

Examination of Flower Bud Initiation and Differentiation in Sweet Cherry and Peach by Scanning Electron Microscope

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Abstract: Flower initiation and development in 0900 Ziraat sweet cherry (*Prunus avium* L.) and Glohaven peach (*Prunus persica* L.) were examined using scanning electron microscopy. The objectives were to determine the timing of floral developmental stages and to achieve a better understanding of the morphological changes during flower formation at the apex of axillary buds of sweet cherry and peach cultivars. In cherry, bud samples were taken every 10 days from June 26 to August 24 and stored in formalin, 70% ethanol, and acetic acid solution (10:50:5, by volume). In peach, bud samples were collected every 10 days from June 28 to September 16 and stored in the same solution. In cherry, on July 5, 85 days after anthesis (DAA), a swelling of the apex signified the initial change from the vegetative to the reproductive stage. Flower primordia differentiated in the axils of bracts on July 15. Sepal primordia were evident on July 25, 15 weeks after anthesis, and petal primordia had formed by August 4. Concentric rings of stamen primordia differentiated between August 4 and 24, followed by the pistil. These organs of the flower attained their normal forms by the end of August. In peach, on July 8, 109 DAA, a flattened apex signified the initial change from the vegetative to the reproductive stage. After this stage, floral primordia developed in the order of sepal, petal, stamen, and carpel. Sepal primordia were evident on August 17, 149 DAA, petal primordia were evident on August 27, 159 DAA, and rings of stamen primordia had formed by September 16, followed by the carpel. These organs of the flower attained their normal forms by the end of September.

Key Words: Floral formation, flower primordia, *Prunus avium*, *Prunus persica*, *Rosaceae*

Kiraz ve Şeftali Çiçek Tomurcuklarında Çiçek Organ Taslaklarının Oluşumu ve Farklılaşmasının Taramalı Elektronik Mikroskopta İncelenmesi

Özet: Kiraz 0900 Ziraat (*Prunus avium* L.) ve şeftali Glohaven (*Prunus persica* L.) çiçek tomurcuklarındaki çiçek organ taslaklarının oluşumu ve gelişimi taramalı elektronik mikroskopta incelenmiştir. Bu çalışmada, kiraz ve şeftali çiçek tomurcuklarındaki çiçek organ taslaklarının gelişim dönemlerini belirlemek ve büyüme konisindeki şekilsel değişimlerin ayrıntılarının ortaya konulması amaçlanmıştır. Kiraz çiçek tomurcukları, 26 Haziran'dan 24 Ağustos tarihine kadar 10'ar gün arayla alınarak, FAA (formalin, etil alkol, asetik asit) (10:50:5) ortamında fikse edilmiştir. Şeftali tomurcukları da 28 Haziran'dan itibaren 16 Eylül tarihine kadar alınarak aynı ortamda muhafaza edilmiştir. 0900 Ziraat kiraz çeşidinin çiçek tomurcuklarında morfolojik ayırım, 5 Temmuz tarihinde anthesisden 85 gün sonra meydana gelmiştir. Çiçek primordiası 15 Temmuz tarihinde şekillenmiştir. Çanak yaprak taslakları 25 Temmuz'da anthesisden 15 hafta sonra, taç yaprak taslakları da 4 Ağustosta oluşmuştur. Erkek organ taslakları 4-24 Ağustos tarihleri arasında farklılaşmıştır. Bütün organ taslakları, Ağustos ayı sonunda şekillenmiştir. Glohaven şeftali çeşidinin çiçek tomurcuklarında morfolojik ayırım 8 Temmuz tarihinde anthesisden 109 gün sonra olmuştur. Morfolojik ayırımdan sonra sırasıyla çanak yaprak, taç yaprak, erkek ve dişi organ taslakları oluşmuştur. Çanak yaprak taslakları 17 Ağustos'ta anthesisden 149 gün sonra, taç yaprak taslakları 27 Ağustos'ta anthesisden 159 gün sonra ve erkek ve dişi organ taslakları 16 Eylül'e kadar oluşmuştur. Çiçek organ taslaklarının tamamı Eylül sonunda normal şekillerini almışlardır.

