Requirements on Budbreak and Flowering Date

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Abstract

Walnut production is limited by late-spring frost in many countries. The current research was carried out to evaluate chilling and heat requirement of catkin and terminal buds break on six selected superior walnut genotypes and cultivars ('C-25', '88-1', '88-2', 'Chandler', 'Jamal' and 'Damavand'). The Utah and accumulation growing degree hours (GDH) models were applied to determine the chilling and heat requirements under field and greenhouse conditions, respectively. 'Damavand' cultivar (650 CU) and 'C-25' genotype (650-800 CU) had the lowest chilling requirement for terminal bud break. 'Jamal' cultivar and 'C-25' genotypes had the lowest chilling requirement to break the dormancy of catkins (650-800 CU). 'Chandler' cultivar and '88-1' and '88-2' genotypes as late-leafing genotypes/cultivars had the highest chilling and heat requirements to break dormancy of terminal buds (800-1100 CU and 11832-12648 GDH) and catkin (800-950 CU and 11484-12180 GDH). In conclusion, late-leafing genotypes/cultivars had the higher heat requirement than early-leafing genotypes/cultivars. Based on the results, a linear and significant relation was observed between chilling requirement and heat accumulation. Therefore, heat accumulation of buds and catkins was reduced by increase in the amount of chilling requirement. Furthermore, the result revealed that heat accumulation is more important than chilling requirements to estimate walnut budbreak date. The GDH of catkins and terminal buds was decreased with increase in the average temperature during heat accumulation.

Keywords: Late-leafing cultivars, Utah model, *Juglans regia*, GDH model, Linear regression.

Introduction

Persian walnut (*Juglans regia* L.) as the world's widely grown nut crop is native to ancient Persia (Vahdati et al., 2014). At present, walnut is cultivated commercially in Asia, Europe, northern Africa, the USA and western parts of South America (Martinez et al., 2010). Walnut is a valuable crop that is largely consumed. Not

only dry fruits (nuts) but also other components of walnuts such as green walnuts, shells, kernels, barks, green walnut husks and leaves are being used in both cosmetic and pharmaceutical industries (Vahdati et al., 2018).

In many countries, production of walnut is limited by late spring frost. On the other hands, climate change in recent years has affected the rates of chilling and heat accumulation, which are vital for flowering

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and production (Guo et al., 2014; Ramírez and Kallarackal, 2015). Early leafing in walnut trees as results of climate change increases the chance of late-spring frost. Therefore, late-leafing is one of the main objectives in fruit breeding programs especially in walnut breeding programs (Akca and Ozogun, 2004; Aygün and Şan, 2005). It is important to determine the factors effected leafing and flowering date. In spite of the decades of research on bud break, the processes leading eventually to break bud dormancy are still not well understood. Chilling requirement and heat accumulation or growing degree hours celsius (GDH °C) are two important factors in leafing and flowering date (Raseira, 1986).

Study of the chilling requirement and heat accumulation of walnut cultivars are very important for the walnut commercial production. The knowledge of the cultivars chilling requirement and heat accumulation plays a fundamental role in selecting the most optimum cultivar for the most suitable areas (Fennell, 1999). The phenomenon is a particular vital in the temperate zone fruit production area, because these species can only be produced where their winter chilling requirements are fulfilled (Chandler, 1942; Lesley, 1944). Previous studies showed that varied rates of chilling and heat accumulation could influence the fulfillment of chilling and heat requirements leading to advance or delay spring phenological events (Campoy et al., 2011; Luedeling et al., 2013).

Several models have been suggested to calculate winter chill, with the development of new models mostly driven by the failure of existing models in a certain growing region (Luedeling et al., 2012). In the Chilling Hours Model, temperatures between 0 and 7.2 °C are assumed to have a chilling effect, with each hour at temperatures between these thresholds contributing one Chilling Hour. Chilling Hours are then summed throughout the dormant season (Bennett, 1949). This model was used by slamarz et al. (2009) for

investigating walnut chilling requirement. The next significant advance in understanding the temperature response of trees during the chilling phase was the discovery that warm temperatures had a negative effect on chill accumulation (Overcash and Campbell, 1955). From this insight arose the Utah Model, which is characterized by differential weighting of temperature ranges, including negative weights for temperatures above 15.9 °C (Richardson et al., 1974). The Dynamic Model (Fishman et al., 1987a; Fishman et al., 1987b) was developed to reflect the results of controlled temperature experiments, indicating that the sequence of cool and warm temperatures was important for describing chilling accumulation (Erez and Couvillon, 1987; Erez et al., 1979a; Erez et al., 1979b) and that moderate temperatures had a chill-enhancing effect (Erez and Couvillon, 1987). Luedeling et al., (2009) used four chilling models (Chilling Hours, Utah Model, Positive Utah Model and Dynamic Model) to explain walnut phenology in California. To date, the Dynamic Model has not been widely adopted, and most fruit and nut growers in subtropical climates still base their choice of cultivars and cultural practices either on the Utah Model or on one of the two versions of the Chilling Hour concept (Luedeling et al., 2012).

