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The influence of vernalization time and day length on flower induction of radish (*Raphanus sativus* L.) under controlled and field conditions

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Abstract: This research was carried out to identify an alternative model plant for the study of vernalization among the cultivars that are commonly grown in Turkey and the influence of vernalization and day length on the flowering of radishes (*Raphanus sativus* L.) under controlled and field conditions throughout 2003, 2004, and 2005. In this study, 6 radish cultivars (*Raphanus sativus* L. cvs. 'Siyah', 'Beyaz', 'Antep', 'İri Kırmızı', 'Red Cherry F₁', and 'Cherry Belle') were used in the laboratory and field experiments as plant material. Under the controlled conditions, 2 day length periods (8 and 16 h light) and 5 vernalization times (0, 5, 10, 15, and 20 days at 5 °C) were studied as the main factors. Under the field conditions, the day length and the changes in vernalization times were provided by different sowing dates. It was determined that flowering did not occur when the vernalization time was less than 15 days during both the long days (LD) and short days (SD) for Siyah. All the same, it was determined that except for Siyah, other cultivars could not flower without being vernalized during SD. Besides this, it was determined that under the field conditions, different sowing dates had an impact on flowering rates. Contrastingly, in early sowings of Siyah, all cultivars showed flowering of up to 88.72%. The study reveals that Antep, Beyaz, İri Kırmızı, Red Cherry F₁, and Cherry Belle have a facultative vernalization response; cold exposure is not required for flowering, but flowering can occur more rapidly after cold treatment. Siyah, in contrast, has an obligate requirement for cold treatment and thus cannot flower without prior cold exposure.

Key words: Flower induction, day length, *Raphanus sativus* L., vernalization

Vernalizasyon süresi ve gün uzunluğunun tarla ve kontrollü şartlarda turp (*Raphanus sativus* L.)'un çiçeklenmesi üzerine etkisi

Özet: Bu araştırma, vernalizasyon ve gün uzunluğunun turp (*Raphanus sativus* L.)'un çiçeklenmesi üzerine etkisini ve ülkemizde yaygın yetiştiriciliği yapılan çeşitler arasından vernalizasyon çalışmaları için alternatif model bir çeşit belirlenmek amacıyla 2003, 2004 ve 2005 yıllarında tarla ve kontrollü şartlarda yürütülmüştür. Araştırmada, laboratuvar ve tarla denemelerinde, bitkisel materyal olarak 6 adet turp çeşidi (*Raphanus sativus* L. cvs. 'Siyah', 'Beyaz', 'Antep', 'İri Kırmızı', 'Red Cherry F₁' ve 'Cherry Belle') kullanılmıştır. Kontrollü şartlarda, iki farklı gün uzunluğu (8 ve 16 saat aydınlık) ve beş farklı vernalizasyon süresi (5 °C'de 0, 5, 10, 15 ve 20 gün) temel faktör olarak kullanılmıştır. Tarla şartlarında ise, gün uzunluğu ve vernalizasyon sürelerindeki değişimler farklı ekim zamanları ile sağlanmıştır. Siyah

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turpta hem uzun günde hem de kısa günde vernalizasyon süresi 15 günden az olduğunda çiçeklenmenin olmadığı tespit edilmiştir. Bununla birlikte, Siyah turp dışındaki çeşitlerin uzun günde vernalize olmadan da çiçeklenebildikleri; kısa günde ise çeşitlerin tamamının vernalize olmaksızın çiçeklenmedikleri belirlenmiştir. Bunun yanında tarla koşullarında, farklı ekim zamanlarının çiçeklenme oranına etki yaptığı, erken dönemde yapılan ekimlerde Siyah turp hariç kullanılan çeşitlerde % 88.72'ye varan çiçeklenme belirlenmiştir. Araştırma sonunda, kullanılan çeşitlerden Siyah turpun çiçeklenmesi için gün uzunluğunun etkili olmadığı, vernalizasyon ihtiyacının zorunlu olduğu; Antep, Beyaz, İri Kırmızı, Red Cherry F₁ ve Cherry Belle çeşitlerinin ise uzun güne bağlı olarak çiçeklendiği, vernalizasyonun çiçeklenmeyi hızlandırdığı ve vernalizasyon ihtiyaçlarının fakültatif olduğu tespit edilmiştir.

Anahtar sözcükler: Çiçeklenme, gün uzunluğu, *Raphanus sativus* L., vernalizasyon

Introduction

The promotion of flowering in response to prolonged exposure to cold temperatures is a useful adaptation for plant species that flower in the spring. This promotion is known as vernalization (Michaels and Amasino 2000). Optimum vernalization temperatures, with only few exceptions, range from 5-8 °C; lower temperatures and freezing periods showed weaker effects (Wiebe 1990). The time required for complete vernalization varies with species (Engelen-Eigles and Erwin 1997). For example, *Allium cepa* L. and *Daucus carota* L. require 12 weeks, and *Brassica oleracea capitata* requires approximately 20 weeks (Wiebe 1990). Complete vernalization of these species takes long periods of time. In contrast, it was determined that the vernalization period of radish, depending on the cultivar, lasted at least 10 days. The maximum effect was obtained after a vernalization period of 20-30 days. The sensibility of the vernalization between the beginning of the germination and the plant becoming 21 days old is constant (Wiebe and Alpers 1983). These simple properties associated with radish make it an ideal candidate for vernalization studies.

The length of vernalization treatment required for complete vernalization is related to whether a species has an obligate or facultative vernalization requirement (Engelen-Eigles and Erwin 1997). Many crops of the biennial plants stay vegetative without cold exposure; the cold requirement is therefore called obligatory. On the other hand, for some species, vernalization has only a furthering effect on flower induction. The cold requirement for those is called facultative (Wiebe 1990; Michaels and Amasino 2000).

