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ASSESSMENT OF TIMING OF BUD DIFFERENTIATION AND DEVELOPMENT STAGES OF FLOWER INFLORESCENCE IN BLACKBERRY (*Rubus* spp.) CULTIVARS IN NORTHERN TURKEY

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ABSTRACT

Blackberry (*Rubus* spp.) production is attracting interest in Turkey's northern part, but information on timing of bud differentiation and developmental stages of flower inflorescence on next season is limited. The objective of this study was to determine the timing of bud differentiation and development stages of flower inflorescence in 4 biennial fruiting blackberry (*Rubus* spp.) cultivars ('Chester', 'Dirksen Thornless', 'Jumbo', 'Bursa 1') grown in northern Turkey. Axillary bud samples were collected from the middle parts of the one year of lateral branches every 7–15 days from September 2008 to May 2010. Ten development stages were identified from the flower bud differentiation to post-bloom period. The transition from the vegetative to reproductive stage occurred during September to October, with the differentiation of the terminal flower occurring on September 18 in 'Bursa 1', October 4–9 in 'Dirksen Thornless', October 16–20 in 'Jumbo', and October 20–22 in 'Chester'. In all the examined cultivars, flower development occurred between September and June and lasted for 193–215 days.

Key words: *Rubus*, blackberry, flower initiation, floral development, paraffin methods, microscopy

INTRODUCTION

Taxonomically, blackberries are classified in *Rubus* subgenus *Rubus* (formerly, *Eubatus*). They are grown in many parts of the world, but they are most productive in regions with mild winters and long, moderate summers [Finn 2008]. Blackberry cultivars greatly differ in their fruit size and plant growth habits, being classified as erect, western trailing, and semi-erect according on their gross morphology [Crandall 1995]. Most blackberry cultivars have biennial fruiting canes (floricane fruiting), however, blackberry germplasm lines with annual fruiting canes (primocane fruiting) are now available [Ballington and Moore 1995].

Flowers and fruits are born in a panicle-like or racemosecymb, with primary fruit ripening prior to secondary, quaternary, or tertiary [Hummer and Janick 2007]. The flower receptacle has multiple pistils (many ovaries, with styles, and stigmas) and is surrounded by white or pink petals; double flowers are not uncommon. The flowers are self-fertile, but each flower needs pollen transferred by a pollinator particularly bee pollination to yield a fully formed, large fruit. After fertilization, an aggregate fruit is produced that consists of the central torus (receptacle) surrounded by a number of fleshy drupelets that each contains a seed (pyrene). The process of flower bud formation is essentially the same in all types of bramble fruits. Fruits are borne on fruit lateral canes, which appear during spring from buds developed on the cane in the

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previous year [Poling 1997]. In thorny blackberry cultivars, flower bud differentiation occurs basipetally within canes and inflorescences, starting with the formation of terminal flower [Daubeny 1996]. In contrast, in eastern thornless cultivars, once the terminal flower has formed, subsequent differentiation occurs at the base of the inflorescence and proceeds acropetally [Takeda 1987].

The onset, progression, and completion of reproductive development appear to be highly variable among blackberry cultivars and are partly influenced by the prevailing environmental conditions and some internal factors [Waldo 1933]. In general, short days and cool temperatures cause flower bud initiation in brambles [Sønsteby and Heide 2009].

Flower initiation and flower bud development have been examined in some blackberry varieties [Waldo 1933, Robertson 1957, Takeda and Wisniewski 1989, Warmund et al. 1992, Takeda et al. 1996, 2002, 2003, Lopez-Medina and Moore 1999]. However, these parameters have not been determined for blackberry cultivars in Turkey, where blackberry cultivation is limited and based on biennial fruiting varieties.

Knowledge of the timing of flower bud differentiation is important for influencing the formation of flower buds through various horticultural practices, particularly pruning and fertilization in various localities [Mathers 1952]. Furthermore, knowledge of the time of onset of flower bud formation and flower development stages in blackberries may be useful for the application of strategies aimed at manipulating fruit production [Hussain et al. 2016]. Therefore, in the present study, we aimed to determine the timing of bud differentiation and development stages of flower inflorescence in blackberry cultivars grown in North Turkey.

MATERIALS AND METHODS

Experimental site. A trial orchard was established in the Horticulture Research Area of Ondokuz Mayis University (41°21'N and 36°11'E) in Samsun, Turkey, in March 2008.