Considering the importance of studies on chilling and heat requirements for their commercial and sustainable production of fruit trees especially for walnut, this study was carried out based on the following hypotheses: I) a specific amount of chill unit followed by heat accumulation prior to budbreak have a significant correlation with budbreak date; II) both chilling and heat accumulations determine budbreak date and budbreak is not determined solely with one of them or based on a calendar date; III) different temperatures have different effects on GDH. IV) the amount of heat accumulation can be predicted based on chilling requirement.

Material and Method

Plant materials

To evaluate chilling requirement and heat accumulation of Persian walnut to break bud dormancy, this study was conducted on 8-years old walnut superior genotypes

and cultivars grafted on seedling rootstock in Horticultural Science Research Institute (HSRI) research orchard (Karaj, Iran) in 2009 (Table 1). These superior genotypes were selected from a large walnut population in the Central Iran (Alborz, Qazvin and Tehran provinces) in 2009.

Table 1. Characteristics of the studied walnut cultivars/genotypes in Karaj condition during 2017

Traits	Cultivars			Genotypes		
	'Chandler'	'Jamal'	'Damavand'	'88-1'	'88-2'	'C-25'
Budbreak date	2 Apr.	24 Mar.	16 Mar.	1 Apr.	2 Apr.	29 Mar.
First pollen shedding date	15 Apr.	3 Apr.	11 Apr.	19 Apr.	19 Apr.	15 Apr.
First female receptivity date	20 Apr.	12 Apr.	6 Apr.	13 Apr.	14 Apr.	13 Apr.
Harvest date	4 Oct.	16 Sep.	10 Aug.	5 Sep.	24 Aug.	3 Sep.
Tree vigor	Low	Medium	Medium	Low	Low	Low
Yield	Medium-high	Medium	Medium	Medium-high	Medium-high	Medium
Bearing habit	Lateral	Terminal	Terminal	Lateral	Lateral	Lateral
Nut weight (g)	9.07	9.87	12.06	9.40	10.11	11.04
Kemel weight (g)	3.83	4.54	5.93	5.51	6.40	5.89
Kemel percentage	45.87	46.52	49.07	59.04	63.33	53.33
Kemel color	Light	Light	Light amber	Light	Light	Light amber

Assessment of chilling requirement

To assess the chilling requirement of the studied walnut cultivars/genotypes, six chilling requirement treatments (650, 800, 950, 1100, 1250 and 1400 Utah Chilling Units (CU)) and three replications including 18 twigs (20-25 cm) were considered. For this purpose, after supplying the required winter chill under field condition, one-year-old twigs with a terminal bud and/or catkin were cut and transferred to the greenhouse.

The Utah model was used to estimate chilling requirement (Richardson et al. 1974). In Utah model, temperatures less than 1.4°C do not have physiological effects on chilling requirement. In contrast, weight of temperatures between 1.4 to 2.4 and 9.1 to 12.4 °C is 0.5. Also, weight of 1, -0.5 and -1 consider for temperatures between 2.4 to 9.1, 15.9 to 18 and more than 18°C, respectively (Richardson et al. 1974; Luedeling et al. 2009).

Meteorological data was used to determine chilling requirement based on Utah model. After different levels of chilling treatments under field conditions, the labeled twigs were cut and placed with

their basal ends in distilled water and forced to grow in the greenhouse with a natural photoperiod and varied temperature between 16 and 23 °C. The basal ends of the cuttings were cut three times a week and the water was replaced daily (Aslamarz et al., 2009). The budbreak time was considered when 50% of buds in twigs in each replication reached the balloon or green tip stages. Data was recorded three times a week for the number of buds reaching the balloon or green tip stage (Citadin et al., 2001). Furthermore, the chilling requirement (CU) and the heat accumulation (buds and catkins) of each genotype/cultivars were evaluated in the greenhouse condition.

Assessment of heat accumulation

Heat accumulation was estimated by Growing Degree Hours (GDH°) model (Richardson et al., 1974). GDH° consider as valid tools to measure heat accumulation (Roltsch, et al., 1999; Ruml, et al., 2010; Covert, 2011) and is defined as one-degree upper threshold temperature (T_{BASE}) for one hour after completing bud breaks chilling requirements. When the T_{BASE} is

below the hourly minimum temperature (T_{HOURLY}), GDH° accumulation is derived from T_{BASE} subtracted from T_{HOURLY} ($\text{GDH} = T_{\text{HOURLY}} - T_{\text{BASE}}$).

