Çukurova Tarım Gıda Bil. Der.



Çukurova J. Agric. Food Sci. 39(1):13-24 doi: 10.36846/CJAFS.2024.129

<u>Research Article</u> **Phenological Growth Stages of Some Olive Cultivars (Olea europaea** *L*.)

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ABSTRACT

In this study, phenological observations were made in Domat, Gemlik and Sarı Ulak olive cultivars in Tarsus region. For this purpose, inflorescences, beginning of blooming, stages of flowering by 25%, 50% and 75%, end of flowering, green and black maturity periods were determined. As a result of the study, it has been determined that there was a period of 10-15 days between the flowering (BBCH=55) and fruit setting (BBCH=69) periods of the cultivars. On average, the time from full blooming to maturity; It was determined as 179 days in Gemlik cultivar, 175 days in Sarı Ulak cultivar and 138 days in Domat cultivar. Flowering and fruit set periods in olive cultivars have a big importance for choosing indigenous cultivar selection. In this study, the effect of climate data of different years on flowering and maturity periods were evaluated. When the climate conditions with flowering and maturity parameters were evaluated together, the best suggestable cultivar for relevant region is became Sarı Ulak.

Keywords: Blooming , phenology, fruit setting, maturity, olive

Bazi Zeytin Çeşitlerine Ait Fenolojik Gelişim Evreleri

ÖΖ

Çalışmada Tarsus yöresinde Domat, Gemlik ve Sarı Ulak zeytin çeşitlerinde fenolojik gözlemler yapılmıştır. Bu amaçla somak oluşumu, çiçeklenme başlangıcı, %25 çiçeklenme, %50 çiçeklenme, %75 çiçeklenme, çiçeklenme sonu, yeşil olum ve siyah olum dönemleri belirlenmiştir. Çalışma sonucunda çeşitlerin çiçek açma ve meyve bağlama dönemleri arasında 10-15 günlük bir süre olduğu saptanmıştır. Tam çiçeklenmeden olgunluğa kadar geçen süre ortalama olarak; Gemlik çeşidinde 179 gün, Sarı Ulak çeşidinde 175 gün ve Domat çeşidinde ise 138 gün olarak tespit edilmiştir. Zeytin çeşitlerinde çiçeklenme ve meyve tutumu dönemlerinin belilenmesi yöreye özgü çeşit seçiminde büyük önem taşımaktadır. Bu çalışmada farklı yıllardaki iklim değerlerinin çiçeklenme ve olgunluk periyotlarını nasıl etkileyeceği değerlendirilmiştir. Çalışmanın yapıldığı bölge iklim şartları ile çiçeklenme ve olgunluk parametreleri değerlendirildiğinde bu bölgeye en iyi önerilebilecek çeşidin Sarı Ulak çeşidi olduğu belirlenmiştir.

Anahtar Kelimeler: Çiçeklenme, fenoloji, meyve tutumu, olgunluk, zeytin

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Yayın Kuruluna Geliş Tarihi: 10.11.2023

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Kabul Tarihi: 29.12.2023

Phenological Growth Stages of Some Olive Cultivars (Olea europaea L.)

Introduction

The distribution area of the olive tree is between 30° and 45° latitude in both the northern and southern hemispheres. Olive is a Mediterranean climate plant characterized by dry and hot summers (Pansiot and Rebour 1964). Loussert and Brousse (1978) stated that the olive tree requires a temperature of 9-10°C in the spring development period, 14-15°C in the inflorescences period, 18-19°C in the flowering period, fertilization occurs at 21-22°C, develop around 15°C and when it reaches 18-19°C this indicates the beginning of flowering. Olive withstands the highest temperature of 40°C. In the winter rest period, the plant is damaged when the temperature drops below -7°C. The development of the olive tree, which grows in places with an average annual precipitation of 200-800 mm, depends on the distribution of the rains throughout the year. The olive is a light-loving plant (Usanmaz et al. 1988; Moltay et al. 1996; Seyran 2009).

According to the phenological calendar of the olive tree in the Mediterranean basin, March is the period when the differentiation of flower buds continues, the flower petals and male organs are formed (Kaya 2006). Pansiot and Rebour (1964) report that the resting period of olives ends in April. In April, the buds begin to open and flower clusters begin to shoot from these buds (Bozkaya 2009). The flowering period, which starts from the first week of May, continues until the middle of June. In June, olive blooming is completed and fruit setting takes place. With the increase in temperature July, the olive grains grow rapidly. In August, fruit growth continues and core hardening is observed (Varol 2006). In September olives starts to gather oil. As the temperatures decrease temperature during flowering.