Selection of cultivars and their characteristics. Four thornless blackberry cultivars ('Chester', 'Dirksen Thornless', 'Jumbo' and 'Bursa 1') with semi-erect growth habits were grown in the orchard. 'Chester' and 'Dirksen Thornless' are of North American origin [Moore and Skirvin 1990], while the origins of 'Jumbo' [Clark et al. 2007] and 'Bursa 1', which was selected from Bursa Province, were unknown. 'Bursa 1' is a very productive cultivar and is more appropriate for use in the industry because of its small fruit size [Demirsoy et al. 2006].

Features of blackberry cultivars. Some features of blackberry cultivars used in our study are summarized as follows:

Chester. The most winter hardy of the thornless varieties with large (individual fruit size up to one inch), sweet, high quality berries with good flavor; plants are quite shrubby [Galletta et al. 1998].

Dirksen Thornless. These cultivars have big, thick clusters of large, sweet, glossy black berries. They are exceptionally vigorous, highly productive, thornless plants with erect, self-pollinating canes and are not particularly winter-hardy [Demirsoy et al. 2006, UMass Extension 2017].

Jumbo and Bursa 1. They are one of the earliest ripening cultivars and having large size of fruits [Gerçekçioğlu and Esmek 2005, Demirsoy et al. 2006, Agaoglu et al. 2007].

Experimental treatment and management. The blackberry plants were planted in 1.7 × 2.1 m plots with 4 replications and 5 plants per replicate. Standard blackberry production practices were followed, such as mulching with black plastic for weed control in the interrow spaces, annual spring application of nitrogen (60 kg·ha^{-1} ammonium sulfate), removal of floricanes after summer harvest, topping of primocanes, trimming of lateral branches, and drip irrigation. Plants were trained to 3 horizontal wires at 0.6, 1.2 and 1.8 m above the ground.

Bud differentiation and development. To examine bud differentiation, 10–20 axillary bud samples were collected from the middle portion of the one year of lateral branches at 15-day intervals from September to May during 2008/09 and once a week from August to May during 2009/10. The bud samples were fixed in a formalin–acetic acid–alcohol (FAA) solution (40% formaldehyde, glacial acetic acid, and 70% alcohol; 5 : 5 : 90, v/v) for at least 24 h. The samples were then transferred into bottles containing 70% ethyl alcohol [Johansen 1940]. The development stages of the blackberry flower buds were examined using the switching on and paraffin methods, as outlined below,

and classified according to Takeda and Wisniewski [1989], with minor adaptations (Tab. 1).

Switching on method. The scales of buds preserved in 70% ethyl alcohol were removed with a scalpel to reveal the apical meristem. The exposed apices were then examined and photographed under a stereomicroscope (model SZ61; Olympus) with an attached camera to examine the morphological differentiation of the meristems and quantify the development stage of each bud [Paydaş 1988, Beyhan 1993].

Table 1. Bud differentiation and development stages of flower inflorescence in blackberry. For detailed description of each developmental stage, refer to Takeda and Wisniewski [1989] with the minor adaptations

Stage	Description
1	Apical meristem differentiation is not started yet. Buds in vegetative phase with leaf primordia encircling flat apical meristem.
2	Apical meristem in terminal flower with a few leaves starts to grow along and bract draft is highlighted. This phase has been considered as morphological differentiation. Axillary flower primordia begin to form around the side of the terminal flower.
3	Apical meristem of terminal flower is enlarge and sepal primordia are evident.
4	Apical meristem of terminal flower gets bigger more. Sepal primordia expand and become three-lobed.
5	Petal primordia are highlighted. Sepals unite and surround the central receptacle.
6	Apical meristem of terminal flower lengthens and shape dome. Sepal primordia expand. Petals and sepals ranked on flower plane.
7	Stamen primordia become evident on the receptacle.
8	Pistil primordia become visible on receptacle. The lengths of stamens extended.
9	Pistils cover the central receptacle. Petals expand and enclose receptacle, stamens and pistils.
10	Anthers and filaments develop, styles and stigmas become visible on pistils.