Assessment of chilling and heat requirements based on meteorological data

In addition to studying chilling and heat requirements under greenhouse condition, chilling requirement and heat accumulation of the studied walnut cultivars/genotypes were evaluated based on meteorological data during 2013-2017. Phenological traits (budbreak date, first pollen shedding date and first female receptivity date) of the studied cultivars/genotypes were evaluated based on IPGRI descriptor during 2013-2017 (IPGRI, 1994). Moreover, chilling units (CU) and growing degree hours (GDH°) were gathered from the IRIMO (Iran Meteorological Organization) website (<http://www.irimo.ir>) for the nearest synoptic station to the HSRI research orchard in Karaj, Iran.

Effect of different temperatures on heat accumulation

To evaluate the effect of different temperatures on heat accumulation, 24 twigs of each genotypes/cultivars (three replications and 8 twigs in each replication) were placed at 10, 13 and 16 °C in growth chamber (16 hours of light:8 hours of dark). The selected twigs were supplied with 1400 CU. Data collection and twigs maintenance were conducted according to Aslamarez et al. (2009).

Statistical analysis

The experiment was conducted as a completely randomized design with three replications and eight twigs per plot. Analysis of variance (ANOVA) was performed using the SAS 9.2 software (SAS Institute, Inc., 2002). Comparison of mean values was performed using the LSD test. Before correlation analysis, the data was normalized by a specific reference sample. The correlation between heat requirement mean and chilling treatments

for all types of buds and catkin of Persian walnut cultivars and genotypes was assessed through regression functions and coefficient of determination.

Results

Chilling requirements and Heat accumulation

The chilling requirements to break terminal buds of six walnut cultivars/genotypes were presented in Figure 1. Generally, budbreak percentage was increased with increase in Utah chill unit. The chilling requirement of terminal buds was significantly varied from 650 to 1100 CU. The lowest chilling requirements were observed in 'Damavand' (650 CU) and 'C-25' (650-800 CU), and the highest was obtained in 'Chandler' (950-1100 CU) cultivar and '88-1' (800-950 CU) and '88-2' (800-950) genotypes (Fig. 1). These results were confirmed in chilling requirement assessment using 5-years meteorological data (Table 3).

Based on the results, a significant difference was observed between six walnut cultivars/genotypes in catkin chilling requirement (Fig 2). The chilling requirement of catkin ranged from 650 CU (in 'C-25' genotype) to 1100 h (in '88-1' genotype). Genotype 'C-25' showed the least amount of catkins and terminal bud chilling requirement. Although, 'Damavand' had the least chilling requirement (650 CU) to break the terminal bud dormancy; its catkin require 800-950 chill Utah unit for pollen shedding. In the other words, 'Damavand' cultivar requires more chill unit than Utah to expand catkin versus terminal bud. Persian walnut are considered as heterodichogamy species; so that, chilling requirements of terminal buds in 'Chandler' and 'Jamal' cultivars (950-1100 and 800-950 CU, respectively) were higher than their catkins (800-950 and 650-800 CU, respectively) confirming that these cultivars are protandrous (Hassani et al., 2014). However, 'Damavand' which is a protogynous (Hassani et al., 2014) cultivar had higher chilling requirement for catkin

(800-950 CU) than terminal bud (650 CU) (Table 2).

The results showed that heat accumulation (GDH) of terminal buds and catkin ranged 10092-12648 h and 10440-12180 h, respectively. A significant difference was observed between the studied walnut cultivars/genotypes in term of heat accumulation of catkin and terminal buds. Although, the lowest chilling requirement of

terminal buds and heat accumulation of terminal buds and of catkins was calculated in ‘Damavand’ cultivar, it had a high amount of chilling requirement for expanding catkins among the other studied cultivars/genotypes (Table 2). The highest chilling requirement and heat accumulation of terminal buds (GDH) were calculated for ‘Chandler’ cultivar and ‘C-25’ genotype, respectively (Table 2).

