Floral differentiation and development in early, middle and late blooming almond cultivars

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Flower initiation and development in almond (Prunus amygdalus L.) were examined with the objectives of determining the timing of floral developmental stages and achieving a better understanding of the morphological changes during flower formation at the apex of axillary buds of early, middle and late blooming almond cultivars. In almond trees, bud samples were taken every 10 days from May 31, 2007. Sample collection ended when more than 50% of the dissected buds had pistils initiated. Bud samples were stored in formalin, 70% ethanol, and acetic acid solution (10:50:5, by volume). Flower induction (flattling of doming meristem) were done in 8, 18 and 31 August of 2007 in middle blooming cultivar (A93), early blooming cultivar (H-2) and late blooming cultivar (Ferragnes), respectively. Sepals primordial were stimulated in A93, H-2 and Ferragnes cultivars in 24 and 31 August and in 13 September, respectively. Petals primordial induction in H-2, A93 and Ferragnes cultivars were done in 13, 15 and 17 September of 2007, respectively. Stamens primordia induction in H-2, A93 and Ferragnes cultivars were done in 1, 10 and 20 October of 2007, respectively. The pistil primordium in H-2, A93 and Ferragnes were evident 10, 21 and 31 October of 2007, respectively. Anthesis was observed in H-2, A93 and Ferragnes in 7 and 21 March and in 5 April 2008, respectively. In general, it was found that process floral formation stages in early, middle and late blooming almond cultivars particularly the timing of flower induction between cultivars was not similar.

Key words: Flower primordia, Prunus amygdalus, flower initiation.

INTRODUCTION

Flowering is a prerequisite for fruit development. Since fruit trees are usually considered to be day neutral plants, flowering is initiated by a complex interaction of factors including environmental (but not day length), cultural and internal chemical signals (Ryugo, 1988; Takeda et al., 2002). Flowers are usually initiated from June to September during the previous year. Understanding the processes of flower induction and flower bud development are important for a lot of horticultural activities particularly in fruit cultivation due to the dependence of fruit formation and for some of breeding programs as well. For example breeding is very important for late flowering. According to the Vasilev and Baev (1967) reported, development of pollen in late cultivar of almond happen later compare as early cultivars. Yablonskii (1972) was reported in almond, peach and apricot that, when developments of flower buds in cultivars are slowly, they are more to cold resistance. Modtolovitsa (1973) understood that in plum, the cultivars that have an early development of pollen cells are damage less than the cultivars that have a late development of pollen cells. 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 fruit set. For example, GA3 application at the beginning of morphological differentiation of flower buds affects the flower thinning and delay the 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, 1986a).

Flower induction occurs with biochemical signals are the cause of changing tissue state from vegetative to reproductive state. It is probably happen by hormones balance such as auxin, gibberellins, cytokines and ethylene (Westwood, 1993).

Differentiation of flower buds in many deciduous fruit and nut trees takes place during the summer or early fall preceding the spring in which the buds open and set fruit. In most of these species under a temperate-zone or Mediterranean climatic conditions, once differentiation has been initiated there is continuous development of floral organs until bloom the following spring (Buban and Faust, 1982; Tufts and Morrow, 1925; Viti and Monteleone, 1991). However, in pistillate flowers of Persian walnut (Juglans regia L.) (Lin et al., 1977) and pistachio (Pistacia vera L.) (Takeda et al., 1979), floral initiation occurred in early summer, but the development of the components of flowers was shown not to be continuous, as there were periods of 3 to 9 months in which little or no growth and development took place. The reason for this discontinuous floral development in these species is not known, but it may be associated with the incomplete complement of floral parts (Takeda et al., 1979). In almond, flower induction happens with sending special message in the special time in some of bud meristems of almond mature trees and it leads to morphological and tissue changing in these meristems. After the meristem induction, start differentiation and flowering of first shapes of flower organs, and during the different stages these organs are made as primordia in flower.