Table 2. Process steps, processing time and number repetition of processes and probable temperatures realized during processes in microwave oven in paraffin method*

Process	Processing time	No. of repetition of the processes	Probable temperatures (ºC)
70% ethyl alcohol	10 seconds	6	49–59
80% ethyl alcohol	10 seconds	8	50–59
90% ethyl alcohol	10 seconds	8	50–62
100% ethyl alcohol	10 seconds	8	55–60
3 unit etyl alcohol + 1 unit xylene	10 seconds	6	56–62
2 unit etyl alcohol + 2 unit xylene	10 seconds	6	55–61
1 unit etyl alcohol + 3 unit xylene	10 seconds	8	54–58
Pure xylene	3 minutes	8	52–60
Pure xylene + piece paraffin	3 minutes	3	62–68
One coat of liquid paraffin	3 minutes	2	60–67
Liquid paraffin	3.5 minutes	4	67–70
Liquid paraffin	3.5 minutes	30–40	65–71

*All the processes were carried in a microwave in 100% power (microwave used in the study has four power level; 100% level is the fourth and maximum level)

Paraffin method. The flower development stages were examined anatomically with the paraffin method described by Johanson [1940], using a microwave oven. The method modified from Demirsoy [1999] who used a microwave oven for dehydration and paraffin infiltration processes for grafting incompatibility works on plum. Main steps of paraffin method such as fixation (in FAA), dehydration (in a graded series of ethyl alcohol and xylol) and paraffin infiltration, were mentioned in Table 2. The bud samples were then embedded in paraffin and sectioned at a thickness of 10 µm by a rotary microtom (model HM310, Microm). The sections were flattened on glass slides coated with glycerin–albumin adhesive and stained with hematoxylin and safranin according to Johanson [1940]. The slides were examined under a light microscope (model DM1000; Leica) and photographed.

RESULTS

The dates of some phenological development stages of flowers in the blackberry cultivars were recorded in the 2009/10 growing season (Tab. 3). 'Bursa 1' was the earliest cultivar in terms of all the phenological stages except of the end bloom in 2010. Secondary buds were observed just below the primary buds (Fig. 1A). These buds were smaller than the primary buds but coarsened in the same manner as the primer buds in spring and sometimes formed a cluster. The process of flower bud formation was essentially the same in all cultivars of blackberry fruits in experiment. The fruit was borne on laterals appearing during spring from buds on canes formed during the previous year. The differentiation pattern described in Table 1 is typical of a terminal flower. Other flowers within the inflorescence also matured in this sequence, as shown in Table 1. The differentiation of the buds within a single inflorescence was clearly acropetal (Fig. 1B).

The terminal flower, which is located at the top of the cluster, differentiated first, following which the axillary flowers in the bud developed acropetally in the inflorescence (Stage 9b, Fig. 3). In the same manner, the opening sequence of flowers in a cluster began with the terminal flower at the end of the cluster and progressed upward, starting from the flowers at the bottom of the cluster (Fig. 1B). Within inflorescences, the mature berries appeared in the same sequence as the flower opening (Fig. 1C). There was no evidence of flower differentiation in the buds of the blackberry cultivars, the apical meristem was flat and small in August, indicating that the buds were vegetative (Fig. 2, stage 1a–b). As the buds transitioned from the vegetative to reproductive stage, the growing point expanded with swelling, the bract primordia became visible around it, and the leaf primordia expanded. This phase was defined as morphological differentiation (Fig. 2, stage 2a–b). During this stage, the axillary flower primordium also became visible on the spiral side directly below the apical meristem. Following the expansion and elongation of the apical meristem, the sepal primordia around the apical meristem started to appear (Fig. 2, stage 3a–b). At this stage, the leaf primordia grew further, allowing the triple structure of the leaves and their formidable margins to be seen (Fig. 2, stage 3a).

At stage 4, the sepal primordia rotated around the terminal flower in a spiral manner and expanded. Following further expansion and elongation of the apical meristem, sepal development was completed (Fig. 2, stage 4a–b). The petal primordia then began to form immediately below the sepals (Fig. 2, stage 5a–b). Once the development of the petal primordia had completed, the apical meristem thoroughly expanded and became dome shaped (Fig. 3, stage 6a–b). The stamen primordia then appeared at the base of the petals in the form of beads, with further expansion of the apical meristem (Fig. 3, stage 7a–b).

Following petal primordial expansion, the stamens and pistils were observed (Fig. 3, stage 8a). The pistil primordia began to growing a spiral manner in the lower parts of the apical meristem, and over time, they continued to form and develop toward the upper part of the growth point (Fig. 3, stage 8a–b). In stage 9 petals grew completely and covered the flower (stage 9b). Thereafter, all the stamens and pistils grew and covered the receptacle (Fig. 3, stage 9a). All the flower structures continued development until the flower bud opened (stage 10a). The filaments and styles then elongated, and the anther sacs and stigmas became visible (Fig. 3, stage 10b). All the flower development stages in the examined blackberry cultivars are summarized in Table 1.