Seif et al. (2015) gave information about olives in their research. A mature olive tree produces about 500,000 flowers. The number of flowers and their distribution on the inflorescences is specific to each cultivar, but can vary from year to year. Flower position on the inflorescences affects gender and opening time. Olive trees have either male or female flowers. Olive flowers are windpollinated; but it is also visited by insects. Critical conditions such as strong and dry winds, rain and high temperature affect pollination and can reduce fruit formation. In a year with normal flowering, 1 to 2% of flowers are sufficient to determine fruit for commercial yield. in October, the rate of photosynthesis decreases. During this period, the olive fruit continues to be oiled and a color change is observed in the fruit. As photosynthesis activities retardation in November, olive fruit continues to turn black and maturity takes place in this month (Kaya 2006; Bozkaya 2009).

Porlingis and Voyiatzis (1999) reported that the germination of olive pollen decreases at high temperatures and early flowering in hot climate is an advantage that prevents damage to fruit set from high temperature. Galán et al. (2005) reported that the best pollination of olives is at about 20-30°C. Cultivars that bloom earlier, pollinate and fertilize at lower temperatures are safer from high temperature damage.

Seif et al. (2008) were examined the opening times of flowers, their gender and the persistence of the petal in Manzanilla, Mission and Frantoio olive cultivars. For each cultivar, 45 inflorescences were inspected each morning during the period from flowering to the shedding of the petal. Hermaphrodite flowers were fundamentally opened at the beginning of the flowering period, while the male flowers were opened later. The flower position on the inflorescencess was found to have a highly significant effect on the day of blooming in all cultivars. It has been established that the position of flowers in Manzanilla and Mission cultivars has a rather significant effect on gender. In the Manzanilla cultivar, hermaphrodite flowers have significantly longer petal persistence than male flowers. According to the results of this study, the persistence of the petal depends on the daily.

In the studies made in olive, the phonological periods are determined but the observations were made subjectively. In this study, the periods for each phenology, which was formed by Sanz-Cortes et al. (2002) in detail, was given clearly that can be observed by researchers. This was carried out as a difference in the study and suggested to be usable for future studies in order to make common markers.

Materials and method

This study was carried out using Gemlik, Sari Ulak and Domat olive cultivars in an olive orchard belonging to a producer in Mersin province, Tarsus district. The study was started at 11th year of Gemlik and 8th year of Sari Ulak and Domat cultivars. Planting distance of the trees were 3×7 .

The orchard was in optimal maintenance conditions and the irrigation, fertigation, pruning and protection processes were made regularly. Phenological observations of these cultivars were made.

According to Sanz-Cortés et al. (2002) the phenological observations of Gemlik, Domat and Sarı Ulak cultivars show the BBCH codes of the beginning of the inflorescences, the beginning of flowering, 25%, 50% and 75% flowering, the end

of flowering, green maturity and black maturity determined (Table 1).

In this way, the number of days after pollination in fruit maturity was also obtained. Sanz-Cortés et al. (2002) determined the following developmental stages in their research on olive phenology.

Code	Explanation
Growth phase 0:	Bud Development
00	Leaf buds on the tops of shoots that developed in the previous crop year are
	completely closed, sharp-pointed, stemless and coloured.
01	Leaf buds start to bulk out and new foliar primordia start to grow.
03	Foliar buds grow and separate from the base.
07	External small leaves open, but not completely separated, remaining joined at
	apices.
09	External small leaves opening further with their tips inter-crossing.
Growth phase 1:	Leaf Development
11	First leaves completely separated. Greenish grey colour.
15	The leaves separate further before they reach their final size. First leaves turn
	greenish on the upperside.
19	The leaves take their typical length and shape.
Growth phase 3:	Shoot Development
31	Shoot reach 10% of their final length.
33	Shoot reach 30% of their final size.
37	Shoot reach 70% of their final size.
Growth phase 5:	Inflorescence emergence
50	Inflorescence buds in the leaf axils are completely closed. They are sharp-
	pointed, stemless and ochre in color.
51	Inflorescence buds begin to swell from the bottom.
52	Inflorescence buds open. Flower cluster development begins.
54	Inflorescence buds grows.
55	Flower cluster are fully expanded. Floral buds begin to open.
57	Corolla green-coloured is longer than calyx.
59	Corolla changes colour from green to white.
Growth phase 6:	Flowering
60	First flowers open.
61	Beginning of flowering: 10% of flowers open.
65	Full flowering: at least 50% of the flowers open.
67	First petals starts to fall.
68	Most of petals fallen or faded.
69	End of blooming, fruit set, non-fertilized ovaries fallen.
Growth phase 7:	Fruit Development
71	Fruit size is approximately 10% of final size.
75	Fruit size is approximately 50% of final size. Stone begins lignified.
79	Fruit size is approximately 90% of final size. Fruit suitable for picking green.
Growth phase 8:	Maturity of Fruit
80	The fruit turns from dark green to yellowish light green.
81	Starts of fruit colouring.
85	Increasing specific fruit colouring.

