Jour. Korean For. Soc. Vol. 94, No. 2, pp. 67~72 (2005)

JOURNAL OF KOREAN FOREST SOCIETY

Identification of Initiation Period and Subsequent Development of Floral Primordia in Black Locust (*Robinia pseudoacacia* L.)

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Abstract : The objectives of this study were to identify the period of initiation of floral primordia in black locust (*Robinia pseudoacacia* L.) and subsequent development of floral buds until following spring. Four mature trees of black locust located in Suwon, Korea were selected. Bud samples were collected from the current-year shoots, starting from mid June to July every week, from August to October and from February to April every month. The buds were fixed in FAA solution, dehydrated, and imbedded in paraffin for microscopic observation. Buds collected on June 16, and 23, 1997, contained primitive primordia that might be interpreted as early floral primordia. By June 30, a bud showed a positive indication of inflorescence primordium with a well-formed shoot apex. All the inflorescence primordia observed throughout the collection periods were always associated with unique hairy appendages around the primordium and enclosed within a sclerenchymatous chamber. By July 7 and 15, a floral apex had early bud scales. By July 22, primitive inflorescence developed into visible arrangement of individual floral primordial By July 29, the inflorescence developed into whirl arrangement of individual floral primordia in a transverse section, but showed little further development until October 15. The inflorescence primordia seemed to over-winter at this stage. Buds collected from February 15 and March 24 the following year also showed no further development of inflorescence primordia. By April 7 the inflorescence started to show further development with elongated axis. At this time individual flowers were easily recognized.

Key words : *flowering, floral primordium, floral apex, inflorescence*

Introduction

Flowering in herbaceous plants is relatively well understood particularly with grasses and cereals (King and Evans, 2003). In contrast flowering in woody plants is poorly understood, because most woody plants show neutrality to day length (Salisbury and Ross, 1992) and their flowering mechanism is less extensively studied due to their large size and long juvenile period.

Black locust (*Robinia pseudoacacia* L.) belongs to a genus of *Robinia* that has about 20 species in tree and shrub forms worldwide. Black locust has a wide range of distribution in the native North America (Hanover *et al.*, 1992), relatively high degree of genetic diversity (Bongarten, 1992; Chang *et al.*, 1998), and is a timber tree of some importance (Harlow *et al.*, 1979). Compared with other broad-leaved trees in temperate zones, it has superior characters in agroforestry (Hanover, 1993). Its potential for timber production has been proved in Hungary where 20% of total forest area has been established by black locust (Redei *et al.*, 2002). In Korea black locust was successfully planted to revegetate about a half million ha of eroded forest lands and to substitute for the fuel requirement of village farmers during the last century.

In addition honey from black locust trees accounts for 70% of total honey production in Korea. Black locust has been considered the most valuable tree in honey production during the last 50 years. For apiculturists prediction of flowering period and flowering abundance of black locust is very important. They still collect honey by continuous moving along the flowering trees from south to north. Prediction of flowering abundance in advance will allow them to plan travelling schedule in spring.

Many studies on the flowering of woody plants have indicated that floral primordia are usually initiated between late spring and mid summer (Matthews, 1963; Kramer *et al.*, 1979) and each species has unique ontogeny for their development. In Korea flowering of black locust has been studied by apiculturists in terms of amounts of flowers and maximum honey production

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(Ministry of Agric. and Forestry, 1999). However, flowering physiology has not been studied in relation to the time of floral bud initiation and to climatic conditions the preceding year to predict abundant flowering the following year.

The objectives of this study were to find out the period of initiation of floral primordia in black locust (*Robinia pseudoacacia* L.) and subsequent development of floral buds until following spring.

Materials and Methods

Black locust (*Robinia pseudoacacia* L.) trees used in this study were located in the old campus of College of Agriculture and Life Sciences, Seoul National University, Suwon, Korea. Suwon is located at 37°15' N latitude. The four mature trees selected were 25 years old and growing under the full sun with abundant flowering every year. Their height ranged from 12 to 15 m.

Black locust has twigs with moderately stout, angular, and somewhat zigzag shape and without terminal buds. Lateral buds are naked and submerged beneath the leaf scar so that none of winter buds are visible on the main shoots. After anthesis in the mid spring actively growing current shoots were selected from the upper part of the tree crown. The buds in the leaf axils were cut in transverse direction from the main shoot and were cut again in longitudinal direction to include the submerged bud primordia. Samplings started from mid June to July every week, from August to October every month. Samplings were resumed next February and continued until April every month.