Once the terminal flowers in the clusters had opened, the flower development stages were completed.

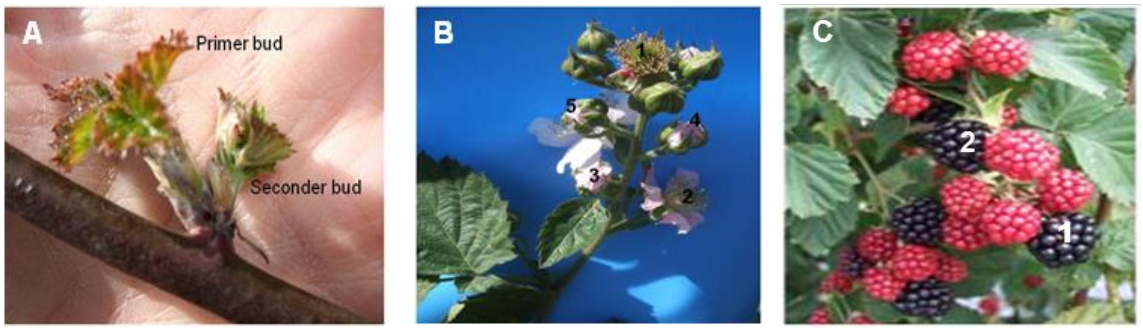

Figure 1. Bud structure of blackberry

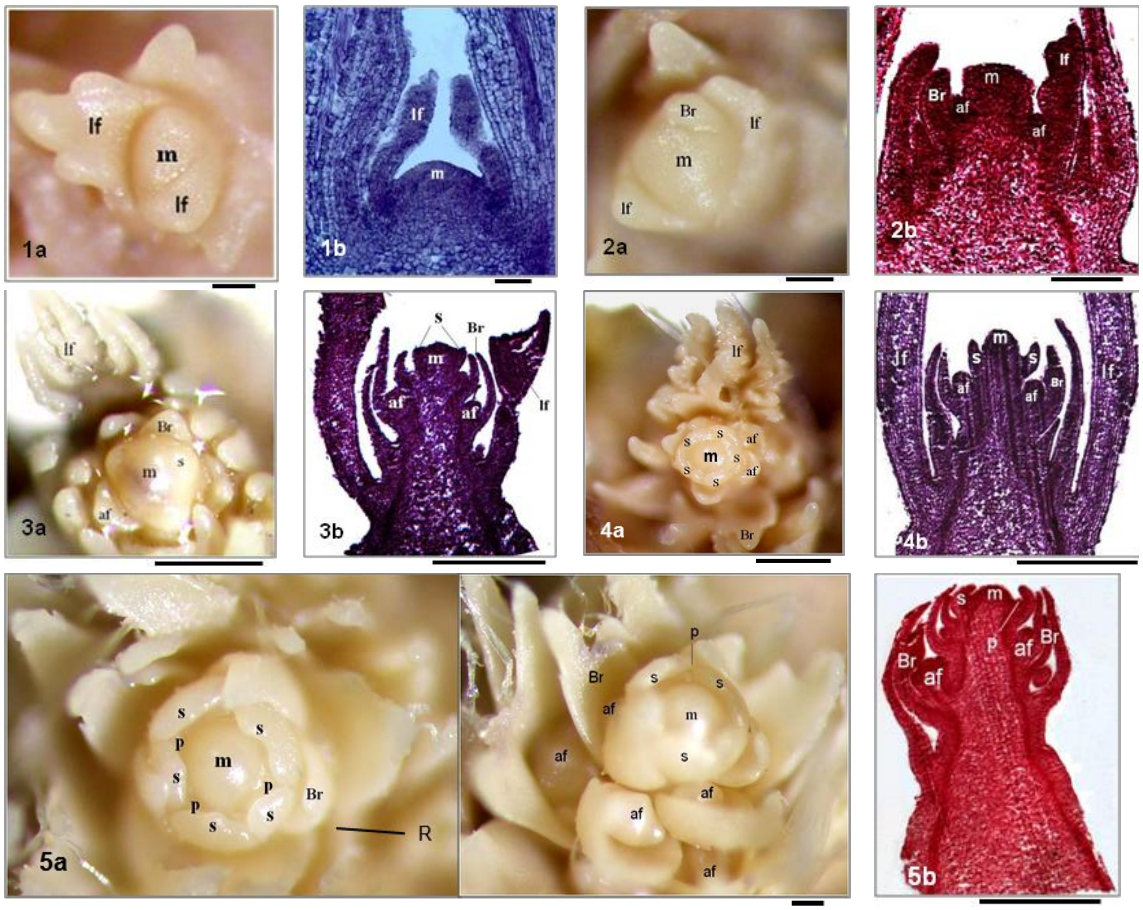

Figure 2. Floral developmental stages of blackberries

Stage 1: apical meristem (m) differentiation has not started yet. Stage 2: apical meristem in terminal flower with a few leaves (lf) starts to grow along and bract (Br) draft is highlighted. This phase has been considered as morphological differentiation. It can be seen that axillary flower primordia (af) are occurred on both sides of the apical meristem. Stage 3: apical meristem of terminal flower is enlarged and sepal primordia (s) are evident. Stage 4: sepal primordia expand and become three-lobed. Apical meristem of terminal flower gets bigger more. Stage 5: sepals unite and surround the central receptacle (R). Petal primordia (p) are highlighted. (1a–5a: stereo microscope views, 1b–5b: light microscope views). Bars = 0.5 mm

