Floral Morphology and Development of 'Sharpblue' Southern Highbush Blueberry in Louisiana

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ABSTRACT. Floral morphology and differentiation of 'Sharpblue' southern highbush blueberry (*Vaccinium corymbosum* L.) were studied under natural growing conditions. There was no rest period during floral development of 'Sharpblue' blueberry in Louisiana. Basal florets were already present within a racemic inflorescence in early September. All floral and reproductive organs were clearly visible in early December. Microspores and pollen grains were observed in mid- and late-January, respectively. Megaspores, two-cell, four-cell, and seven-cell embryo sacs were found to be simultaneously present in developing ovules in late January, suggesting that megasporogenesis and megagametogenesis in 'Sharpblue' blueberry are asynchronous.

Floral development of lowbush blueberry (*Vaccinium angustifolium* var. *laevifolium* Ait.) was reported previously, and the histology of floral differentiation and development was described using drawings instead of photographs (Bell and Burchill, 1955). Histological studies of vegetative and reproductive growth in northern highbush blueberry (*Vaccinium corymbosum* L.) were published in the late 1970s (Gough et al., 1978a, 1978b). However, numerous developmental differences exist between these two species (Table 1). There is very little information available on the anatomy of floral development of rabbiteye blueberries (*Vaccinium ashei* Reade) (Spiers, 1978).

The genetic background of southern highbush blueberries (SHB) involves several blueberry species, including *V. corymbosum* (tetraploid), *V. angustifolium* (tetraploid), *V. ashei* (hexaploid), and *V. tenellum* Ait. and *V. darrowi* Camp. (diploids) (Lang, 1993). SHB hybrids are principally grown in the southern United States. Their genetic diversity and environmental niche could affect floral development. Very limited information is available on SHB floral morphology and development. Flower development and growth of 'Sharpblue' blueberry are described in this study.

Materials and Methods

Plants were maintained in outdoor beds of a 1 sand : 1 bark : 2 peat medium at the Louisiana State Univ. Horticulture Farm, Baton Rouge. Floral buds were collected from September 1995 to January 1996. For 19 out of 151 d, the minimum temperature was below 0 °C but above -5.5 °C. There were no visible freezing injury symptoms on the samples. Samples for anatomical studies were collected on the summer-season shoots located at the middle to outer canopies of 8-year-old 'Sharpblue' plants. Buds of racemic inflorescences at the first node below the apex were collected monthly from early September to early December 1995. Basal florets within the first-node racemes were collected at 10-d intervals from late December 1995 to early February 1996. Slides for

microscopic examination were prepared according to the procedures described by Berlyn and Miksche (1976). Samples were fixed in formalin-acetic acid alcohol (FAA), dehydrated in a tertiary butyl alcohol series and embedded in Paraplast wax. Paraplast-embedded material was microtomed at 10-µm thickness, stained with safranin-O, and counterstained with alcian blue. To minimize variation due to bud and floret locations, the descriptions of floral development were limited to the first-node buds below the apex and the basal florets within these buds.

Results

Basal florets were already present in early September (Fig. 1A). These florets had well-developed sepals and early petal primordia (Fig. 1A1). The average width and length of a basal floret were 0.66 and 1.14 mm, respectively (Fig. 2). Floret differentiation within the raceme was acropetal and was not completed until early November (Fig. 1B and C). Basal florets measured 1.5 mm in width and 1.8 mm in length in early October and 2.2 mm in width and 2.6 mm in length in early November (Fig. 2). Florets in early October showed developing pistil primordia with ovary initials and stamen primordia with recognizable anthers and filaments. The reproductive parts were completely covered by well-formed petal primordia (Fig. 1B1). Florets in early November presented ovary locules and immature anthers with early differentiation of sporogenous tissues (Fig. 1C1).

By the beginning of December, complete flower structures were apparent in a floret (Fig. 3A). Florets averaged 2.55 mm in width and 3.25 mm in length (Fig. 2). Developing tapetal cells and primary sporogenous cells were clearly evident within the immature anthers (Fig. 3B). Placental tissues with developing ovules were formed within the ovaries. At this time, integuments were not yet initiated (Fig. 3C).

Florets and inflorescences started to enlarge rapidly by the end of December. By the end of January, florets reached 7.15 mm in width and 10.75 mm in length (Fig. 2). Developing anthers were characterized by slightly more developed sporogenous cells and tapetal cells at the beginning of January (Fig. 4A1). Anthers were at an early meiotic stage in mid-January, showing well-formed tapetal cells and developing microspores (Fig. 4B1). Mature pollen tetrads were present in late January (Fig. 4C1). By this time, the tapetal cells were degenerating.

Ovules were enlarged and developed, showing outer integuments and developing embryo sacs by the end of January (Fig.