The buds were fixed in FAA (formalin/acetic acid/ethyl alcohol) solution (Berlyn and Miksche, 1976) until all the samples were collected in April. The samples were dehydrated, imbedded in paraffin, and cut into thin sections of 10 μm thickness with a microtome (Jensen, 1962). After being mounted on glass slides, the sections were stained with safranin and fast green. The permanent slides were examined under a light microscope for floral structures in reverse order of collection dates to ensure the development of the floral initials from positively identifiable floral structures in the samples in April. The first indication of floral bud initiation was determined from positive identification of primitive buds on the slide.

Results

Figure 1 shows the first indication of formation of primitive buds collected on June 16, 1997. The shoot apex was densely stained by safranin, indicating active cell divisions in this region. Figure 2 shows a picture taken from buds collected on June 23, with further development of primitive buds into well-organized shoot apex. By June 30 the primitive bud expanded further in longitudinal direction to form a distinctive axis (Figure

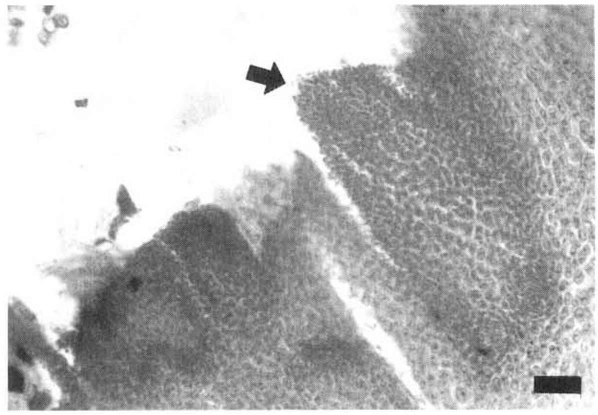

Figure 1. The first indication of formation of primitive buds (length of bar=32 μm). (June 16, 1997)

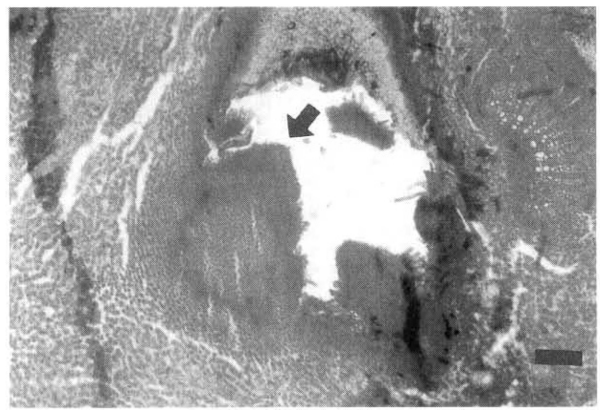

Figure 2. Further development of primitive buds into organized shoot apex (length of bar=160 μm). (June 23)

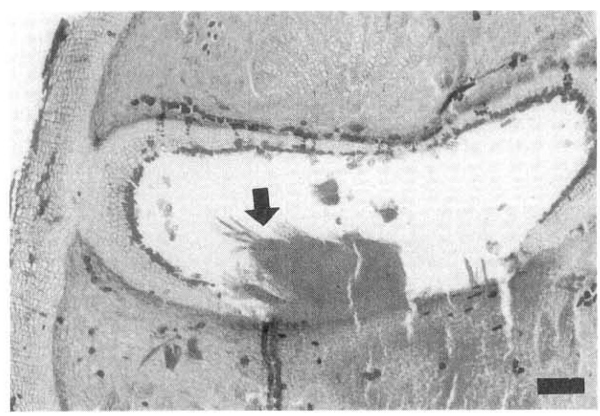

Figure 3. The primitive bud expanded further in longitudinal direction to form a distinctive axis. Hairy appendages and sclerenchymatous multicellular layers around the bud are visible (length of bar=160 μm). (June 30)

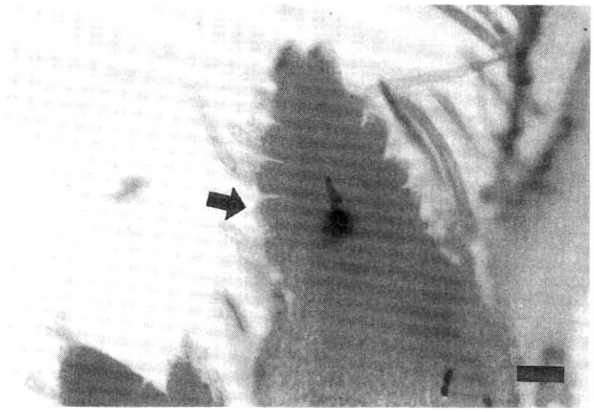

Figure 4. A floral apex has early bud scales in the longitudinal section (length of bar=64 μm). (July 7)