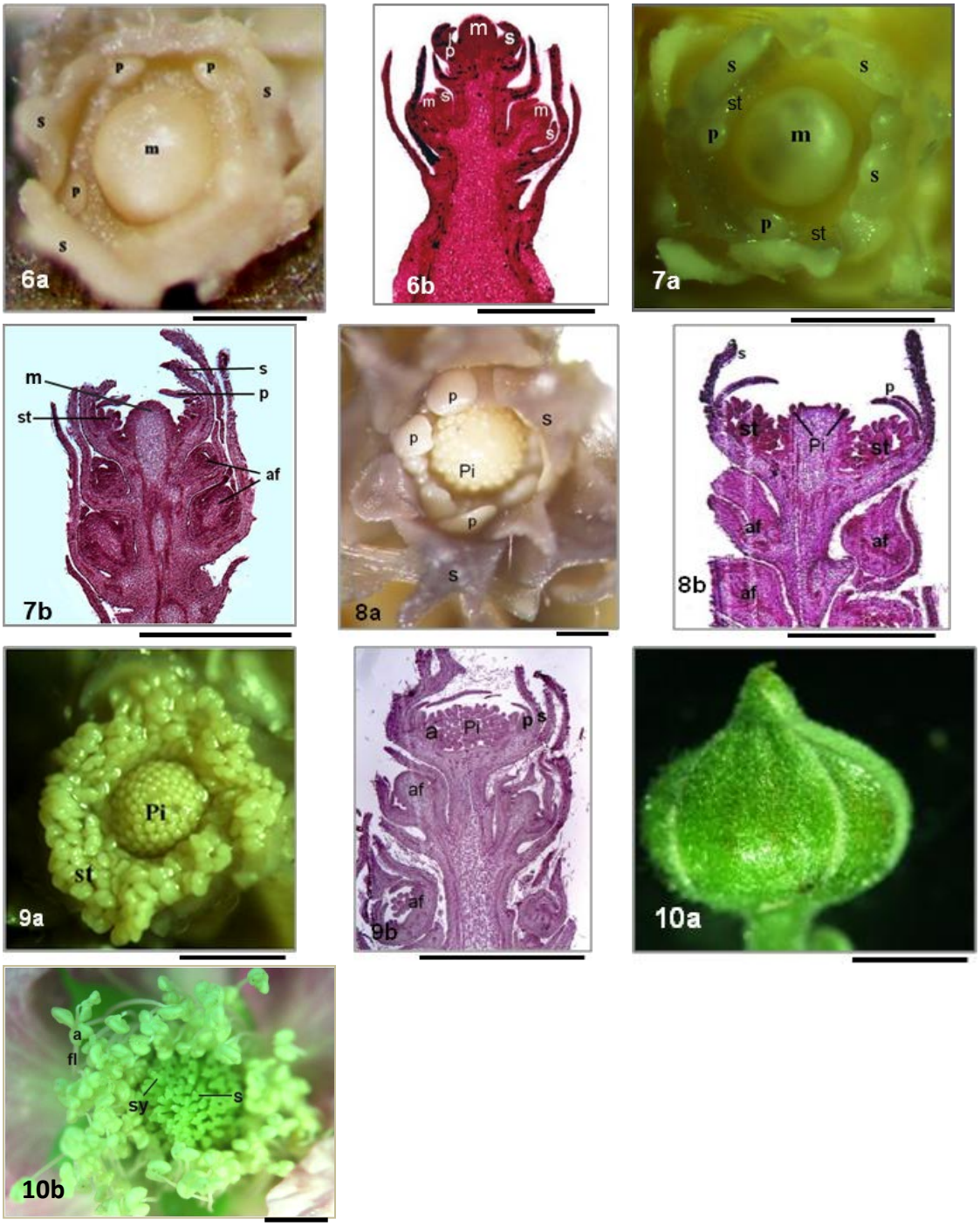

Figure 3. Floral developmental stages of blackberries

Stage 6: apical meristem of terminal flower lengthens and shape dome. Sepal primordia expand. Petals and sepals ranked on flower plane. Stage 7: stamen primordia (st) become evident on the receptacle. Stage 8: pistil primordia (Pi) become visible on receptacle. The lengths of stamen extended. Stage 9: gynoecia (Pi) cover the central receptacle. Petals expand and enclose receptacle, stamen and pistil. Stage 10: in unopened flower bud (10a) anthers (a) and filaments (fl) develop; style (sy) and stigma (S) become visible on pistil. (6a–10a and 10b: stereo microscope views, 6b–9b: light microscope views). Bars = 0.5 mm

Table 3. Phenological development dates of flower in 'Jumbo', 'Chester', 'Dirksen Thornless' and 'Bursa 1' blackberries growing in 2009–2010

Varieties	Bud swelling		Bud break		Inflorescence emergence		First bloom		Full bloom		End of bloom		First ripen fruits	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Jumbo	18/3	20/3	6/4	3/4	14/5	15/5	20/5	19/5	1/6	1/6	17/6	14/6	3/7	7/7
Chester	19/3	19/3	5/4	2/4	8/5	10/5	18/5	17/5	31/5	1/6	15/6	15/6	6/7	7/7
DTL*	21/3	22/3	6/4	29/3	15/5	12/5	22/5	12/5	4/6	1/6	17/6	9 /6	3/7	5/7
Bursa 1	12/3	14/3	30/3	25/3	3/5	1/5	11/5	6/5	22/5	27/5	6/6	12/6	30/6	1/7

***** DTL – Dirksen Thornless

Depending on the weather conditions, the flowers continued to open gradually from the bottom to the top (Fig. 1B). Following the completion of pollen development, the pollen sacs containing the pollen opened (Fig. 4A). Pollen was then dispersed to reach the stigma and pistil was maturated with the style, stigma and ovary on receptacle (Fig. 4B). Pollination and fertilization occurred by