Anahtar Sözcükler: Çiçek oluşumu, çiçek organ taslağı, *Prunus avium*, *Prunus persica*, *Rosaceae*

Introduction

Some of the most important Turkish cherry cultivars frequently have low yield, which affects total productivity

year by year. 0900 Ziraat, the most popular cultivar in Turkey on account of its excellent fruit quality, is particularly susceptible to this problem, especially when

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grown in the western part of the country. Flower bud initiation has great importance in fruit cultivation due to the dependence of fruit formation. Flower bud initiation occurs through a biochemical signal. This biochemical signal makes it possible for the tissue to change from vegetative to reproductive state in a programmed term (Faust, 1989). It occurs as a result of the balance of GA₃, auxin, cytokinins, and ethylene-like hormones (Westwood, 1993). Floral initiation in sweet cherry occurs after harvest. Sepals, petals, stamens, and pistil differentiate sequentially (Westwood, 1993).

Glohaven is an early peach cultivar that yields small fruits unless heavily thinned. Gibberellins are known to inhibit flower initiation in *Prunus* species (Bradley and Crane, 1960). Peach cultivars can be thinned by gibberellin application in the previous summer (Corgan and Widmoyer, 1971; Pallas et al., 2001). Experiments carried out by Taylor and Geisler-Taylor (1998) showed that flower inhibition in peach depended upon the date of GA₃ spray. Knowledge about the timing of different stages of flower bud initiation in peach is important in order to increase the flowering location in the canopy and to obtain regular yield every year.

It may be important to know the timing of floral initiation and developmental changes for developing management strategies in order to enhance flowering and ultimately regulate fruit crop loads. For example, GA₃ application at the beginning of morphological differentiation of flower buds affects the flower thinning and delay in flowering in some peach cultivars (Mizutani et al., 1996). In pear and sweet cherry trees, growth retardants such as daminozide (SADH) used for inhibiting the biosynthesis of GA₃ were found to encourage flower bud initiation (Ryugo, 1986). Sweet cherry buds are most sensitive to the induction of abnormal flower primordia at high temperatures at the transition stage from sepal to petal differentiation and forcing culture can be applied to sweet cherry production in warm areas to reduce abnormal flower formation by avoiding the exposure of buds to high temperatures while the buds are still in the sensitive period (Beppu et al., 2001). High temperature interfered with floral initiation and differentiation in some species of *Prunus* (Ryugo, 1988; Shen et al., 1999), and so it is important to know the timing of sweet cherry floral initiation and developmental changes to avoid high temperatures during routine cultural practices, such as use of over-tree sprinkler irrigation or applying artificial shading.

The presence of floral bud initiation and developmental changes in sweet cherry and peach trees helps one to choose the correct time for cultural practices. The timing of floral initiation and developmental changes has not been determined for any sweet cherry or peach cultivars in Turkey yet. Taking into account this situation, in the present investigation, the major objectives were to determine the timing of these stages and differentiation and to achieve a better understanding of the morphological changes at the apex of axillary buds of sweet cherry and peach during flower formation. Thus, sweet cherry and peach researchers and orchard managers will have reliable information on the phenology of flower development.

In this study, the timing of sweet cherry and peach floral initiation and developmental changes was determined in western Turkey. Furthermore, the morphological changes of 0900 Ziraat sweet cherry and Glohaven peach flower buds were examined by scanning electron microscopy (SEM).

Materials and Methods

This study was conducted in the 14-year-old sweet cherry 0900 Ziraat and 10-year-old peach Glohaven orchard located at the Department of Horticulture, Faculty of Agriculture, Ege University, İzmir. Monthly average temperatures and total rainfall during the year of the study (2004) for the location are presented in the Table.