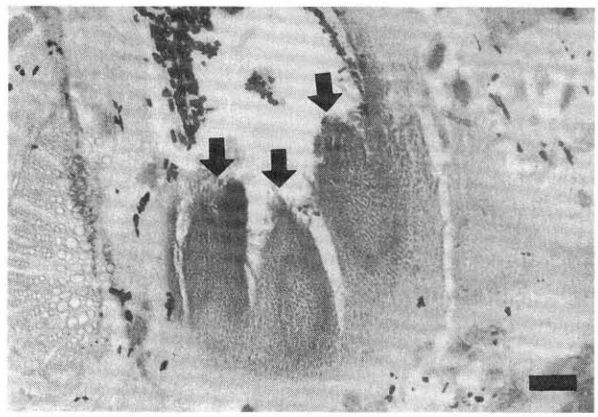

Figure 5. Primordia of three buds. It may become a mixed bud containing one inflorescence primordium and two vegetative shoot primordia, which is common in black locust (length of bar=160 μm). (July 15)

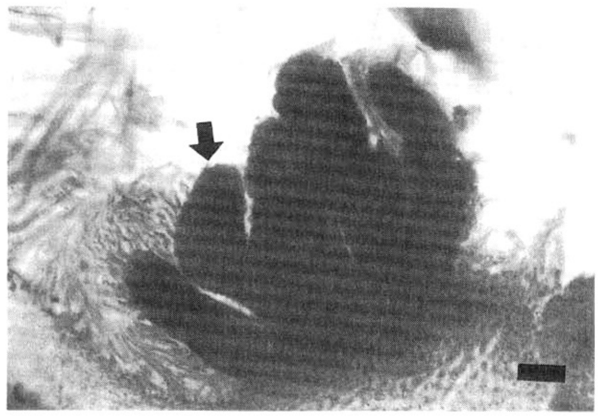

Figure 6. A visible arrangement of individual floral primordium with very short central axis in a longitudinal section (length of bar=64 μm). (July 22)

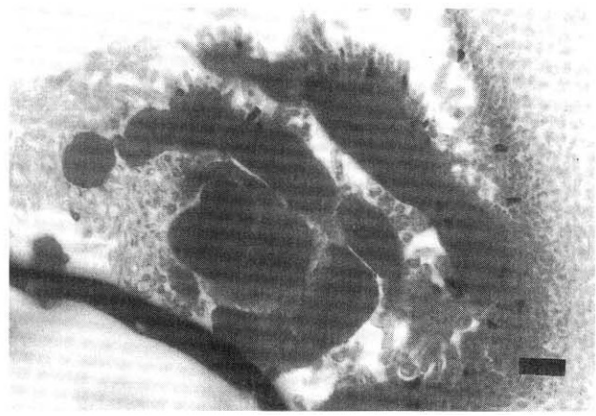

Figure 7. An inflorescence developed into whirl arrangement of individual floral primordia in a transverse section (length of bar=64 μm). (July 29)

3). The size of the primitive buds was about 200 μm in diameter and 300 μm in length. All the inflorescence primordia observed throughout the collection periods were always associated with unique hairy appendages on and around the buds and protected by sclerenchymatous multicellular layers same as the protective outer layers of the buds. By July 7, a floral apex had early bud scales in the longitudinal section (Figure 4). The unique hairy appendages around the shoot apex were clearly visible in this picture. Comparing the bud samples collected from June 16 or 23 with the subsequent samples from June 30 or July 7, it was assumed that inflorescence primordia would be first initiated in early mid June in Suwon area, Korea.

Figure 5 shows primordia of three buds collected on July 15. A winter bud of black locust as shown in this picture may be a mixed bud containing one inflorescence primordium and two vegetative shoot primordia. By July 22, primitive inflorescence (Figure 6) developed into clearly visible arrangement of individual floral primordia with very short central axis in a longitudinal section. By July 29, the inflorescence developed into whirl arrangement of individual floral primordia in a transverse section (Figure 7). However, The primitive inflorescence on July 29 seemed to show little further development by August 19 (Figure 8). The arrangement of buds in Figure 8 was essentially the same as in Figure 7. The buds in Figure 9 (from September 17) and Figure 10 (from October 15) also showed very little changes compared with that of July 29.