pollen transfer from the anther to the stigma (Fig. 4C, D). Following fertilization, each pistil developed into a miniature fruit (drupe) (Fig. 4E). The number of fruit cells increased and then miniature fruits grew bigger (Fig. 4F). Following maturation, the first fruit became red, while the later fruits varied in color depending on the cultivar (Fig. 1C). The date of each development stage in each cultivar was recorded for 2 years in Table 4.

DISCUSSION

In the present study, we investigated the flower development stages, bud structure, and phenological development of flowers in the 4 main blackberry cultivars grown in Turkey. It was investigated from the results that the secondary buds of these cultivars were smaller than the primary buds and located just below them and sometimes they form clusters. Blackberries and raspberries usually have secondary buds and occasionally have tertiary buds, lying dormant just below the primary buds. Secondary buds develop in the event of injury to the primary bud [Warmund and George 1990, Warmund et al. 2008]. Keep [1969] showed that these secondary buds were very small and usually vegetative and that their clusters were smaller and weaker than those of primary buds. When they occur, flower bud differentiation in the secondary buds followed that in the primary bud and appeared to be stimulated by injury to the primary bud.

In all the evaluated cultivars, firstly the terminal flower and later the flower at the base of the inflorescence differentiated following which the differentiation proceeded acropetally. These findings are similar to those obtained in previous studies on thornless cultivars of blackberries [Takeda 1987, Takeda and Wisniewski 1989, Takeda et al. 2002].