89

92

Harvest maturity: fruits achieve the typical cultivar colour, remain turgid and are suitable for oil extraction.

Growth phase 9: Senescence

Overripe: fruits lose turgidity and begin to fall (Sanz-Cortés vd. 2002).

Results and discussion

The flowering and fruit maturity times obtained as a result of phenological observations in the olive cultivars included in the experiment are shown in table 1; The time differences of different phenological development periods according to the full flowering period are given in Table 2. The date of full flowering was accepted as 50% flowering period (Sanz-Cortes et al. 2001).

As a result of the observations made in 2017; The earliest flowering was seen in Sarı Ulak cultivar, followed by Domat and Gemlik cultivars (Table 2). The time from full bloom to maturity was 183 days of Gemlik cultivar (black maturity), 180 days of Sarı Ulak cultivar (black maturity) and 142 days of Domat cultivar (green maturity) (Table 3).

In 2018, the first bunch initiation and flowering was seen in Sarı Ulak cultivar. The flowers of the Gemlik cultivar started to bloom after a few days. On the other hand, in Domat cultivar, it was observed in the period in this trial year and flowering did not occur (Table 2). The time from full bloom to maturity was found to be 174 days in Gemlik cultivar and 171 days in Sarı Ulak cultivar (Table 3).

As a result of the observations made in 2019; inflorescences onset and flowering were observed at the earliest in the Sarı Ulak cultivar, followed by the Gemlik cultivar, and these developmental stages were realized in the latest Domat cultivar (Table 2). The period of full bloom to maturity was determined as 181 days in Gemlik cultivar (black maturity), 173 days in Sarı Ulak cultivar (black maturity) and 134 days in Domat cultivar (green maturity) (Table 3). The location of the BBCH codes of the phenological observations made in the olive cultivars included in the experiment according to the months is given in table 3.

Within the scope of phenological observations, in 2017, 2018 and 2019, the earliest inflorescences start and flowering were observed in the Sarı Ulak cultivar; The latest flowering took place in Gemlik in 2017 and 2018, and in Domat in 2019. In 2018, higher temperatures were detected in February and March compared to other years (Table 2, 3 and 4),

which brought the bunching period earlier. The earliest inflorescences onset was observed on March 30 in 2018 in the Sarı Ulak cultivar due to high temperature, while it was detected on April 2 in 2019 and on April 3 in 2017.

Barut et al. (2002) as a result of the examinations of the Gemlik olive cultivar in Bursa province, it determined that the morphological was differentiation in the buds was in the first week of February, and the emergence of the ovules in the middle of April. Bolat and Güleryüz (1995) determined the first inflorescences formation in the Coruh valley in the second week of May in the Butko cultivar, and stated that the first flowering in the cultivars was observed in the first week of June and the full flowering was observed in the second week of June. Since Mersin (Tarsus) shows milder conditions compared to the regions where these studies were conducted. inflorescences formation of our cultivars was observed in early April, while flowering was completed in the second week of May at the latest.

Karadağ et al. (2007) reported that the beginning of flowering was the earliest in Edincik Su and Labib (28 April), and the latest in Domat (03 May) and Erkence (06 May) cultivars. While flowering started on April 29 in Gemlik cultivar, this date was May 2 in Sarı Ulak cultivar. The end of flowering was determined as 19 May in Gemlik cultivar and 20 May in Domat and Sarı Ulak cultivars. A period of 17-20 days was observed between the beginning of flowering and the end of flowering. The flowering and fruit maturity results obtained in our study has partly similarity with the results of the researchers.