The inflorescence primordia seemed to over-winter at this stage. The first picture taken on February 15 (Figure 11) during the wintering indicated the same stage of development as on October 15. March 24 also witnessed no further development of inflorescence primordia. The weather in Suwon, Korea would have mild weather in March and day temperature would rise above 10 degree Celsius. Black locust showed little further development in the inflorescence during March. However, by April 7 the inflorescence showed further development with elon-

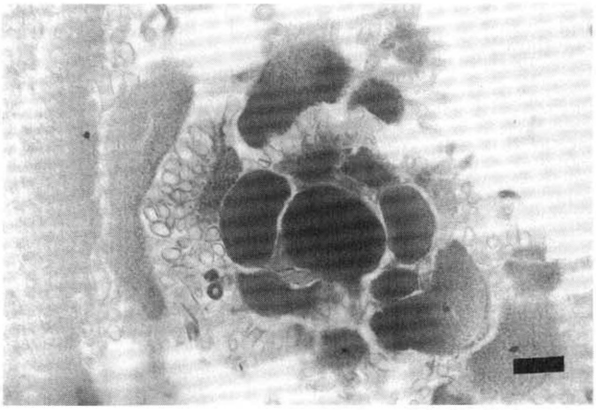

Figure 8. The primitive inflorescence in Fig. 7 (July 29) seemed to show little further development (length of bar=64 μm). (August 19)

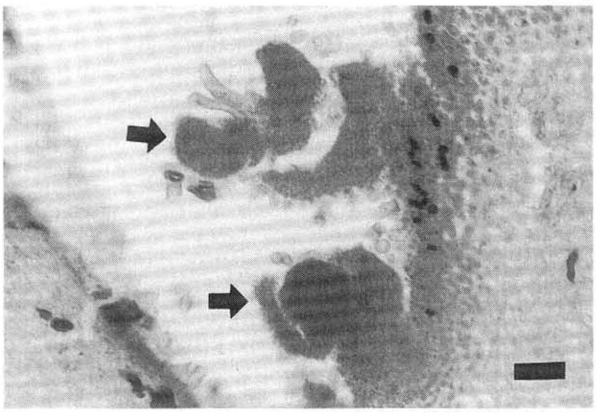

Figure 9. A similar stage of development to that of Fig. 7 (length of bar=160 μm). (September 17)

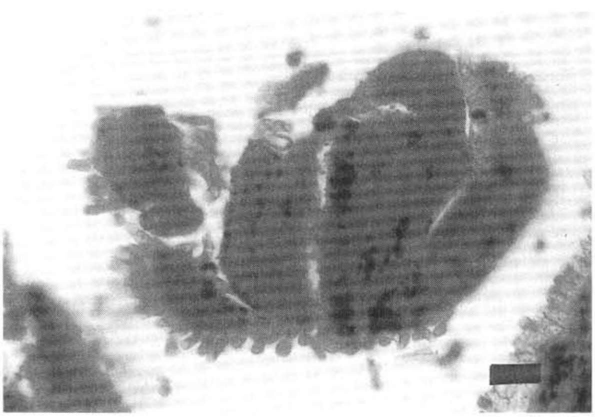

Figure 10. No further development of bud compared with that of July 29 (length of bar=64 μm). (October 15)

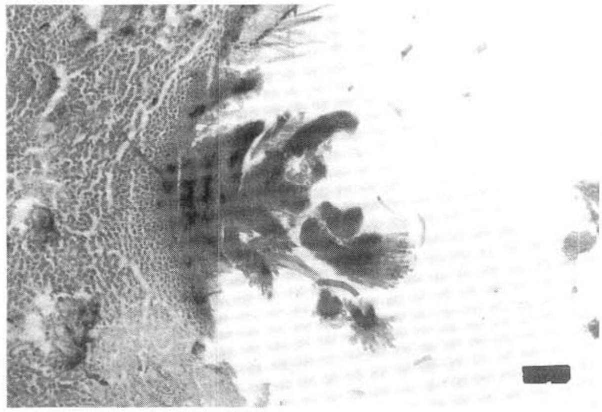

Figure 11. A wintering bud indicated the same stage of development as on October 15 (length of bar = 160 μm). (February 15, 1998)

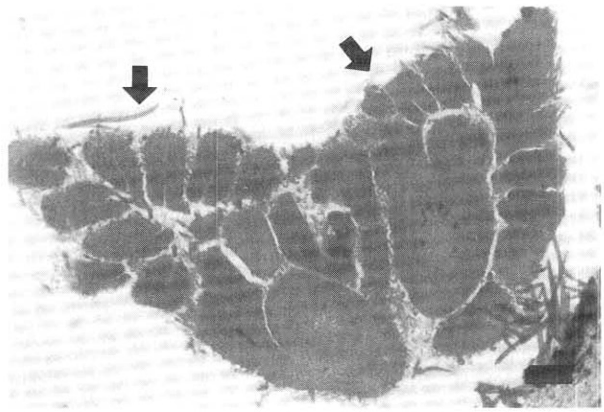

Figure 12. Two inflorescences showed further development with elongated axis. At this time individual flowers were easily recognized (length of bar = 160 μm). (April 7)