In August, the buds of blackberries studied were quite small. However, as the sampling progressed, buds developed, especially buds in the morphological differentiation phase were more evident than those of the vegetative phase. The buds of all the cultivars were observed to be vegetative until the middle of September (Tab. 4). Similarly, Takeda et al. [2003] found that the buds of 'Boysen', 'Marion', and 'Cherokee' blackberry cultivars in Oregon and Arkansas were vegetative in August. Examination of the blackberry buds under a stereomicroscope revealed the time at which the buds changed from a vegetative to a reproductive state, which is marked by the enlargement of the apex [Takeda and Wisniewski 1989]. The first morphological indication of the transition from the vegetative to reproductive stage was the change in the shape of the

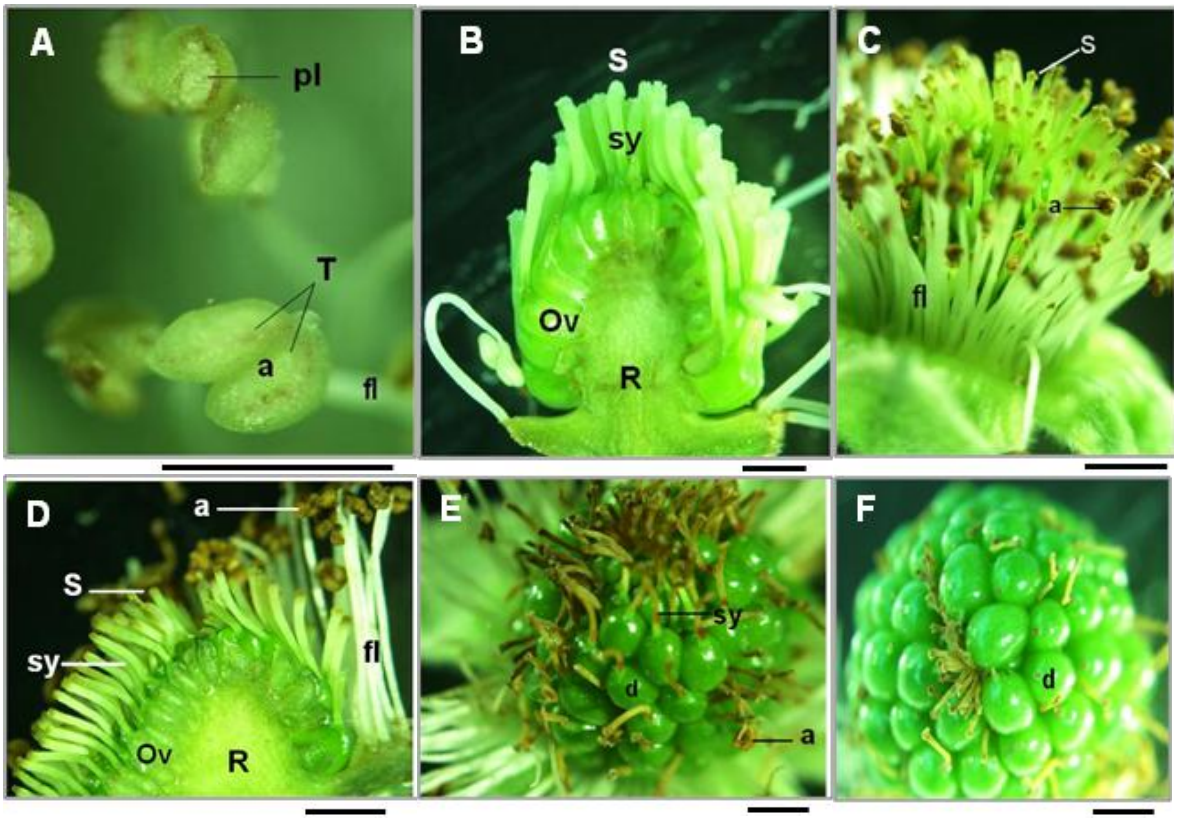

Figure 4. Flowers and frut-sets of blackberries

A, B – first flowers open anthers consists of (a) two thecae (T) with pollen grains (pl) and ovaries (Ov) are visible. Stigma (S), style (sy), Receptacle (R), Flament (fl). C, D – the occurrence of full bloom and fertilization. E – the end of blooming. Small drupelets (d) are visible. F – the number of drupelet (fruit) cells increased and then grew bigger (fig. A–F: stereo microscope views). Bars = 0.5 mm

Table 4. Bud differentiation and flower inflorescence development stage dates of 'Jumbo', 'Chester', 'Dirksen Thornless' and 'Bursa 1' blackberries growing in 2009–2010