Fifteen buds uniform in size and vigor were collected every 10 days from 5 trees. Cherry bud samples were taken from June 26 to August 24, 2004. Peach bud samples were taken from June 28 to September 16, 2004. Sample collection ended when more than 50% of the dissected buds had pistils initiated.

The samples were fixed and stored in solution of formalin, 70% ethanol (EtOH), and acetic acid (10:50:5, by volume) (McLaughlin and Greene, 1991). Fifteen buds from each sample were dissected using an Olympus SZ 60 stereomicroscope before processing for SEM. Buds were rinsed twice (10 min each) in 50% EtOH to remove the FAA from the plant tissue and were kept in 50% EtOH during dissection to prevent desiccation. Later the samples were dehydrated in EtOH series (one time 50%, 70%, and 95% and then twice in 100% for 10 min) (Guimond et al., 1998). The samples were dried with a

Table. Monthly average temperature (°C) and total rainfall (mm) values (2004).

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Average temperature (°C)												
2004	7.1	8.2	12.2	15.7	20.3	26.5	29	27.8	23.8	19.8	13.2	10.7
Total rainfall (mm)												
2004	189	26.8	12.9	29.6	10.7	1.6	1.8	0.0	0.0	1.6	72.6	45.7

critical-point dryer and stored in a desiccator over anhydrous CaSO₄. Then, the samples were mounted on stainless – steel stubs with carbon tape before being gold coated with a sputter coater (Polaron SC 502). The samples were examined with a scanning electron microscope (JEOL ISM 5200). Morphological differentiations of the investigated materials were photographed with the camera attached to the microscope.

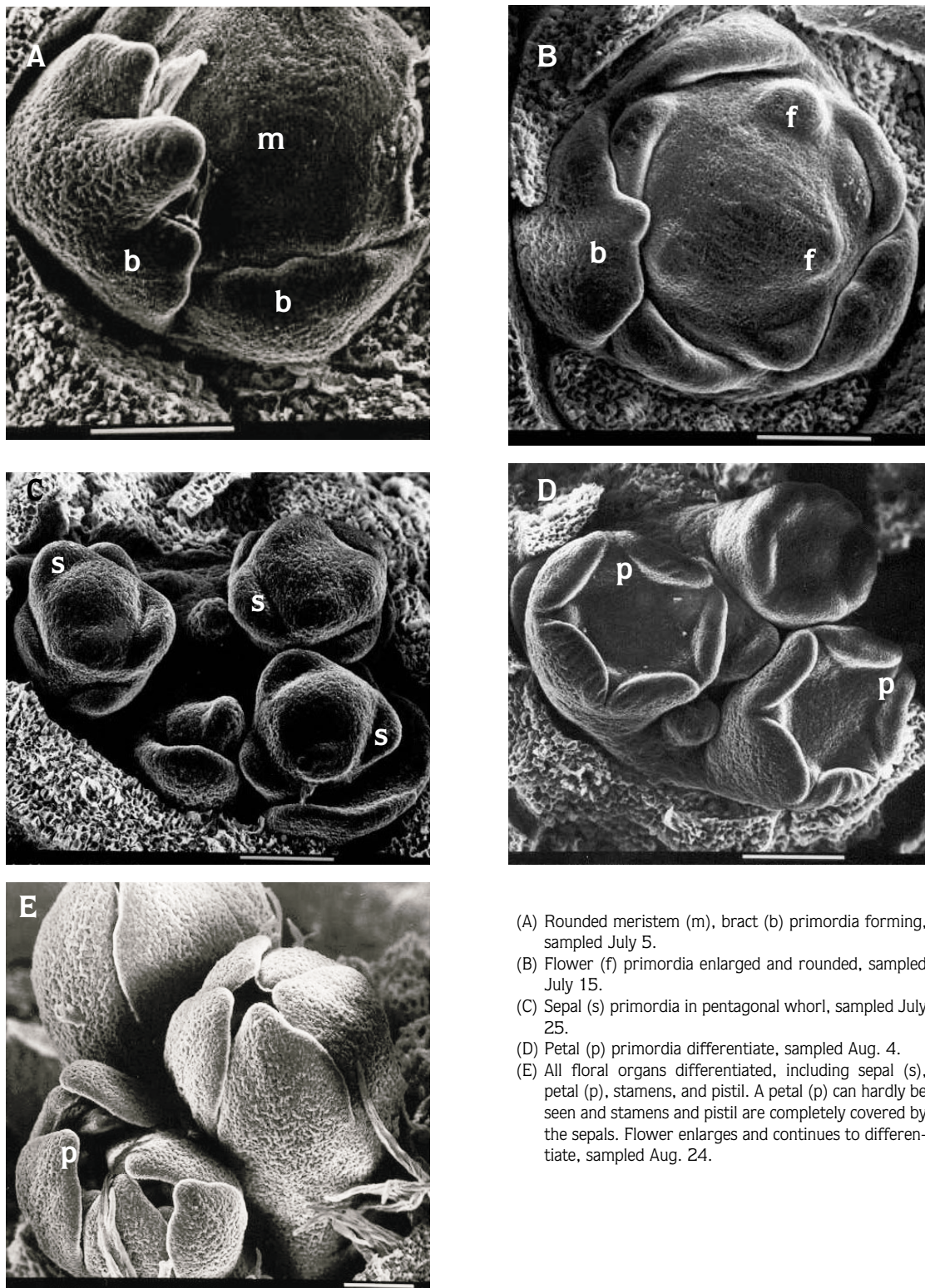
For each collection, the records were taken when the number of buds at a defined stage exceeded 50% of the total buds. Fifteen buds from 5 trees were dissected and fixed; however, the number of observed buds varied due to inevitable losses during preparation. Buds that were obviously necrotic or aborted were not scored.