Extensive breeding studies have rapidly produced many cultivars in the last few decades. Moreover, it is known that Turkey has 2 (Near East and Mediterranean) of 8 important meeting places of gene centers. Besides these 2 gene centers, Turkey also has a genetic diversity center of many cultivated forms of annual and biennial herbaceous plants (Ercisli 2004). Along with China and Asia, Anatolia is also the center of origin of the radish (Günay 2005). Therefore, the goal of this research was to identify the influence of vernalization and day length on the flowering of radish (*Raphanus sativus* L.) cultivars that are grown commonly in Turkey. This will characterize a model plant among these cultivars for future vernalization studies. Hence, an alternative model plant for vernalization studies can be found.

Materials and methods

This study was conducted both under field conditions and in growth chambers in Erzurum in 2003, 2004, and 2005.

Erzurum is located at 40°57'N and 39°10'N latitude, and 40°15'E and 42°35'E longitude, 1850 m above sea level. It had an average air temperature of 14.7 °C in 2003, 13.9 °C in 2004, and 14.3 °C in 2005 (May-October). Detailed information about temperature is given in Figure 1. The soil of the experimental area in 2003-2005 had a loamy texture of 30.42% sand, 43.75% silt, and 25.83% clay. Some of the soil chemical characteristics were as follows: soil pH, 7.81, 7.54, and 7.29; organic matter, 1.71, 1.76, and 1.80%; available P₂O₅, 35.7, 36.1, and 36.9 kg ha⁻¹; and exchangeable K, 2.70, 2.70, and 2.78 meq 100 g⁻¹ soil, in 2003, 2004, and 2005, respectively.

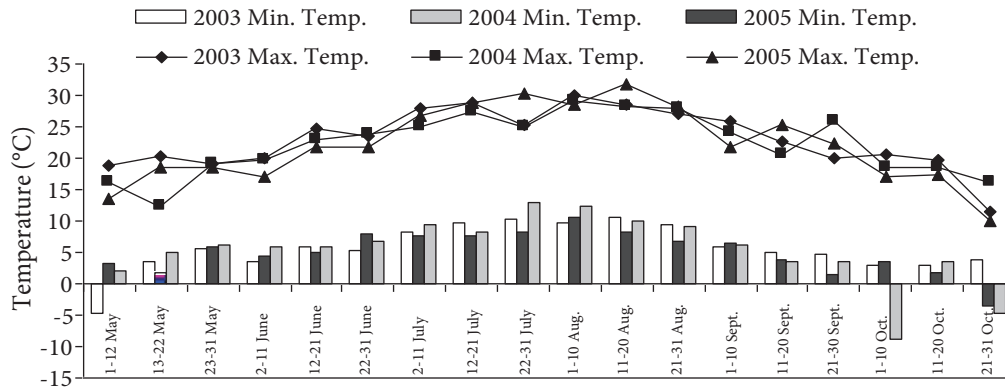


Figure 1. The maximum and minimum air temperature (°C) of the experimental area during the May-October period.

In the study, the seeds of 6 radish cultivars (*Raphanus sativus* L), namely cvs. Siyah, Beyaz, Antep, İri Kırmızı, Red Cherry F₁, and Cherry Belle, were used, supplied by Turkish seed companies MayAgro Seed and SETO Corporations. İri Kırmızı was added to the experiments in 2004. In all experiments, days to anthesis and flowering ratio were evaluated.

Experiment 1

The field experiment (Exp. 1) was conducted at the Agriculture Experimental Station of Atatürk University in 2003-2005. Seeds were sown in 10 days intervals, starting from the middle of May, to investigate the effect of day length and temperature under field conditions. The sowing dates were 13 May (1st), 23 May (2nd), 2 June (3rd), 12 June (4th), 22 June (5th), 2 July (6th), and 12 July (7th) for all experiment years. Seeds were sown on plots of 6 m², in rows 280 cm long, with a distance between rows of 40 cm and between plants of 20 cm for Siyah, Beyaz, Antep, and İri Kırmızı, and a distance between rows of 30 cm and between plants of 20 cm for Red Cherry F₁ and Cherry Belle (Güvenç 1995; Daşgan et al. 1996; Ara et al. 1999), respectively. When the seedlings formed 4-5 true leaves, thinning was done by hand. The plants were irrigated with furrow irrigation. During the development phases, all of the plant care practices were irrespectively applied to the plants in each plot.

All plots received 100 kg ha⁻¹ of N and 80 kg ha⁻¹ of P₂O₅ (Güvenç 1996; Thapa et al. 2003) as calcium ammonium nitrate and triple super phosphate, respectively. All of the P₂O₅ and half of the N fertilizer

were applied uniformly prior to sowing onto the soil surface by hand and incorporated. The remaining half of N was given 20 days after emergence (Srinivas and Naik 1990) in Siyah, Beyaz, Antep, and İri Kırmızı. On the other hand, before Red Cherry F₁ and Cherry Belle were sown, all of the P₂O₅ and N fertilizer were applied.

The experimental design was a completely randomized block design with 4 replications. Main variables were cultivar (6 levels) and sowing dates (7 levels). ANOVA was applied on the data obtained in this study and the differences between means were compared using Duncan's multiple range test.

Experiment 2

Experiment 2 (Exp. 2) was conducted in growth chambers to investigate the effect of day length and vernalization under controlled conditions. Therefore, seeds were imbibed for 24 h in the dark at 25 ± 1 °C in 9 cm petri dishes on moist filter paper (Engelen-Eigles and Erwin 1997; Kaymak et al. 2004). The emerging radicle was approximately 1-2 mm long after imbibition, when vernalization treatments were initiated (Engelen-Eigles and Erwin 1997).