Stages	Jumbo		Chester		DTL*		Bursa 1	
	2009	2010	2009	2010	2009	2010	2009	2010
1	4/10	2/10	4/10	2/10	18/9	18/9	3/9	3/9
2	20/10	16/10	20/10	22/10	4/10	9/10	18/9	18/9
3	4/11	13/11	19/11	13/11	20/10	22/10	4/10	2/10
4	4/12	8/12	4/12	8/12	19/11	23/11	20/10	30/10
5	20/1	22/1	20/1	22/1	19/12	8/12	19/11	13/12
6	19/2	23/2	19/2	8/2	20/1	22/1	20/1	22/1
7	6/3	10/3	6/3	23/2	19/2	23/2	19/2	8/2
8	23/3	24/3	23/3	19/3	6/3	10/3	6/3	10/3
9	6/4	7/4	6/4	7/4	6/4	1/4	23/3	24/3
10	6/5	3/5	6/5	3/5	6/5	26/4	21/4	19/4

***** DTL – Dirksen Thornless

apex from flat to dome, as expressing by Foster et al. [2003]. In both 2008/09 and 2009/10, 'Bursa 1' exhibited the earliest morphological differentiation (September 18), followed by 'Dirksen Thornless' (October 4–9), 'Jumbo' (October 16–20), and 'Chester' (October 20–22). Thus, in general, morphological differentiation occurred in fall (September–October) – Table 4.

In temperate regions, shoot extension diminishes in late summer or fall and an irreversible process termed flower induction occurs in the axillary buds, wherein some parts of the meristem are programed to form flowers [Bernier et al. 1981]. The timing of flower bud formation has shown difference greatly depending on varieties and locations. Some other blackberry varieties also underwent morphological differentiation in fall. For instance, morphological differentiation occurred in September in 'Eldorado' and a wild blackberry (*R. canadensis* L.) in Maryland [Waldo 1933] and in the erect-growing 'Cherokee' in Arkansas and Oregon [Takeda et al. 2003]. This process occurred in October in 'Austin Thornless', 'Loganberry', 'Mommoth', and a wild blackberry (*R. macropetalus* Dougl.) in Oregon [Waldo 1933]; 'Young Dewberry' in Maryland and Oregon [Waldo 1933]; 'Boysen' and 'Marion' trailing blackberries in Oregon and Arkansas [Takeda et al. 2003] and 'Black Satin' in West Virginia [Takeda and Wisniewski 1989]. However, other varieties underwent morphological differentiation in spring [Waldo 1933, Warmund et al. 1988, 1992, Takeda and Wisniewski 1989, Takeda et al. 1996, 2002].

'Bursa 1', which has the third best performance in terms of yield, fruit strength, taste, and shoot growth among 12 blackberry varieties cultivated in North Anatolia [Demirsoy et al. 2006], underwent the earliest morphological differentiation and provided earliness the most phenological data among the studied cultivars. In turn 'Dirksen Thornless' showed latest in first bloom and full bloom (Tab. 3) and the time of blooming was the most shorter in this cultivar. 'Dirksen Thornless' had got weaker shoot growth than the other cultivars.

Flower bud differentiation was found to occur in September and October in the blackberry cultivars 'Jumbo', 'Dirksen Thornless', 'Chester', and 'Bursa 1' in North Anatolia. This indicates that the potential yield could be influenced by nutritional factors and that the application of manures may be useful in late summer. Because 'Bursa 1' undergoing early bud differentiation in autumn, early autumn nitrogen fertilization may be applied safely in this cultivation.

CONCLUSION

This study aimed to determine the timing of bud differentiation and development stages of flower inflorescence in 'Chester', 'Dirksen Thornless', 'Jumbo' and 'Bursa 1' blackberry cultivars grown in northern Turkey. Ten development stages were identified from the flower bud differentiation to post-bloom period. The transition from the vegetative to reproductive stage occurred on mid-autumn in all examined cultivars. Flower development occurred between September and June and lasted for 193–215 days.

An understanding of the timing of flower initiation and differentiation helps in selecting the correct time for cultivation practices, including the application of plant growth regulators and nitrogen fertilizers, desuckering and the use of row covers. In particular, it is likely that determining the timing of flower bud differentiation will the most valuable factor for increasing the yield and quality of blackberries in agro climatic condition of Turkey.

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