Results and Discussion

In sweet cherry buds, the use of SEM showed the time frame for initiation of visual changes from a vegetative to a reproductive state. Figure 1 illustrates the stages of development. Flattening of the apex marks the change from vegetative to the reproductive phase (Ryugo, 1986). In this study, the first morphological indication of the transition from vegetative to reproductive development was the change in the shape of the apex from flattened to domed. This phase had occurred on July 5, 2004, 85 DAA (Figure 1A). Westwood (1978) reported that this phase is affected by annual environmental conditions, especially temperature. In the sweet cherry tree, this phase has been reported to occur during July. Similar results were recorded in the present study. In our study, subsequent events were the development of the growing point (visually manifested by doming of the apex), the production of flower primordia, and the differentiation of flower parts. The youngest bract primordia occurred near the center of the

meristem, indicating a centripetal pattern of differentiation. Flower primordia initially formed in the axils of bracts on July 15 (Figure 1B). Floral organogenesis began with the initiation of 5 sepals. The sepal primordia arose in spiral phyllotaxis at the periphery of the terminal apex. This phase occurred on July 25 (Figure 1C). Petal primordia also initiated in spiral phyllotaxy, and arose alternate to the sepals within the calyx. This phase occurred on August 4 (Figure 1D). Differentiation of the stamen and pistil could be observed 135 DAA in longitudinal sections of individual flowers (not shown). Stamen primordia became visible between August 4 and 24 and completed its development by forming 1st, 2nd, and 3rd lines of stamen primordia. On August 24, 2004, pistil primordia initiated within the floral cup (Figure 1E) in which pistil primordia could be seen with removed sepals and petals. Our observations suggested that pistil primordia are formed in a short period of time. Diaz et al. (1981) investigated the timing of flower development in sour cherry (*Prunus cerasus* L.), where the inflorescence meristem initiated flower primordia at the end of June, and the first signs of pistil initiation were evident by mid-September. In another experiment, in which flower development in Bing sweet cherry was studied and compared pruned and nonpruned shoots, no difference was found in the timing of some developmental events involved in flowering. Furthermore, it was noted that differences possibly existed among cultivars and among locations (Guimond et al., 1998).