Seedlings were vernalized at 5 °C under either 8 [short days, (SD)] or 16 h [long days, (LD)] day lengths in a growth chamber. Petri dishes were randomized within the chamber. Seedlings were vernalized for 5, 10, 15, and 20 days for both SD and LD. Light intensity of the growth chambers was 8850 lux.

Following vernalization, seedlings were planted in standard plastic pots (650 cm³) in a soilless medium (75% sphagnum peat and 25% perlite). Planted seedlings were then placed in another growth chamber maintained at a constant 18 ± 1 °C for either 8 (SD) or 16 h (LD) day lengths to investigate the effect of day length and vernalization together. Plants were watered daily and fertilized 3 or 4 times a week with CaNO₃ and KNO₃ (7.2 mM N and 2.6 mM K). Plants were also fertilized with MgSO₄ (0.35 mM Mg) supplemented with essential micronutrients once a month (Engelen-Eigles and Erwin 1997).

Data were collected on days to anthesis (DTA) (Engelen-Eigles and Erwin 1997) and flowering ratio. Days to anthesis was the number of days from the end of the vernalization treatment to anthesis (Engelen-Eigles and Erwin 1997; Hawlader et al. 1997; Wang et al. 2003). Flowering ratio was the ratio of flowering plants to nonflowering plants (Wang et al. 2003). The experimental design was a 3-way factorial. Main factors were cultivar (6 levels), day length (2 levels), and vernalization time (4 levels). Seedlings not exposed to a low temperature (18 °C under SD and LD) served as controls.

Experiment 3

Experiment 3 (Exp. 3) was conducted at the experimental areas of the Horticulture Department and growth chambers in 2004 and 2005, to investigate the effect of field and controlled conditions on day length and vernalization together. Hence, Experiment 3 combined both controlled and field conditions. Seedlings were vernalized at 5 °C under either 8 (SD) or 16 h (LD) in a growth chamber, but were then planted outside under natural temperatures and day length. Postvernalization day length was composed of natural light for both late June sowing (SD, 24 June-3 September) and early May sowing (LD, 4 May-15 August) in 2004 and 2005.

During the development phases, all of the plant care practices, such as watering and fertilization, were applied to the plants in each plot, like in Experiment 2.

Data were analyzed using an analysis of variance procedure (SPSS 1999) in Experiment 2 and 3. Arcsine transformation was done before statistical analysis for percentage data. ANOVA was applied to

the data obtained in this study and the differences between means were compared using Duncan's multiple range test.

Results

Days to anthesis changed according to cultivars and sowing dates in Experiment 1 (Table 1). The effect of sowing dates on days to anthesis was statistically significant ($P < 0.01$). On the other hand, days to anthesis was shorter in early sowings than late sowings.

The effect of vernalization time and day length on days to anthesis was statistically significant in Experiment 2. Increasing vernalization time decreased days to anthesis across cultivars with both LD and SD in Experiment 2. Increasing vernalization time from 0 to 15 days under LD and SD decreased days to anthesis in Cherry Belle more than other cultivars in Experiment 2. On the other hand, when vernalization time was below 15 days, Siyah did not flower. Increasing the vernalization time beyond 15 days under LD and SD decreased days to anthesis in Siyah and other cultivars (Table 2). The effect of day length on days to anthesis varied with vernalization time. For instance, days to anthesis were shorter under LD than SD in all cultivars when vernalized from 5 to 20 days (Table 2). Cultivars varied in their vernalization requirements. Beyaz, Antep, İri Kırmızı, Red Cherry F₁, and Cherry Belle did not require vernalization for flowering (Table 2); they flowered after 73.00, 55.67, 54.67, 58.67, and 56.67 days, respectively, when grown at 18 ± 1 °C without a cold treatment. In contrast, vernalization was essential for the flowering of Siyah (Table 2).

In Experiment 3, increasing vernalization time decreased days to anthesis both for early May sowing (LD) and late June sowing (SD) in 2004 and 2005 (Table 3). While the effect of day length on days to anthesis varied with vernalization time, cultivars varied in their vernalization requirements. The results of Experiment 3 were similar to Experiment 2 and the results of Experiment 2 and 3 confirmed each other in regards to day length and vernalization time.

In the field experiment, as seen in Table 4, the highest flowering rate was seen with the first sowing date for all cultivars in the 3 experiment years.

Table 1. The effect of sowing dates on days to anthesis in different cultivars in Experiment 1 (day).