 Table 1. Images of development periods are taken as a basis the phenological observations and shown

 by BBCH codes

Periods	BBCH Codes	Pictures
Beginning of inflorescence	55	
25% Blooming	63	
50% Blooming*	65	
75% Blooming	67	
End of Blooming	69	

*Full blooming, has been recognized as a 50% blooming period.

	BBC		Domat			Gemlik	Σ.	S	Sarı Ulak	
Periods	H Code	2017	2018	2019	2017	2018	2019	2017	2018	2019
Beginning			-							
of	55	06.Ap		10.A	10.A	03.A	04 Apr	03 Apr	30.Ma	02.Ap
infloresce	55	r		pr	pr	pr	04.Api	05.Api	rch	r
nce										
25%	63	27.Ap	-	28.A	30.A	19.A	26 Apr	25 Apr	17 Apr	25.Ap
Blooming	05	r		pr	pr	pr	20.Api	23.Api	17.Api	r
50%		01 M	-	02 M	04 M	24 4	$02 M_{\odot}$			01 Mo
Blooming	65			05.101	04.101	24.A	02.101a	30.Apr	21.Apr	
*		ay		ay	ay	рг	У	_	_	У
75%	67	04.M	-	09.M	07.M	29.A	07.Ma	02 May	27 Apr	06.Ma
Blooming	07	ay		ay	ay	pr	у	02.1viay	27.Api	у
End of	60	07.M	-	14.M	09.M	02.M	11.Ma	04 May	01.Ma	09.Ma
Blooming	09	ay		ay	ay	ay	у	04.May	У	у
Green	80	20.Se	-	14.Se	03.Oc	12.Se	22 Sam	20 5	01 Sam	20.Se
maturity	80	р		р	t	р	25.Sep	29.Sep	01.Sep	р
Black	00		-		05.Ka	15.Oc	20.0*	27 Oct	05.0 at	20.Oc
maturity	88	-		-	s	t	29.0ct	27.0ct	05.0ct	t

Table 2. Flowering and fruit maturity times determined as a result of phenological observations in the olive cultivars included in the study

Table 3. The time differences of the different phenological development periods determined in the olive cultivars used in the study compared to the full flowering period

Dominda	BBCH		Domat			Gemlik		S	Sarı Ula	k
Perious	Code	2017	2018	2019	2017	2018	2019	2017	2018	2019
Beginning of	55	24		22	25	21	27	27	22	20
inflorescence	55	-24		-23	-23	-21	-27	-27	-23	-28
25% Blooming	63	-4		-5	-5	-5	-6	-5	-4	-6
50% Blooming *	65	0*		0*	0*	0*	0*	0*	0*	0*
75% Blooming	67	+3		+6	+3	+5	+6	+2	+6	+5
End of Blooming	69	+6		+11	+5	+8	+9	+4	+10	+8
Green maturity	80	+142		+134	+151	+141	+145	+152	+136	+143
Black maturity	88	-		-	+183	+174	+181	+180	+171	+173

* full bloom is considered to be "0"

	M	arc	h															1																					N	Лav						
2017	101	30	31	1	2	3 4	5	6	7	8	9	10	11	12	13	14	15	10	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	Γ,-	0	لر	10	11	12	13 1	4 15
Domat								<mark>55</mark>																					63				65			67			69							
Gemlik												55																				63				65			67		69					
Sarı Ula	k				5	5																						63				65		67		69										
																																							F							
	М	[arc	h	April																					May	/																				
2018			-																																											
Domat																																														
Gemlik					5	<mark>5</mark>															63					65					67			69												
Sarı Ula	ık <mark>5</mark>	55																	63				65						67				69													
																																							F							
	M	larc	h														Ap	ril																					N	Лау						
2019																																														
Domat												55																		63					65						67				6	9
Gemlik						55	5																						63					65					67				69			
Sarı Ula	k			4	55																						63						65					67			69					

Table 4. Placement of BBCH codes of phenological observations made in olive cultivars included in the experiment by months