Table 2. Chilling requirement (CU) and heat accumulation (GDH) of terminal bud and catkins in the studied walnut cultivars/genotypes obtained from 5-years meteorological data

Cultivars/ Genotypes	Chilling requirement (CU)		Heat accumulation (GDH)	
	Terminal bud	Catkin	Terminal bud	Catkin
‘88-1’	800-950	800-950	12180	11484
‘Chandler’	950-1100	800-950	11832	12180
‘88-2’	800-950	800-950	12648	11832
‘Jamal’	800-950	650-800	11532	11484
‘Damavand’	650	800-950	10092	10440
‘C-25’	650-800	650-800	12276	12180

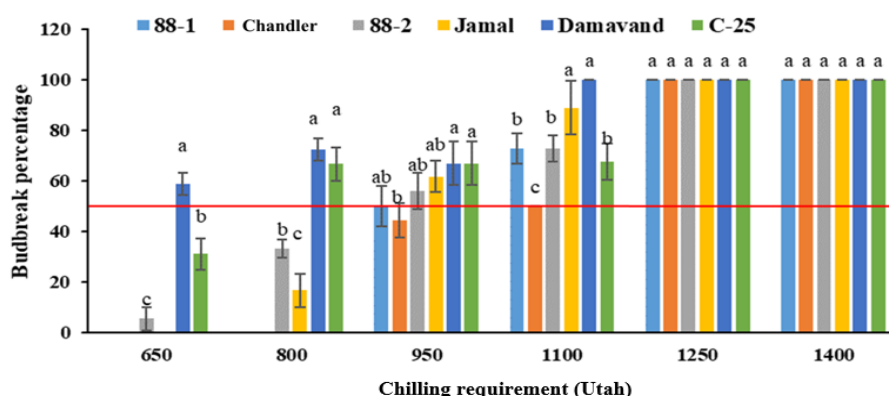


Fig. 1. Effect of different Utah chill unit on budbreak date of the studied walnut cultivars/genotypes (Red line showed that 50% of the twigs reached the balloon or green tip stage). The bars show means ± SE.

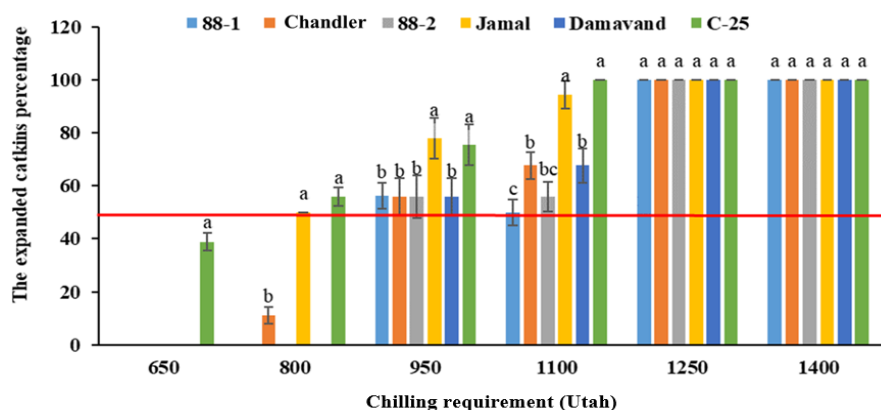


Fig. 2. Effect of different Utah chill unit on pollen shedding date of the studied walnut cultivars/genotypes (Red line showed that 50% of the twigs reached the balloon or green tip stage). The bars show means ± SE.

Phenological traits

Phenological characteristics of the studied walnut genotypes/cultivars are presented in Table 3. Based on the obtained results, '88-1', '88-2' and 'Chandler' were late leafing and late flowering genotypes/cultivars (budbreak on 31Mar. to 13Apr. 2013-2017) and seemed to be appropriate for cultivation in the regions with late winter or early spring frosts. While, 'Jamal' and 'Damavand' were early leafing and early flowering genotypes (budbreak on 16 to 28 Mar. 2013-2017) that are not suitable for these areas. The results showed that the heat requirements were variable in

different years per each genotypes/cultivars. According to the results, late-leafing genotypes/cultivars required more heat accumulation. In contrast, 'Jamal' and 'Damavand' as early leafing cultivars required less heat accumulation to bud break and female and male flowering. In general, a strong and positive correlation was observed between heat accumulation and bud break, male and female flowering time. In general, it can be concluded that the heat accumulation plays an important role in determination of budbreak and flowering date (Table 3).

Table 3. Phenological characteristics and heat requirement (GDH) in terminal bud break and flowering stage of the studied walnut genotypes/cultivars during 2013-2017