Cultivars	Sowing Date	2003	2004	2005	Mean (Year)	Mean
Siyah	1st (13 May)	-	62.00	67.00	64.50	64.50 (A) ¹
	2nd (23 May)	-	-	-	-	
	3rd (2 June)	-	-	-	-	
	4th (12 June)	-	-	-	-	
	5th (22 June)	-	-	-	-	
	6th (2 July)	-	-	-	-	
	7th (12 July)	-	-	-	-	
Beyaz	1st (13 May)	62.00 b**	48.50 b**	51.00 d**	53.83 d**	58.31 (B)
	2nd (23 May)	66.75 ab	55.00 ab	48.50 cd	56.75 cd	
	3rd (2 June)	69.50 a	56.50 ab	57.50 b	61.17 ab	
	4th (12 June)	64.50 ab	60.00 a	54.25 bc	59.58 bc	
	5th (22 June)	-	-	64.00 a	64.00 a	
	6th (2 July)	-	-	-	-	
	7th (12 July)	-	-	-	-	
Antep	1st (13 May)	59.50 b*	46.25 c**	51.00 bc**	52.25 d**	57.72 (B)
	2nd (23 May)	66.75 a	56.75 ab	46.25 b	56.58 c	
	3rd (2 June)	62.00 ab	52.25 bc	51.50 bc	55.25 cd	
	4th (12 June)	64.50 ab	62.75 a	55.50 ab	60.92 ab	
	5th (22 June)	66.50 a	55.00 b	52.00 bc	57.83 bc	
	6th (2 July)	66.25 a	62.00 a	62.25 a	63.50 a	
	7th (12 July)	-	-	-	-	
İri Kırmızı	1st (13 May)	-	47.75 c**	48.00 ab**	47.88 c**	52.75 (C)
	2nd (23 May)	-	53.25 bc	41.50 b	47.38 c	
	3rd (2 June)	-	53.25 bc	50.75 a	52.00 bc	
	4th (12 June)	-	57.25 b	52.75 a	55.00 b	
	5th (22 June)	-	55.00 bc	52.75 a	53.88 b	
	6th (2 July)	-	67.25 a	53.50 a	60.38 a	
	7th (12 July)	-	-	-	-	
Red Cherry F ₁	1st (13 May)	61.25	53.50	54.25	56.33	56.13 (B)
	2nd (23 May)	55.50	-	-	55.50	
	3rd (2 June)	-	-	-	-	
	4th (12 June)	-	-	-	-	
	5th (22 June)	-	-	-	-	
	6th (2 July)	-	-	-	-	
	7th (12 July)	-	-	-	-	
Cherry Belle	1st (13 May)	55.00	43.25	46.50	48.25	48.94 (D)
	2nd (23 May)	51.00	-	-	51.00	
	3rd (2 June)	-	-	-	-	
	4th (12 June)	-	-	-	-	
	5th (22 June)	-	-	-	-	
	6th (2 July)	-	-	-	-	
	7th (12 July)	-	-	-	-	
Mean (Sowing Date)	1st (13 May)	59.44	50.21	52.96	53.55 D**	
	2nd (23 May)	60.00	55.00	45.42	54.13 D	
	3rd (2 June)	65.75	54.00	53.25	56.66 C	
	4th (12 June)	64.50	60.00	54.17	58.94 B	
	5th (22 June)	66.50	55.00	56.25	57.54 BC	
	6th (2 July)	66.25	64.63	57.88	62.25 A	
	7th (12 July)	-	-	-	-	

1: Letters in parentheses are comparisons of each cultivar across sowing dates.

'-' indicates that plants did not flower, i.e. data could not be collected.

** : Significant at $P < 0.01$, * : Significant at $P < 0.05$.

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Table 2. The effect of vernalization time and day length on days to anthesis in Experiment 2 (day).

Cultivars	Vernalization Time	Day Length		Day Length (Mean)	Mean
		LD (16 h)	SD (8 h)		
Siyah	0 day	-	-	-	56.75 (A) ¹
	5 days	-	-	-	
	10 days	-	-	-	
	15 days	64.00	58.00	61.00	
	20 days	53.33	51.67	52.50	
Mean		58.67 A ²	54.83 B		
Beyaz	0 day	73.00 a**	-	73.00 a**	53.89 (B)
	5 days	48.33 b	77.33 a**	62.83 b	
	10 days	37.33 c	73.00 b	55.17 c	
	15 days	36.00 c	55.67 c	45.83 d	
	20 days	41.67 bc	42.67 d	42.17 d	
Mean		47.27 B	62.17 A		
Antep	0 day	55.67 a**	-	55.67 ab**	48.89 (D)
	5 days	43.67 b	74.00 a**	58.83 a	
	10 days	43.00 b	60.00 b	51.50 bc	
	15 days	35.33 bc	57.00 b	46.17 c	
	20 days	31.00 c	40.33 c	35.67 d	
Mean		41.73 B	57.83 A		
İri Kırmızı	0 day	54.67 a**	-	54.67 a**	46.93 (E)
	5 days	42.00 b	75.00 a**	58.50 a	
	10 days	35.33 c	58.67 b	47.00 b	
	15 days	35.00 c	53.67 b	44.33 b	
	20 days	28.33 d	39.67 c	34.00 c	
Mean		39.07 B	56.75 A		
Red Cherry F ₁	0 day	58.67 a**	-	58.67 b**	51.96 (C)
	5 days	46.67 b	78.00 a**	62.33 a	
	10 days	38.67 c	62.33 b	50.50 c	
	15 days	36.00 c	56.00 c	46.00 d	
	20 days	38.33 c	53.00 d	45.67 d	
Mean		43.67 B	62.33 A		
Cherry Belle	0 day	56.67 a**	-	56.67 a**	46.30 (E)
	5 days	42.67 b	73.33 a**	58.00 a	
	10 days	36.33 c	52.33 b	44.33 b	
	15 days	33.33 cd	47.33 b	40.33 bc	
	20 days	28.33 d	46.33 b	37.33 c	
Mean		39.47 B	54.83 A		
Vernalization Time (Mean)	0 day	59.73	-	59.73 A**	
	5 days	44.67	75.53	60.10 A	
	10 days	38.13	61.27	49.70 B	
	15 days	39.94	54.61	47.28 C	
	20 days	36.83	45.61	41.22 D	
Day Length Mean		43.46 B	58.42 A		

1: Letters in parentheses in columns are comparisons of each cultivar across vernalization time.

2: Letters not in parentheses in lines are comparisons of day length.

'-' indicates that plants did not flower, i.e. data could not be collected.

** : Significant at P < 0.01, * : Significant at P < 0.05.

Table 3. The effect of vernalization time and day length on days to anthesis in Experiment 3 (day).