Septe														em	be	r		_					_					_									_	_						_		С	Oct	ob	ber	l						_		_		_		_	_		N	lov	em	ıbe	r	ľ			
2017	1	2	3	4	5	6	7	8	9	10) 1	1 1	2	13	14	15	10	17	18	19	20	21	22	23	2	4 1	25	26	27	2	8 2	9 3	0	1	2	3	4	5	6	7	7	8	9	10	11	12	13	3 1	4 1	15	16	17	18	19	20	21	22	23	24	25	26	5 27	28	8 2	9 3	30 2	31	1	2 3	5 4	÷ []	, T e	-
Domat																					80																																																				
Gemlik																																				80																																	Т		8	8	٦
Sarı Ulal	ĸ																														8	80																														8	3							L			_
		September												r																													-	С	Oct	ob	ber															_	N	lov	em	ıbe	r						
2018	1	2	3	4	5	6	7	8	9	10) 1	1 1	2 1	13	14	15	16	17	18	19	20	21	22	23	2	4	25	26	27	2	8 2	9 3	0	1	2	3	4	5	6	7	7	8	9	10	11	12	13	3 14	4 1	15	16	17	18	19	20	21	22	23	24	25	26	5 23	28	8 2	9 3	30 3	31	1	2 3	3 4	4 5	5 6	Ī
Domat																																																																									٦
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Sarı Ulal	x 80)																																				88																													٦.						
															Se	pte	em	be	r																													-	С	Oct	ob	ber															_	N	lov	em	ıbe	r	
2019	1	2	3	4	5	6	7	8	9	10) 1	1 1	2 1	13	14	15	16	17	18	19	20	21	22	23	2	4	25	26	27	2	8 2	9 3	0	1	2	3	4	5	6	7	7	8	9	10	11	12	13	3 14	4 1	15	16	17	18	19	20	21	22	23	24	25	26	5 23	28	8 2	9 3	30 3	31	1	2 3	3 4	4 5	; (
Domat															80																																																T				٦						
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Baktır et al. (1995) reported that the inflorescences formation took place between March 22 and April 4, the first flowering was on April 23 in the "Uslu" cultivar, and the last flowering was on the May 9 in the Domat cultivar. They observed that the earliest fruit maturity was on 12 December in "Uslu" and "Edincik Su" cultivars, and the latest on January 4 in "Memecik" and "Sarı Yaprak" cultivars. Toplu (2000) states that bunching begins in the first week of April; the earliest bunching was observed in "Gemlik" cultivar, and the latest in "Savrani" cultivar; reported that flowering took place in May and the flowering period lasted 11-15 days. In addition, it was determined that the number of days from full flowering to greening maturity was between 143-153 days. In this study, the time from the formation of the inflorescencess to fruit set between 31-37 days on average for 3 years. The average temperature is around 20°C during flowering and pollination periods. Time from full bloom to maturity; It was found to be 183 days in 2017, 174 days in 2018 and 181 days in 2019 in Gemlik cultivar. It was determined that this period was 180 days in 2017, 171 days in 2018 and 173 days in 2019 in Sarı Ulak cultivar. According to meteorological evaluations, while the maximum temperatures were lower during the maturity period in 2017, they were higher and stable in 2018 and 2019 respectively (Table 2, 3 and 4).

Selak et al. (2013) reported that high temperatures during full bloom shortened the flowering period by 1 day in Oblica trees. Laaribi et al. (2013) found that the time from the formation of inflorescence to fruit set of Chemlali Sfax cultivar was between 35-63 days. The full bloom period was between April 3 and May 15 on average. Due to the favorable temperature increase until the last ten days of March, it has been determined that bud burst occurs early. Ay (2018) reported that the beginning of flowering occurs in the last week of April, and the full flowering period occurs in the middle of May.

Özdağ and Koyuncu (2017) reported that the formation of the inflorescences of "Çiltopak" cultivar started on March 20, and the bursting of flower buds started on April 20. Özdağ and Koyuncu (2017)have determined that the beginning of flowering is on April 15-25, the full flowering period is on May 01-07, and the end of flowering is on May 20-22. Green stage; While it was found between 13 October (Çiltopak)-20

October (Domat) in 2015, it was detected as 04 September (Çiltopak)-10 September (Domat) in 2016. The black stage period, which was 30 November in 2015, was determined as 09 October in 2016. Gemlik cultivar, the most important native olive cultivar, turned black on October 25 (Özdağ and Koyuncu, 2017).

Sanchez-Estrada and Cuevas (2019) reported that the flowering periods of Manzanilla and Barouni cultivars coincided in two years in their phenological observations in northern Mexico. In contrast to these cultivars, Sevillano trees showed slightly later flowering and a shorter overlap with the flowering time of the Manzanilla cultivar. The beginning of flowering of Manzanilla and Barouni cultivars was on March 14, full flowering was found on March 19 and flowering had ended 1 week later on March 26. The beginning of flowering in 2017 began 5 days after 2016 and ended on April 5th.