Genotypes/ Cultivars	Years	GDH on terminal budbreak	GDH on Female Flowering	GDH on pollen shedding	Tim of Bud break (day of years)	Time of female bloom (day of years)	Time of Pollen shedding (day of years)
'88-1'	2013	4864.8	10411.2	10831.2	93	108	110
	2014	4329.6	7221.6	10750.8	101	111	116
	2015	4194	7162.8	7844.4	96	105	112
	2016	4889.6	10660	10014.8	94	107	115
	2017	3883.2	7732.8	5956.2	96	107	114
'Chandler'	2013	5174.4	11217.6	9652.8	94	111	103
	2014	5092.8	10201.2	9477.6	104	119	112
	2015	4620	10136.4	8066.4	97	114	104
	2016	5112	11620.8	9687.6	96	119	110
	2017	4082.4	10188	8566.8	97	116	109
'88-2'	2013	4824.8	9064.8	10296	122	104	107
	2014	4802.4	7755.6	9700.8	103	112	116
	2015	4620	7750.8	9333.6	97	106	113
	2016	5112	9509.2	11720.4	96	109	115
	2017	4333.2	7744	9669.2	98	108	114
'Jamal'	2013	5056.8	9746.4	8872.8	88	104	109
	2014	4258.8	9541.2	7267.2	88	111	101
	2015	2624.6	8497.2	6906	89	106	99
	2016	4876.6	9824.8	8637.6	79	102	97
	2017	3600	8246.4	6250	87	108	97
'Damavand'	2013	5820	7538.4	9924	86	93	104
	2014	4616.4	7267.2	8912.4	87	101	109
	2015	4764	6906	8373.6	85	98	106
	2016	6073.6	8406	9091.2	76	95	108
	2017	3541.6	6060	7978.8	81	101	106
'C-25'	2013	6582.4	8947.2	10428	90	100	105
	2014	6313.2	8577.6	10272	99	108	113
	2015	5041.2	9020.4	9334.8	92	105	110
	2016	6804	10042.8	10422	87	104	113
	2017	4630.8	8566.8	9696	95	109	111

The relationship between chilling and heat requirement

The chilling requirement and heat accumulation interact to control budbreak and flowering time (Richardson et al., 1974; Couvillon and Erez, 1985; Gibson and Reighard, 2002; Okie and Blackburn, 2011). Our results showed that the chilling requirement of terminal buds and catkins decreased with increase in GDH. Generally, a negative correlation was observed between chilling requirement and heat accumulation (Fig. 3 and Fig. 4). The logical relationship between heat

accumulation and chilling requirements was achieved with a linear regression (Fig 3). Heat accumulation of buds and catkins in all studied cultivars (except Chandler) was reduced by increase in the amount of chilling requirement. In this study, we found that late leafing walnut trees (Chandler cultivar and ‘88-1’ and ‘88-2’ genotypes) required a larger number of growing degree days (high heat accumulation) for bud break than early leafing walnut trees (‘C-25’ genotype, ‘Damavand’ and ‘Jamal’ cultivars).