Cultivars	Vern. Time	2004			2005		
		LD	SD	Day Length (Mean)	LD	SD	Day Length (Mean)
Siyah	0 day	-	-	-	-	-	-
	5 days	65.33 a**	-	65.33 a**	65.67 a**	-	65.67 a**
	10 days	54.33 b	45.00 a**	49.67 b	56.67 ab	-	56.67 b
	15 days	44.00 c	37.00 c	40.50 b	50.00 b	56.33	53.17 bc
	20 days	43.67 c	40.33 b	42.00 c	47.00 b	47.33	47.17 c
Mean		51.83 A ²	40.78 B	47.10 (A) ¹	54.83 ^{NS-2}	51.83	53.83 (A) ¹
Beyaz	0 day	63.33 a**	-	63.33 a**	67.00 a**	-	67.00 a**
	5 days	52.67 b	36.00 a**	44.33 b	47.00 b	46.67 a**	46.83 b
	10 days	44.33 c	26.67 b	35.50 c	43.33 bc	30.00 b	36.67 c
	15 days	41.33 c	24.00 b	32.67 c	39.00 cd	27.00 b	33.00 cd
	20 days	40.00 c	28.00 b	34.00 c	37.33 d	26.00 b	31.67 c
Mean		48.33 A	28.67 B	39.59 (B)	46.73 A	32.42 B	40.37 (B)
Antep	0 day	60.00 a**	-	60.00 a**	51.67 a**	-	51.67 a**
	5 days	41.00 b	36.67 a**	38.83 b	44.67 b	44.67 a**	44.67 b
	10 days	34.33 c	33.00 ab	33.67 c	35.67 c	28.67 b	32.17 c
	15 days	33.67 c	25.33 c	29.50 c	32.00 cd	25.33 b	28.67 c
	20 days	29.67 c	28.67 bc	29.17 c	29.00 d	24.33 b	26.67 c
Mean		39.73 A	30.92 B	35.81 (C)	38.60 A	30.75 B	35.11 (D)
İri Kırmızı	0 day	-	-	-	47.33 a**	-	47.33 a**
	5 days	-	30.00 a**	30.00 a**	42.00 b	48.33 a**	45.17 a
	10 days	-	28.00 a	28.00 a	30.33 c	33.00 b	31.67 b
	15 days	-	28.00 a	28.00 a	28.67 cd	30.00 b	29.33 bc
	20 days	-	24.67 b	24.67 b	25.00 d	30.67 b	27.83 c
Mean			27.67	27.67 (D)	34.67 B	35.50 A	35.04 (D)
Red Cherry F ₁	0 day	55.67 a**	-	55.67 a**	52.00 a**	-	52.00 a**
	5 days	42.33 b	36.33 a**	39.33 b	45.67 b	40.00 a**	42.83 b
	10 days	35.00 c	26.00 b	30.50 c	42.33 c	33.00 b	37.67 c
	15 days	34.67 c	24.33 b	29.50 c	38.67 d	24.00 c	31.33 d
	20 days	30.67 c	28.00 b	29.33 c	36.00 d	29.67 b	32.83 d
Mean		39.67 A	28.67 B	34.78 (C)	42.93 A	31.67 B	37.93 (C)
Cherry Belle	0 day	56.67 a**	-	56.67 a**	50.67 a**	-	50.67 a**
	5 days	46.67 b	38.00 a**	42.33 b	47.33 a	40.33 a**	43.83 b
	10 days	32.67 c	28.00 b	30.33 c	37.67 b	28.67 b	33.17 c
	15 days	32.67 c	25.33 b	29.00 c	36.00 b	23.33 b	29.67 cd
	20 days	30.33 c	24.67 b	27.50 c	28.67 c	23.67 b	26.17 d
Mean		39.80 A	29.00 B	35.00 (C)	40.07 A	29.00 B	35.15 (D)
Vern. Time (Mean)	0 day	58.92	-	58.92 A**	53.73	-	53.73 A**
	5 days	49.60	35.40	42.50 B	48.72	44.00	46.58 B
	10 days	40.13	31.11	35.21 C	41.00	30.67	36.30 C
	15 days	37.27	27.33	31.85 D	37.39	31.00	34.19 D
	20 days	34.87	29.06	31.70 D	33.83	30.28	32.06 E
Day Length (Mean)		43.54 A	30.52 B		42.56 A	33.68 B	

1: Letters in parentheses in columns are comparisons of each cultivar across day length.

2: Letters not in parentheses in lines are comparisons of day length.

‘-’ indicates that plants did not flower, i.e. data could not be collected.

** : Significant at $P < 0.01$, * : Significant at $P < 0.05$, NS: Not significant at $P < 0.05$.

Table 4. The effect of sowing dates on flowering rates in different cultivars in Experiment 1 (%).