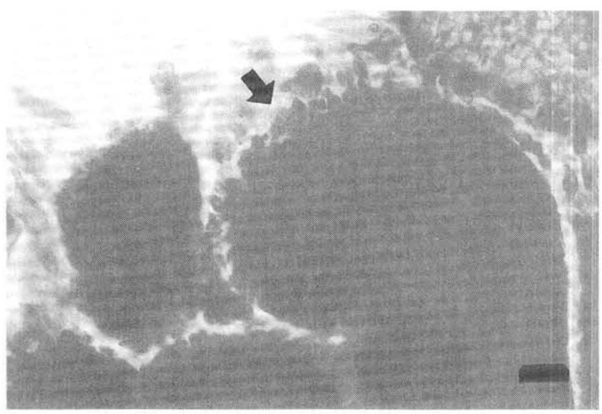

Figure 13. Individual flowers did not show any sign of more advanced arrangement for differentiation into floral parts (length of bar=32 μm). (April 7)

gated axis as shown in Figure 12. At this time individual flowers were easily recognized under the microscope and had a relatively small dimension of 1.5mm long and 0.8mm wide. However, winter buds did not show any sign of opening by April 7. They are not still visible by the naked eyes and still submerged in the leaf axil. Figure 13 from April 7 collection indicated that the inflorescence was still in the early stage in which individual flowers did not show any sign of more advanced arrangement for differentiation into floral parts.

The air temperature in early April would rise up to 10 to 15 degree in Celsius. Black locust normally flowers in mid May in Suwon area. The last picture taken on April 7 (Figure 12) showed that inflorescence of black locust were still in the undifferentiated stage in early April, and suggested that the further development of the inflorescence would be quite fast during the last one month before the anthesis in mid May.

Discussion

Woody plants which flower in early spring normally form floral primordia during the preceding year. Floral initiation in many woody plants starts from the late spring to early summer depending on the species. In angiosperm woody plants with monoecious and monosexual flowers, floral primordia are first initiated in late May through July. In gymnosperms with monoecious and bisexual flowers, male flowers are initiated earlier than female flowers (Lee, 1993).

Initiation and development of floral primordium in black locust seemed to be similar to other woody plants in general. *Vitis* is known to initiate floral primordia as early as late May. *Pyrus* initiates them in mid to late June, while *Malus* and *Prunus* initiate them in mid July and early August, respectively (Kim, 1986). *Quercus* whose flowering was intensively studied by Merkle *et al.* (1980) using scanning electron microscopy initiated staminate inflorescence primordia in late May and pistillate inflorescence primordia in late July. The difference in the period of floral primordium initiation between staminate and pistillate flowers, and earlier initiation of staminate flowers than pistillate flowers in bisexual flowers are observed in many woody plants including gymnosperms (Owens and Molder, 1974).

The main objective of this study was to identify the period of floral bud initiation in black locust. Present study confirmed that floral primordia of black locust were initiated as early as mid June in central part of Korea. Considering the late opening of black locust winter buds in late April compared with other neighboring trees in the same forest, it was unexpected that initiation of floral buds would start in mid June about one month after anthesis. Identification of the period is necessary to evaluate the influence of weather of the preceding year on the flowering abundance the following year. Many studies on the flowering of woody plants have indicated that flowering abundance of woody species is related to climatic conditions during the period of floral bud initiation in the preceding year. However, there are conflicting reports on this issue and rainfall during the floral bud initiation may positively or negatively affect the floral bud formation. For example, mild drought during the period of floral bud initiation in previous summer may increase the flowering abundance (Bonnet-Masimbert, 1978), while abundant or normal flowering has been reported to be associated with average precipitation during the period of floral bud initiation (Lee, 1979).

The present study indicated that initiation of floral bud primordia in black locust occurred in mid June in Suwon, Korea. Korea has a relatively dry weather in May through June due to its geographic location as a peninsula in eastern end of Asian continent. After a long spell of dry spring starting April through June, a short rainy season of about three to four weeks may start as early as mid June or as late as early July. The present study suggested that the beginning period of rainy season in summer in Korea may influence the flowering abundance of black locust in the following year. Based on this study, we may analyze the already accumulated data on flowering abundance at each province or city to give much information to the apiculturists on the predicted flowering abundance in the coming year.

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(Received March 29, 2004; Accepted November 25, 2004)