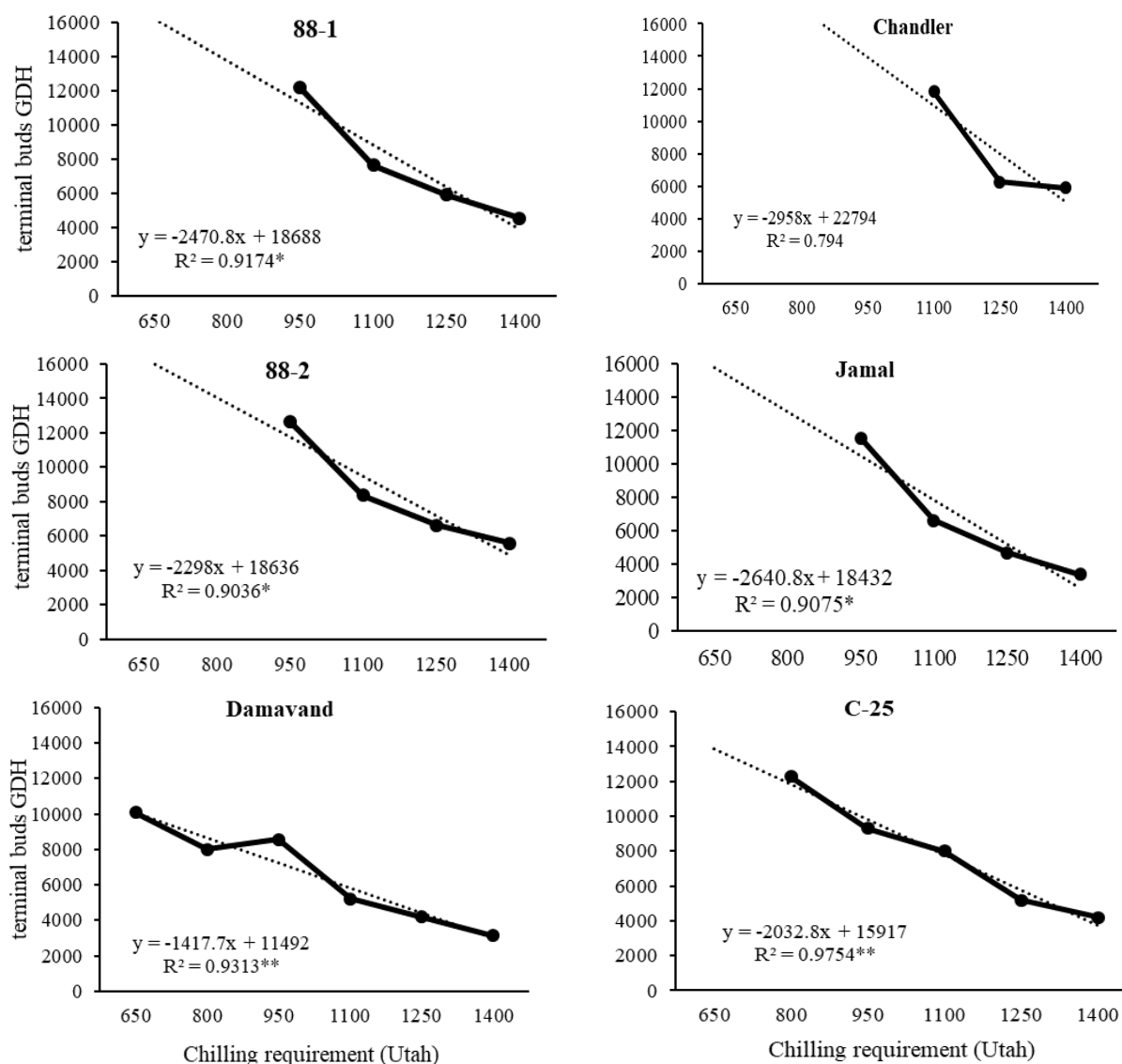


Fig. 3. Linear regression between chilling requirement (y) and heat accumulation (x) of terminal buds in the studied walnut genotypes/cultivars (* and ** significant difference at 5 and 1 % levels, respectively).