Cultivar	Sowing Date	2003	2004	2005	Years (Mean)	Mean
Siyah	1st (13 May)	1.28 ^{NS}	25.75 a**	8.67 a**	11.90 a**	2.80 (E) ¹
	2nd (23 May)	1.28	1.28 b	1.28 b	1.28 b	
	3rd (2 June)	1.28	1.28 b	1.28 b	1.28 b	
	4th (12 June)	1.28	1.28 b	1.28 b	1.28 b	
	5th (22 June)	1.28	1.28 b	1.28 b	1.28 b	
	6th (2 July)	1.28	1.28 b	1.28 b	1.28 b	
	7th (12 July)	1.28	1.28 b	1.28 b	1.28 b	
Beyaz	1st (13 May)	54.68 a**	88.72 a**	88.72 a**	77.37 a**	22.15 (C)
	2nd (23 May)	46.51 b	26.31 b	60.40 b	44.41 b	
	3rd (2 June)	11.22 c	19.57 c	13.97 c	14.92 c	
	4th (12 June)	10.15 c	15.93 d	9.25 c	11.78 c	
	5th (22 June)	1.28 d	1.28 e	9.46 c	4.01 d	
	6th (2 July)	1.28 d	1.28 e	1.28 d	1.28 d	
	7th (12 July)	1.28 d	1.28 e	1.28 d	1.28 d	
Antep	1st (13 May)	65.44 a**	88.72 a**	88.72 a**	80.96 a**	36.37 (B)
	2nd (23 May)	65.10 a	59.71 b	88.72 a	71.18 b	
	3rd (2 June)	36.68 b	46.35 c	40.46 b	41.16 c	
	4th (12 June)	21.52 c	28.85 d	29.93 c	26.77 d	
	5th (22 June)	18.40 cd	19.44 e	29.69 c	22.51 e	
	6th (2 July)	11.27 d	9.41 f	11.62 d	10.77 f	
	7th (12 July)	1.28 e	1.28 g	1.28 e	1.28 g	
İri Kırmızı	1st (13 May)		88.72 a**	88.72 a**	88.72 a**	48.32 (A)
	2nd (23 May)		88.72 a	88.72 a	88.72 a	
	3rd (2 June)		64.56 b	50.01 b	57.28 b	
	4th (12 June)		42.92 c	41.29 b	42.10 c	
	5th (22 June)		38.44 c	41.10 b	39.77 c	
	6th (2 July)		19.56 d	21.21 c	20.38 d	
	7th (12 July)		1.28 e	1.28 d	1.28 e	
Red Cherry F ₁	1st (13 May)	88.72 a**	88.72 a**	88.72 a**	88.72 a**	15.89 (D)
	2nd (23 May)	45.72 b	1.28 b	1.28 b	16.09 b	
	3rd (2 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	4th (12 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	5th (22 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	6th (2 July)	1.28 c	1.28 b	1.28 b	1.28 c	
	7th (12 July)	1.28 c	1.28 b	1.28 b	1.28 c	
Cherry Belle	1st (13 May)	88.72 a**	88.72 a**	88.72 a**	88.72 a**	16.87 (D)
	2nd (23 May)	66.39 b	1.28 b	1.28 b	22.98 b	
	3rd (2 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	4th (12 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	5th (22 June)	1.28 c	1.28 b	1.28 b	1.28 c	
	6th (2 July)	1.28 c	1.28 b	1.28 b	1.28 c	
	7th (12 July)	1.28 c	1.28 b	1.28 b	1.28 c	
Sowing Date (Mean)	1st (13 May)	59.77	78.22	75.38	71.79 A**	
	2nd (23 May)	44.99	29.77	40.28	37.96 B	
	3rd (2 June)	10.35	22.39	18.05	17.31 C	
	4th (12 June)	7.10	15.26	14.05	12.43 D	
	5th (22 June)	4.71	10.50	14.02	10.04 E	
	6th (2 July)	3.28	5.68	6.33	5.20 F	
	7th (12 July)	1.28	1.28	1.28	1.28 G	

1: Letters in parentheses are comparisons of each cultivar across day length.
 ** : Significant at P < 0.01, * : Significant at P < 0.05, NS: Not significant at P < 0.05.

Delaying the sowing date decreased the flowering rate. After the 4th sowing date in 2003 and 2004 and the 5th sowing date in 2005, flowering was not observed for Beyaz. It was noted that flowering went on through the last sowing date for Antep and İri Kırmızı.

While flowering was not seen from all sowing dates in 2003, a low flowering rate was determined only from the 1st sowing date in 2004 (25.75%) and 2005 (8.67%) for Siyah. That flowering rate was also similar to those of Red Cherry F₁ and Cherry Belle. Additionally, flowering was not determined from the last (7th) sowing date across the cultivars in all experiment years (Table 4).

The effect of different vernalization times and day lengths on flowering rates in radish cultivars was significant ($P < 0.01$) in Experiment 2 (Table 5). For all vernalization time treatments, it was determined that the flowering rate was higher with LD than SD for Antep, Red Cherry F₁, and Cherry Belle. While the flowering rate was determined to be 88.72% for Beyaz and İri Kırmızı in 20 days vernalization time treatments with both SD and LD, it was seen that the flowering rate was higher under LD than SD in other vernalization time treatments. The flowering rate in vernalization times of 5 and 10 days was not significant under either LD or SD in Siyah. In addition, it was observed that the most effective vernalization time was 15 and 20 days under LD and SD. In contrast to Siyah, other cultivars flowered, depending on day length, without vernalization under LD, and did not flower without vernalization under SD (Table 5).

Increasing vernalization time increased the flowering rate across cultivars under SD. However, it was determined that the effect of vernalization time on flowering rates under LD was not significant for İri Kırmızı, Red Cherry F₁, and Cherry Belle (except for 5 days), but the flowering rate was increased by using low temperature treatments for Siyah, Beyaz, and Antep. In contrast to other cultivars, Siyah did not flower when 5 or 10 days of vernalization time were used (Table 5).

The effect of different vernalization time and day length on flowering rates was found to be statistically

significant ($P < 0.01$) across cultivars in both experiment years in Experiment 3 (Table 6). It was determined that the flowering rate was higher under LD than SD by using 0, 5, and 10 days of vernalization time treatments in both experiment years. In all vernalization treatments, except for the control, the flowering rate of Siyah was higher under LD than SD. In addition, Siyah did not flower without vernalization under LD, but all of the other cultivars flowered depending on day length without vernalization in both experiment years. On the other hand, Siyah did not flower under SD with 0 and 5 or 0, 5, and 10 days of vernalization treatments in 2004 and 2005, respectively. Moreover, the flowering rate was not determined in controls under SD in all cultivars (Table 6).

It was observed that when increasing the vernalization time, the flowering rate increased under LD and SD in all treatments. However, in contrast to Siyah, the effect of vernalization time in other cultivars was not found to be statistically significant. Red Cherry F₁ flowered under LD (88.72%) in all vernalization times in 2004 and 2005 (Table 6).