In 2017, during the flowering and pollination period, the maximum temperature average was 26.8° C, the minimum temperature average was 14° C, the average temperature was 20.3° C and the relative humidity was 63.2° . Particular attention was paid to the absence of precipitation on the days of pollination . In the fruit maturity period (BBCH: 80-85), the maximum temperature average was 27.6° C, the minimum temperature average was 16.7° C, the average temperature was 21.8° C and the relative humidity was 69.4° (Figure 1,2,3).

In 2018, during the flowering period, the maximum temperature average was 27° C, the minimum temperature average was 14.1° C, the average temperature was 20° C and the relative humidity was 66.8%. In the fruit maturity period (BBCH: 80-85), the maximum temperature average is 34° C, the minimum temperature average is 18.7° C, the average temperature is 25.5° C and the relative humidity is 61.5% (Figure 1,2,3).

In 2019, during the flowering and pollination period, the maximum temperature average was 26.9° C, the minimum temperature average was 13.7° C, the average temperature was 20.2° C and the relative humidity was 66.46%. In the fruit maturity period, the maximum temperature average was 30° C, the minimum temperature average was 17.4° C, the average temperature was

Phenological Growth Stages of Some Olive Cultivars (Olea europaea L.)

 22.9° C and the relative humidity was 67.1% (Figure 1,2,3).



Figure 1. In 2017-2019, maximum average temperature (°C) values during the period from inflorescences to fruit maturity



Figure 2. In 2017-2019, minimum temperature (°C) values during the period from inflorescences to fruit maturity



Figure 3. In 2017-2019, Relative Humidity (%) values during the period from inflorescences to fruit maturity

Conclusion

The study was carried out on Gemlik, Sarı Ulak and Domat olive cultivars which are the most popular cultivars in the region, planted in the same orchard in Tarsus / Mersin Province. Phenological evaluation was made for each cultivar and its relationship with climate was tried to be revealed. As a result of the observations, the olive cultivars in question for Mersin-Tarsus-Yenice region;

• It has been found that the most suitable flowering and fertilization times are between April 15 and May 15.

• The period from the formation of the inflorescences to the fruit set between 31-37 days on average for 3 years.

• The average temperature is around 20°C during flowering and pollination periods.

• It has been determined that the period from the opening of the flowers of the cultivars to the fruit setting stage covers a period of 10 to 15 days.

According to meteorological evaluations, while the maximum temperatures were lower during the maturity period in 2017, they were observed to be higher and stable in 2018 and 2019. The indicated situation was prolonged the maturity process in 2017 and as a result, the harvest time was delayed. Apart from that, the processes were affected similarly to each other in different years. Time from full bloom to maturity;

• It was found to be 183 days in 2017, 174 days in 2018 and 181 days in 2019 in Gemlik cultivar.

• This period was determined as 180 days in 2017, 171 days in 2018 and 173 days in 2019 in Sarı Ulak cultivar.

• In the Domat cultivar, it was determined that there were 142 days in 2017 and 134 days in 2019.

Two factors that should be taken into account during flowering and fruit keeping periods for the Mersin region are high temperature and relative humidity. Especially in 2017, the decrease in temperatures up to 5 degrees at the beginning of somaklama shows that low temperature is a risk factor, although it does not create a risk for the Mersin region.

The minimum temperatures of 5.9-22.2 degrees, the maximum temperatures of 15.6-37.6 degrees and the relative humidity of 35.8-90.4% have been evaluated as suitable values for fruit set. When the climate conditions with flowering and maturity

parameters were evaluated together, the best suggestable cultivar for relevant region is became Sarı Ulak. This study will create an example for phenological evaluations of different cultivars using BBCH codes. In the studies made in olive, the phonological periods are determined but the observations were made subjectively. In this study, the periods for each phenology, which was formed by Sanz-Cortes et al. (2002) in detail, was given clearly that can be observed by researchers. This was carried out as a difference in the study and suggested to be usable for future studies in order to make common markers.

Acknowledgements

This study was produced from the PhD thesis of Researches On Reproductive Biology of Gemlik, Sarı Ulak and Domat Olive Cultivars. Authors are grateful to Barış Gürbüz for providing plants.

This work was supported by Ankara University Scientific Research Projects [Project number 18L0447008].

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