As is known, flower initiation and differentiation may vary according to cultivar and climate. This study indicates the timing of the stages of sweet cherry flower bud formation. For 0900 Ziraat in western Turkey, initiation occurs at the beginning of July. Similarly, in Japan, sweet cherry flower initiation is generally thought to occur on June 30 (Watanabe, 1983), in Columbia and



(A) Rounded meristem (m), bract (b) primordia forming, sampled July 5.
 (B) Flower (f) primordia enlarged and rounded, sampled July 15.
 (C) Sepal (s) primordia in pentagonal whorl, sampled July 25.
 (D) Petal (p) primordia differentiate, sampled Aug. 4.
 (E) All floral organs differentiated, including sepal (s), petal (p), stamens, and pistil. A petal (p) can hardly be seen and stamens and pistil are completely covered by the sepals. Flower enlarges and continues to differentiate, sampled Aug. 24.

Figure 1. SEM micrographs of 0900 Ziraat sweet cherry buds, showing developmental changes from initiation to differentiation. Bar = 100 µm.

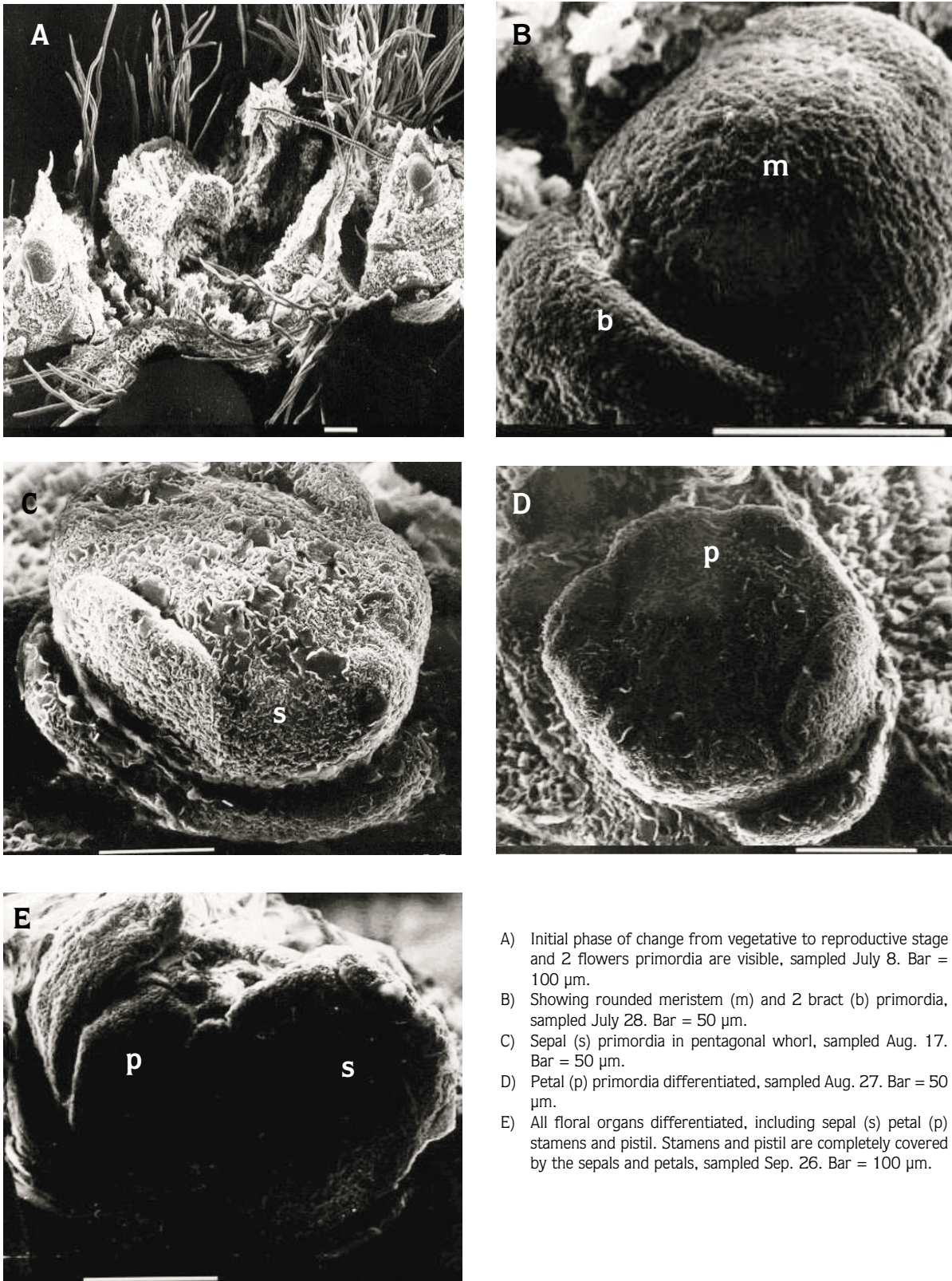


Figure 2. SEM micrographs of Glohaven peach buds, showing developmental changes during flower initiation.

Canada in late June to early July (Kappel et al., 1990), and in central Washington in mid- to late July (Guimond et al., 1998). Orchard management and cultural practices at this critical time can be optimized in order to favor floral initiation.

In peach buds, the use of SEM showed the time frame for initiation of visual changes from a vegetative to a reproductive state. Figure 2 illustrates the developmental stages. Flower initiation first became apparent at the shoot apex with an increase in meristem size on July 8 (Figure 2A), 109 DAA. The apex then became broadened and thickened to form an elongated, broad dome that produced bract primordia on the periphery of the apex on July 28 (Figure 2B). Bracts were observed only in buds where doming of the apex was already evident and were absent in vegetative buds. The sepal primordia changed to pentagonal whorl (Figure 2C). Petal primordia were evident on August 27 (Figure 2D). Stamen primordia became visible at the beginning of September and it completed its development by forming 1st, 2nd, and 3rd lines of stamen primordia. At the final stage the pistil developed on September 16 (Figure 2E) in which pistil primordia could be seen with removed sepals and petals.