Discussion

It was determined that days to anthesis, considering Beyaz, Antep, and İri Kırmızı in Experiment 1, was short from the first sowing date but increased progressively through the last sowing date. Furthermore, on considering the mean of experiment years and all cultivars, similar results appeared (Table 1). Radishes could supply their vernalization requirements more easily from the 1st sowing date than other sowing dates in Experiment 1, owing to the low air temperatures. This can be an advantage for seed production for practical applications. In other periods, it is possible that the effect of vernalization removed devernalization because of air temperatures (Figure 1) (MBMK 2003, 2004, 2005). Similarly, some researchers (Shin et al. 1989; Sheen et al. 2000; Cheon and Saito 2003) reported the effect of high temperatures on devernalization in radish. Thus, delaying of days to anthesis is an expected result. The obtained results coincide with Experiments 2 and 3 and preceding findings, which say that vernalization time extends

Table 5. The effect of vernalization time and day length on flowering rates in Experiment 2 (%).

Cultivars	Vernalization Time	Day Length		Day Length (Mean)	Mean
		LD (16 h)	SD (8 h)		
Siyah	0 day	1.28 c**	1.28 c**	1.28 c**	14.99 (D) ¹
	5 days	1.28 c	1.28 c	1.28 c	
	10 days	1.28 c	1.28 c	1.28 c	
	15 days	27.63 b	31.57 b	29.60 b	
	20 days	46.83 a	36.22 a	41.52 a	
Mean		15.66 ^{NS-2}	14.33		
Beyaz	0 day	27.33 d**	1.28 c**	14.30 d**	51.68 (C)
	5 days	48.08 c	40.20 b	44.14 c	
	10 days	64.82 c	41.55 b	53.19 b	
	15 days	66.13 b	50.00 b	58.07 b	
	20 days	88.72 a	88.72 a	88.72 a	
Mean		59.02 A	44.35 B		
Antep	0 day	44.80 b**	1.28 c**	23.04 c**	61.53 (B)
	5 days	73.99 a	44.50 b	59.24 b	
	10 days	82.25 a	62.63 ab	72.44 a	
	15 days	88.72 a	61.33 ab	75.03 a	
	20 days	88.72 a	67.09 a	77.90 a	
Mean		75.70 A	47.37 B		
İri Kırmızı	0 day	72.67 b*	1.28 c**	36.98 c**	72.87 (A)
	5 days	88.72 a	55.35 b	72.04 b	
	10 days	88.72 a	72.26 ab	80.49 ab	
	15 days	88.72 a	83.50 a	86.11 a	
	20 days	88.72 a	88.72 a	88.72 a	
Mean		85.51 A	60.22 B		
Red Cherry F ₁	0 day	56.18 b**	1.28 c**	28.73 c**	62.46 (B)
	5 days	88.72 a	33.74 b	61.23 b	
	10 days	88.72 a	50.08 ab	69.40 ab	
	15 days	88.72 a	60.27 a	74.50 a	
	20 days	88.72 a	68.18 a	78.45 a	
Mean		82.21 A	42.71 B		
Cherry Belle	0 day	49.81 b**	1.28 b**	25.54 c**	64.03 (B)
	5 days	65.13 ab	52.06 a	58.59 b	
	10 days	88.72 a	56.82 a	72.77 ab	
	15 days	88.72 a	75.06 a	81.89 a	
	20 days	88.72 a	74.00 a	81.36 a	
Mean		76.22 A	51.84 B		
Vernalization Time (Mean)	0 day	42.01	1.28	21.65 (E)	
	5 days	60.99	37.85	49.42 (D)	
	10 days	69.08	47.44	58.26 (C)	
	15 days	74.77	60.29	67.53 (B)	
	20 days	81.74	70.49	76.12 (A)	
Day Length (Mean)		65.88 A	43.47 B		

1: Letters in parentheses in columns are comparisons of each cultivar across day length.

2: Letters not in parentheses in lines are comparisons of day length.

** : Significant at P < 0.01, * : Significant at P < 0.05, NS: Not significant at P < 0.05.

Table 6. The effect of vernalization time and day length on flowering rates in Experiment 3 (%).