Almond flower bud should pass the special stages for getting the flowering trigger. It happens when the shoot vegetative growth stop or decrease during the leave maturity, among of source carbohydrates around the bud meristem is enough, hormones balance start, prepare good environmental condition such as (light intensity, photoperiods, air temperature and humidity (Bustamante-Garcia, 1980; Buban and Faust, 1982; Ryugo, 1986a). So, when these factors prepare in buds, vegetative meristem can change to reproductive meristem in this 4 stages: flower initiation, differentiation, development and anthesis Taylor et al., 1997). Warriner et al. (1985) studied the timing of flower initiation and the effects of environment and cultural practices on peach [Prunus persica (L.) Batch (Peach Group)] flower initiation and development in Perkins, Oklahoma. He observed no differentiation in flower buds by 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. The research of Guimond et al. (1998) in 'Bing' sweet cherry (Prunus avium L.) shows that flower induction occur the middle of July whereas the pistil induction occur by the end of August Almond flower buds are made in long shoots and short spurs like laterally in leaves axillaries. Each bud makes a flower, bud in some cultivars from each bud 2 flowers are make like Tunuo almond. Tufts and Morrow (1925) reported that 'Nonpareil' almond flowers were initiated at the beginning of September, and sepal initiation did not occur until mid-September. Brooks (1940) examined the process of almond flower initiation and organogenesis in 'Nonpareil' almond trees growing in Davis. The investigation of Briget et al. (2001) shows that changing the vegetative to reproductive states, it can be different in climate conditions and kinds of almond variety. From the time of flowering, almond varieties are different from other fruit trees such as (Prunoeidea) have different times of flowering (Imani, 1997). Almond varieties are changed in 3 types: early cultivar, middle cultivar, late cultivar. While in some (Prunoeidea) fruit trees this variety is less saw. The aim of this study investigation of process floral formation stages in early, middle and late blooming almond cultivars, which show us is the timing of flower induction between that cultivars are same or not?

MATERIALS AND METHODS

This study was conducted in the 10-year-old almond orchard located at the Kamal-Shahr Collection Orchard, Seed and Plant Improvement Institute (SPII), 50 km west of Tehran, Iran. Kamal-Abad station enjoys specific geographical conditions such as benefiting from approximately 250 sunny days per year, minimum temperature, maximum temperature and altitude (above sea level), -15 and 38°C and 1258 M respectively. More than 150 almond varieties are available in the kamal-abad collection orchards. The different stages of this research were done in following:

1. Collected and stabilized samples. Fifteen buds uniform in size and vigor were collected every 10 days from 5 trees in early blooming cultivar (H-2), middle blooming cultivar (A93) and late blooming cultivar (Ferragnes). Almond bud samples were taken from May 31, 2007. Sample collection ended when more than 50% of the dissected buds had pistils initiated. At first they were studied with binocular in laboratory, then after remove the scales the samples were fixed and stored in a solution of FAA (30 Formalin, 60 Ethanol, and 10 Glacial Acetic Acid) (Imani, 1997).
2. Washed, took water and lucider of samples. In order to send out fixation from tissues, samples were washed with water. Then took water was done with series of alcohols (50, 70, 90, 96 and 100%), then for lucid they were put on tolune (Imani, 1997).
3. Provided moulds of Paraffin. For sent out Paraffin from tissues, and influenced Paraffin into them, buds were put in Paraffin-Toluene for a day in incubator 60°C. Then buds were put in Paraffin 1, 2, 3, 4, 5 for 5 days for influence of Paraffin into them. After that they were molded.
4. Provided them for saw on microscope. Micrometre is used to cutting samples to 6 micron thick. Cuttings put on lam and after dried were colored. In order to coloring tolune was used for take out Paraffin, then used the decreasing degree of alcohols, after that Hematoxilin and Eosin were used for coloring, then increasing degree of alcohols were used. The samples were examined with optical microscope and to take photos.

RESULTS AND DISCUSSION

Different development stages of almond bud flowers (Prunus amygdalus L.) were considered in 8 stages (Figures 1A to H), also bud morphology (Figure 2A) and
Figure 1. Different development stages of almond bud flowers: (a) leaf appendage, (f) leaves primordium (m) meristem, (s) sepal, (c) petals, (e) stamens and (p) pistil, during the followed dates of 2007: A) sampled July, 31; B) sampled August, 8; C) sampled August, 31; D) sampled September, 13; E) sampled October, 1 and F) sampled October, 10; G) sampled October, 19 and H) sampled in November, 12.

Figure 2. Macroscopic view of pistil, stamens (A) and anthers (B) in November, 12.

macroscopic view (Figures 2B).