Warriner et al. (1985) studied the timing of flower initiation and effects of environment on peach flower initiation and development in Perkins, Oklahoma, USA. It was stated that there was no differentiation in flower buds until mid-July. The first evidence of initiation was in mid- to late August. This was followed by flower organogenesis with pistil initiation occurring in mid-October. In our study, the first indications of floral

initiation were observed in about mid-July and pistil initiation in mid-September, 1 month earlier than Warriner et al. reported (1985). In a study that compared flower bud initiation in peach between the northern and southern hemispheres, flower initiation occurred at approximately the beginning of summer in both hemispheres (Raseira and Moore, 1986).

Consequently, it was seen that in both *Prunus* species (*Prunus avium* and *Prunus persica*), the morphological progress of flower bud differentiation is similar. Flower initiation is manifested by changes in the size and shape of the shoot apical meristem, which takes the form of a broad, low dome as it undergoes transition from a vegetative to a reproductive meristem. This morphological stage is marked by a shift in organogenetic activity from bud production to the sequential initiation of bracts at the periphery of the meristem. In *Prunus* species with inflorescences containing several flowers, such as sour cherry (Diaz et al., 1981) and sweet cherry, lateral flower primordia emerge in the axils of the bracts. In the solitary-flowered peach bud, the shoot apical meristem is converted to a terminal floral meristem with no developmental activity apparent in the axils of the bracts. Detailed information on the timing of floral development is also useful for tree crop research and management as put forward by the studies by Whiting et al. (2006) in sweet cherry and Bridget et al. (2001) in almond. We are hopeful that, as a result of the data presented here, sweet cherry and peach researchers can design and interpret experiments with a clearer understanding of the stages of flower bud development and differentiation that may be affected by their experimental manipulations.

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