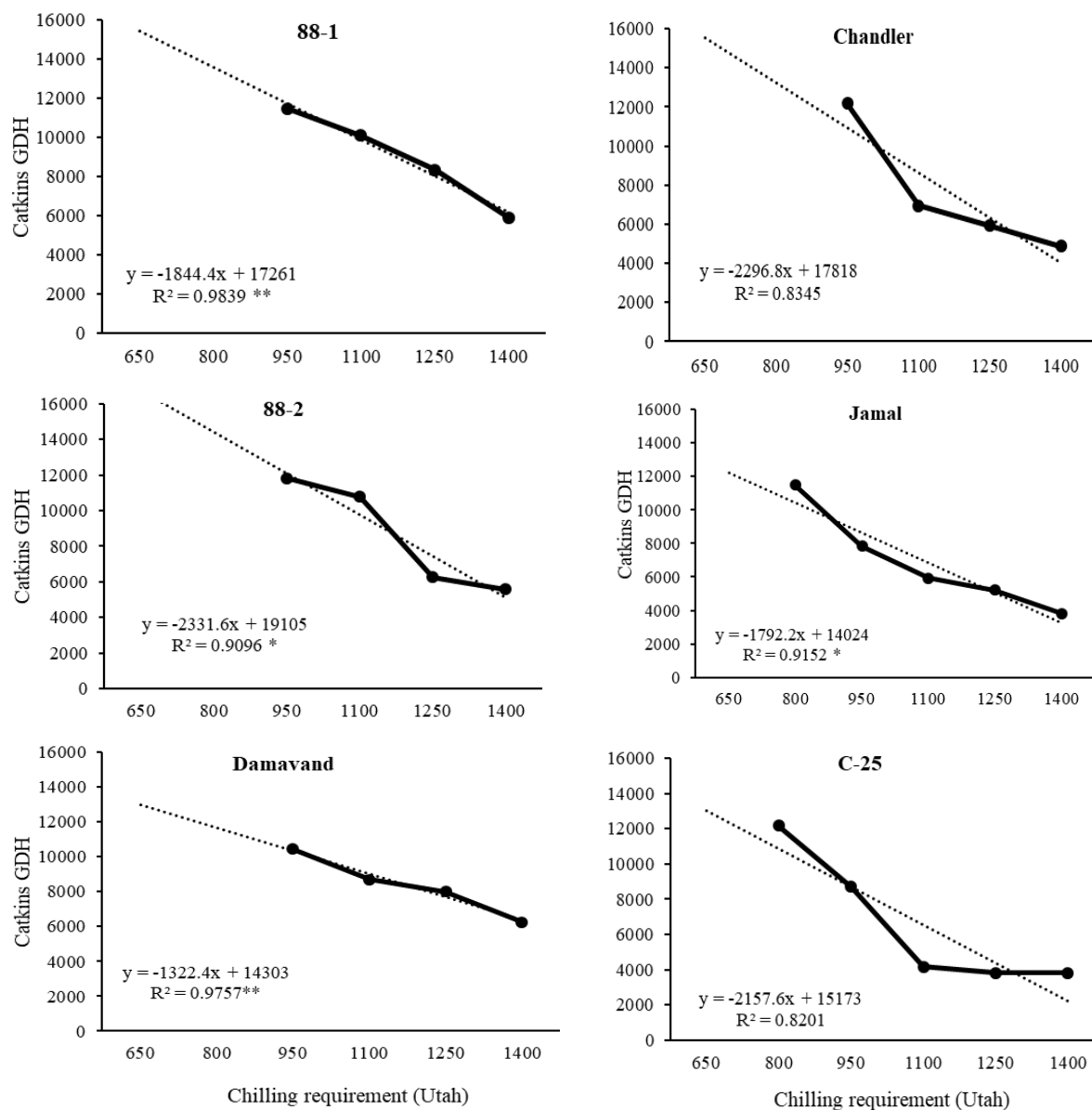


Fig. 4. Linear regression between chilling requirement (y) and heat accumulation (x) of catkins in the studied walnut genotypes/cultivars (* and ** significant difference at 5 and 1 % levels, respectively).

Effect of different temperatures on GDH, catkin and terminal bud break

A separate experiment was conducted on the studied walnut cultivars/genotype to determine the effect of the average temperature on GDH. The results showed that there is not significant difference between Chandler cultivar, '88-1' and '88-2' genotypes in term of terminal buds GDH. Except '88-1' and 'Chandler', terminal buds GDH decreased with increase in the average temperature during

heat accumulation (Fig. 5). In addition to terminal bud, catkin GDH of all studied walnut genotypes/cultivars was decreased with increase in the average temperature during heat accumulation (Fig. 6). With increase in the average temperature from 10 °C to 16 °C, catkin and terminal buds break were occurred earlier. For instance, catkin and terminal buds break in 16 °C temperature were occurred earlier than 10 °C temperature (Fig. 5 and Fig. 6).

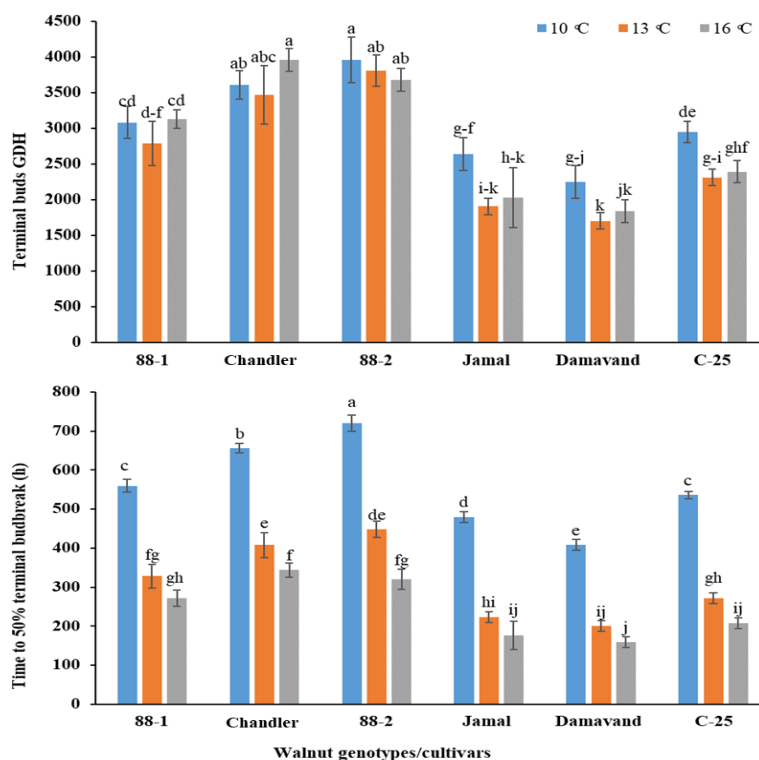


Fig. 5. Effect of different temperatures on terminal bud GDH and budbreak time of the studied walnut genotypes/cultivars. The bars show means \pm SE..