Flattening of the apex marks the change from vegetative to the reproductive phase (Ryugo, 1986b). 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. Flower induction (flattening of doming meristem) (Figure 1B) were done in late blooming cultivar variety (Ferragnes) in 31 August 2007 while in early blooming cultivar variety (H-2) in 8 August and in middle blooming cultivar (A93) it occurred in 18 August. Sepal’s primordia (Figure 1C) were stimulated in early blooming cultivar
Table 1. Floral stages time (initiation, differentiation, development and anthesis) in 3 Almond cultivars early cultivar (H-2), middle cultivar (A93) and late cultivar (Ferragnes) during the year of 2007 and 2008.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Flower induction</th>
<th>Sepals primordial stimulate</th>
<th>Petals primordial stimulate</th>
<th>Stamen primordial stimulate</th>
<th>Pistil primordial stimulate</th>
<th>Flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late flower cultivar (Ferragnes)</td>
<td>31 August, 2007</td>
<td>13 September, 2007</td>
<td>17 September, 2007</td>
<td>20 October, 2007</td>
<td>31 October, 2007</td>
<td>5 April, 2008</td>
</tr>
</tbody>
</table>

(H-2), middle blooming cultivar (A93) and late blooming cultivar (Ferragnes) in 31 August, 24 August and 13 September respectively. Petals primordial induction (Figure 1D) in early blooming cultivar (H-2), middle blooming cultivar (A93) and late blooming cultivar (Ferragnes) were done in 13 September, 15 September and 17 September 2007 respectively. Stamens primordia (Figure 1E) induction in early blooming cultivar (H-2), middle blooming cultivar (A93) and late blooming cultivar (Ferragnes) were evident in that order 1 October, 10 October and 20 October 2007 while Pistil primordium (Figure 1F) were evident respectively 10 October, 21 October and 31 October 2007. Anthesis (Table 1) was observed in early blooming cultivar (H-2), middle blooming cultivar (A93) and late blooming cultivar (Ferragnes) in 7 March, 21 March and 5 April 2008 respectively. Flower induction start in apex when meristem enlargement that lead to doming. After this stage apex change from doming to flat shape (Figure 1B). Then from outside to inside it shapes and in continues organogenesis (Floral primordium) start from margins and sepal primordium were evident as a first of flower (Figure 1C part s).

After sepal primordium differentiation, petals primordium are stimulated (Figure 1D part c). Subsequent to this process, stamen primordium are evident inside the petal primordium (Figure 1E part e). With progress of petals, sepal, stamens primordium, in the base of them, hypanthium is shaped. On the hypanthium, pistil primordium is shaped as projection (Figure 1F part p). During progress of differentiation, perfect pistil is appearing (Figure 1F to H part p).

Appearance of flower bud, pistil and stamens is shown in Figure 2. Different stages of flower progress in cultivars with different flowering time are in the Table 1.

Morphological progress in flower bud differentiation in almond is like another porunoidea species have similar pattern. It means that 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 organogenesis activity from bud production to the sequential initiation of bracts at the periphery of the meristem. This morphological change such as transition from product bud scales to differentiation 3 brackets around of meristem (Hakan and Unal. 2007). In Prunus such as sour cherry (Prunus cerasus L.), Diaz et al. (1981) investigated the its timing of flower development, 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). Our observations on the timing of flower development are like observations with electron microscope that done with Bridget et al. (2001). Tefts and Morrow (1925) reported time of flowering of Nonpareil in Davis was 1 September, Bridget et al. (2001) were reported at the same time of flowering in same area 14 July 1997 and 17 August 1998.

In this study different among cultivars were observed in flower development that can use for orchard management, because almond reproduction biology is an important factor in orchard management. Almond contrary to another fruit trees, does not need to thin. Almond crop production for next year starts from the time of flower induction and managing orchard management and cultural practices at this critical time can be optimized in order to favor floral initiation (Polito, 1997). For example water stress in this time can be lead to decrease product in next year. Also timing of flower induction is different among different cultivars, so knowing the time of flower induction in cultivars and having a good management specially manage of water stress in this time lead to increase product in next year (Sedgley and Gharifain, 1989; Castro, 2003). So, in this study, it was found that process floral formation stages in early, middle and late blooming almond cultivars...
particular the timing of flower induction between cultivars was not similar.

One of the benefits of these studies is for management of irrigation. For example when we don't have enough water for orchard irrigation, stress management during flower development stages is very important for prevent of product decrease in next year.

REFERENCES