Cultivars	Vern. Time	2004			2005		Day Length (Mean)
		LD	SD	Day Length (Mean)	LD	SD	
Siyah	0 day	1.28 d**	1.28 b**	1.28 e**	1.28 c**	1.28 c**	1.28 d**
	5 days	48.57 c	1.28 b	24.93 d	29.04 b	1.28 c	15.16 c
	10 days	67.35 b	16.96 b	42.15 c	82.25 a	1.28 c	41.76 b
	15 days	88.72 a	49.67 a	80.27 a	82.25 a	31.76 b	57.00 a
	20 days	88.72 a	71.81 a	69.19 b	88.72 a	40.69 a	64.71 a
	Mean		58.93 A ²	28.20 B	43.56 (C) ¹	56.71 A ²	15.26 B
Beyaz	0 day	39.94 b**	1.28 c**	20.61 c**	41.83 b**	1.28 d**	21.56 d**
	5 days	88.72 a	62.24 b	75.48 b	88.72 a	28.36 c	58.54 c
	10 days	88.72 a	75.77 ab	82.25 ab	88.72 a	65.63 b	77.18 b
	15 days	88.72 a	88.72 a	88.72 a	88.72 a	66.09 a	77.41 b
	20 days	88.72 a	88.72 a	88.72 a	88.72 a	88.72 a	88.72 a
	Mean		78.97 A	63.35 B	71.16 (B)	79.34 A	50.02 B
Antep	0 day	58.10 b**	1.28 b**	29.69 b**	70.88 b*	1.28 c**	36.08 c**
	5 days	88.72 a	67.67 a	78.20 a	88.72 a	47.41 b	68.07 b
	10 days	88.72 a	82.25 a	85.48 a	88.72 a	82.25 a	85.48 a
	15 days	88.72 a	88.72 a	88.72 a	88.72 a	83.00 a	85.86 a
	20 days	88.72 a	88.72 a	88.72 a	88.72 a	88.72 a	88.72 a
	Mean		82.60 A	65.73 B	74.16 (B)	85.15 A	60.53 B
İri Kırmızı	0 day		1.28 b**	1.28 b**	88.72 ^{NS}	1.28 b**	45.00 c**
	5 days		88.72 a	88.72 a	88.72	69.06 a	78.89 b
	10 days		88.72 a	88.72 a	88.72	88.72 a	88.72 a
	15 days		88.72 a	88.72 a	88.72	88.72 a	88.72 a
	20 days		88.72 a	88.72 a	88.72	88.72 a	88.72 a
	Mean			71.23	71.23 (B)	88.72 A	67.30 B
Red Cherry F ₁	0 day	88.72 ^{NS}	1.28 b**	45.00 b**	88.72 ^{NS}	1.28 b**	45.00 c**
	5 days	88.72	82.25 a	85.48 a	88.72	75.00 a	81.86 b
	10 days	88.72	88.72 a	88.72 a	88.72	88.72 a	88.72 a
	15 days	88.72	88.72 a	88.72 a	88.72	88.72 a	88.72 a
	20 days	88.72	88.72 a	88.72 a	88.72	88.72 a	88.72 a
	Mean		88.72 A	69.94 B	79.33(A)	88.72 A	68.49 B
Cherry Belle	0 day	82.25 ^{NS}	1.28 b**	41.76 b**	82.25 ^{NS}	1.28 c**	41.76 c**
	5 days	88.72	88.72 a	88.72 a	88.72	52.35 b	70.54 b
	10 days	88.72	88.72 a	88.72 a	88.72	80.29 a	84.51 a
	15 days	88.72	88.72 a	88.72 a	88.72	88.72 a	88.72 a
	20 days	88.72	88.72 a	88.72 a	88.72	88.72 a	88.72 a
	Mean		87.43 A	71.23 B	79.33 (A)	87.43 A	62.27 B
Vern. Time (Mean)	0 day	54.06	1.28	25.27 D**	62.28	1.28	31.78 D**
	5 days	80.69	65.15	72.21 C	78.77	45.58	62.18 C
	10 days	84.45	73.52	78.49 B	87.64	67.82	77.73 B
	15 days	88.72	82.21	85.17 A	87.64	74.50	81.07 AB
	20 days	88.72	85.90	87.18 A	88.72	80.72	84.72 A
Day Length (Mean)		79.33 A	61.61 B		81.01 A	53.98 B	

1: Letters in parentheses in columns are comparisons of each cultivar across day length.

2: Letters not in parentheses in lines are comparisons of day length.

** : Significant at P < 0.01, * : Significant at P < 0.05, NS: Not significant at P < 0.05.

when days to anthesis shorten (Yoo and Uemoto 1976; Yoo 1977; Engelen-Eigles and Erwin 1997; Hawlader et al. 1997; Wang et al. 2003).

When the results of the 3 experiments were evaluated, it appeared that the flowering rate changed according to cultivars, day length, vernalization time, and sowing date. It was determined that by delaying the sowing date, the flowering rate of all cultivars decreased in all experiment years in Experiment 1. None of the cultivars flowered after the last sowing date in Experiment 1. This may be the result of the lack of fulfillment of vernalization due to the increase in temperature, delay in sowing date, and decrease in day length (MBMK 2003, 2004, 2005). Flowering was not seen in the control groups for all cultivars under SD in either Experiment 2 or 3. With respect to these results, it was determined that increasing the vernalization time increased the flowering rate. In Experiments 2 and 3, the flowering of Siyah was not seen when it had less than 15 days of vernalization time under both SD and LD. It was also found that the flowering of Siyah could occur with LD if vernalization occurred. Moreover, various proportions of Siyah flowered in other cultivars when the day length increased. For other radish cultivars, under LD and SD, all of the vernalization times increased the flowering rate and reduced the days to anthesis. In contrast to Siyah, all cultivars flowered without vernalization, depending on the day length under LD; however, they did not flower without

vernalization under SD. Vernalization appears as obligatory and facultative in plants (Wiebe 1990; Michaels and Amasino 2000). In addition, it was found that flowering depended on day length or was affected by either day length or low temperatures in radishes (Yoo and Uemoto 1976; Yoo 1977; Wiebe 1985). Moreover, Wang et al. (2003) reported that the flowering of radish varieties was promoted by low temperature pretreatment, and the promoting effects were better when the germinated seeds were subjected to longer treatment duration.

In conclusion, it was clearly determined that Antep, Beyaz, İri Kırmızı, Red Cherry F₁, and Cherry Belle have a facultative vernalization response; cold exposure is not required for flowering, but flowering will occur more rapidly after cold treatment. Siyah, in contrast, has an obligate requirement for cold treatment and thus cannot flower without prior cold exposure. Moreover, Siyah may be a new and alternative model plant to *R. sativus* L. cv. 'Chinese Radish Jumbo Scarlet,' *A. thaliana*, *L. biennis*, or *A. graveolens* (Engelen-Eigles and Erwin 1997) for vernalization studies. As reported about *R. sativus* L. cv. 'Chinese Radish Jumbo Scarlet' by Engelen-Eigles and Erwin (1997), *R. sativus* L. cv. 'Siyah' may be a more applicable plant for future vernalization studies since it has an obligate vernalization requirement and is rapidly vernalized, receptive to vernalization early in development, day-length insensitive during vernalization, and available from seed.

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