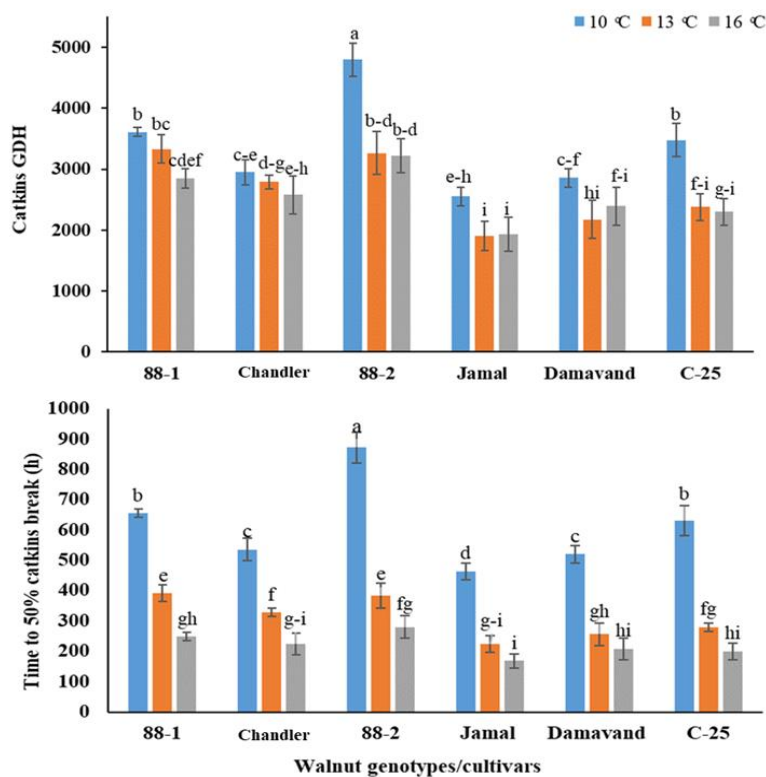


Fig. 6. Effect of different temperature on catkin GDH and male flowering time of the studied walnut genotypes/cultivars. The bars show means \pm SE.

Discussion

Previous studies have shown that the chilling requirement of walnut cultivars varied between 400 to 1500 chill hours in temperatures below 7°C (Aslamarz et al., 2009; Chandler et al., 1937). Our results also confirmed that the chilling requirement of the studied walnut genotypes/cultivars varied from 650 to 1100 hours to break catkin and terminal bud dormancy.

Similar to the results reported by Aslamarz et al., (2009), our results showed that late-leafing walnut genotypes/cultivars require higher chilling (CU) and heat hours than early-leafing genotypes. Late-leafing cultivars are suitable to cultivate in mountain areas where the late spring frosts are frequent (Akca and Ozongun, 2004; Aslamarz et al., 2009). Gianfagna and Mehlenbacher (1985) reported that GDH is more important than the chilling requirement to determine leafing and flowering time. Our results also revealed that heat accumulation plays an important role in determination of bud break and flowering date.

The interaction between chilling and heat requirement determines flowering time in different walnut cultivars. Increase in Chilling Unit reduces heat requirement for floral budbreak in walnut (Okie and Blackburn, 2011). Therefore, depending on the chilling requirement provided in different years, the need for heat accumulation will be different. Considering the high cold season in the studied area, it seems that the heat accumulation in this area is more effective factor than the chilling requirement of different genotypes/cultivars. Therefore, the difference in the time of bud break and flowering in the studied genotypes/cultivars may be due to the difference in heat requirements (GDH). Generally, the leafing and flowering time depends on the heat requirement of the cultivars, GDH accumulation, during endodormancy (Arnold, 1959). Our results showed that after 1400 Utah chill unit, increase in temperature from 10 to 16 °C, increased the speed of the

catkins development more than the expected. In the other words, the study of phenological data showed that Utah and GDH models were suitable for determining habit and approximate flowering time of walnut cultivars (Luedeling et al., 2009; aslamars et al, 2009; Luedeling et al., 2011). However, using these models, it is possible to predict the estimated budbreak time. Some factors affecting the chilling requirement are not considered by using temperature data (Synoptic station). According to the earlier researchers, dormancy and subsequently chilling requirements are affected by atmospheric and soil condition such as nutrition (Almond and Young, 1990), temperature (Welling, 2003), light, rainfall (Buchanan et al., 1977), and water stress (Benzioni et al., 1992) and could change in different years. Furthermore, the mentioned factors can affect the GDH. An accurate model for predicting the exact time of flowering will be achieved by examining the chilling and heat requirements of each cultivar in different regions and years.

In conclusion, chilling requirement of catkin in protogynous walnut cultivars was higher than terminal buds. Late-leafing genotypes/cultivars (such as '88-1', '88-2', 'Chandler') had higher chilling and heat requirement than early-leafing genotypes/cultivars (such as 'Damavand' and 'Jamal'). A linear and negative relation was observed between chilling requirement and heat accumulation. Heat accumulation plays an important role in determination of bud break and flowering date and with increase in winter chill unit, heat accumulation of walnut genotypes/cultivars was decreased. Our results also showed that temperature during heat accumulation had significant effect on budbreak and flowering date. In the other words, leafing and flowering date were occurred earlier with increase in the average temperature during heat accumulation period.

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