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Table 1. Comparison of floral developmental events of lowbush (*Vaccinium angustifolium* Ait.) and northern highbush (*Vaccinium corymbosum* L.) blueberries.^z

Developmental event	Time sequence	
	Lowbush	Highbush
Onset of floral differentiation	Late June	Late August
Primordia of all floral parts	Late July	Early October
Formation of micro mother cells	Early April	Early November
Fully formed pollen grains	Late May	Middle April
Mature female gametophytes	Late May	Middle April

^zAdapted from H.P. Bell et al. (1955) and R.E. Gough et al. (1978b).

4C2). Megasporogenesis and megagametogenesis were asynchronous within a floret. Ovules shown in Fig. 5 were from the same floret. However, these four ovules presented four totally different developmental stages: a single large megasporocyte in A, two-cell stage in B, four megaspores in C, and a mature embryo sac in D.

Discussion

Initiation of racemic inflorescences in rabbiteye (*Vaccinium ashei*) blueberry is basipetal (Lyrene, 1984). Florets within the raceme are initiated and differentiated acropetally in lowbush and northern highbush (Aalders and Hall, 1961; Gough et al., 1978b). The same floral development pattern was observed in SHB cultivars.

Floral differentiation and development of blueberries exhibits great variation among species and locations. Lowbush and northern highbush blueberries undergo a few months of dormancy under northeastern climatic environments (Bell et al., 1955; and Gough et al., 1978b). Flower buds of 'Bluecrop' northern highbush blueberry had no increase in size from early October to early March and no apparent progress in floral differentiation from November to early March in Kingston, R.I., However, flower buds of 'Sharpblue' SHB blueberry in Louisiana experienced a continuous increase in size and a gradual progress in floral differentiation from September to December. This gradual progress was immediately followed by a rapid growth in size and development of pollen and ovules (Fig. 2). 'Sharpblue' has the lowest chilling requirement (≈ 250 h below 7 °C) for breaking dormancy among the SHB blueberry cultivars (Lang, 1993). Its chilling requirement was satisfied by the end of November during Winter 1995–96 in Baton Rouge. Budbreak could occur if sufficient heat were provided immedi-

ately following the chilling process. Obviously, breakdown of dormancy at this time was irregular, since its floral development was immature (Fig. 3) and mature microgametophytes and macrogametophytes were only evident by the end of January (Fig. 4 C1 and C2).

Fig. 1. Early events in floral development of 'Sharpblue' blueberry. (A) longitudinal section of inflorescence presents initiated florets in early September. (A1) Enlarged proximal floret from A shows sepal and petal primordia. (B) Longitudinal section of inflorescence in early October. (B1) Enlarged proximal floret from B shows pistil initial (PI), ovary initial (OI), and stamen initials (SI). (C) Longitudinal section of inflorescence in early November shows more florets. (C1) Enlarged proximal floret shows two ovary locules and immature anthers with early differentiation of sporogenous tissue (ST).

Ovule abortion is a common phenomena for all blueberry species (Vander Kloet and Lyrene, 1987; Vander Kloet, 1983). It also occurs in self- and cross-pollinated 'Sharpblue' fruit (Huang, 1995). The possible explanations for ovule abortion previously suggested include 1) incompatible pollen (Vander Kloet and Lyrene, 1987), 2) nutrition competition among developing berries on a single inflorescence (Gorchov, 1985), and 3) poor-quality pollen deposited on the stigma (Vander Kloet, 1983). We found that the ovules from a single floret, ≈ 1 week before anthesis, were at the asynchronous stages of megasporogenesis and megagametogenesis in 'Sharpblue' floral development (Fig. 5). We hypothesize that the asynchrony of embryo sac formation is a possible mechanism to account for ovule abortion occurring in blueberry fruit development. The rationale is that, at anthesis, ovules without a completely developed embryo sac will abort immediately after pollination because they lack functional egg cells and polar nuclei.

Literature Cited

- Aalders, L.E. and I.V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. *Can. J. Genet. Cytol.* 3:300-307.
 Bell, H.P. and J. Burchill. 1955. Flower development in lowbush blueberry. *Can. J. Bot.* 33:251-258.
 Berlyn, G.P. and J.P. Miksche. 1976. Botanical microtechnique and cytochemistry. Iowa State Univ. Press, Ames.
 Gorchov, D.L. 1985. Fruit ripening asynchrony is related to variable seed

- number in *Amelanchier* and *Vaccinium*. *Amer. J. Bot.* 72:1939-1943.
 Gough, R.E., V.G. Shutak, and R.L. Hauke. 1978a. Growth and development of highbush blueberry. I. Vegetative growth. *J. Amer. Soc. Hort. Sci.* 103:94-97.
 Gough, R.E., V.G. Shutak, and R.L. Hauke. 1978b. Growth and development of highbush blueberry. II. Reproductive growth, histological studies. *J. Amer. Soc. Hort. Sci.* 103:476-479.
 Huang, Y.H. 1995. Pollen influence on developmental physiology of southern highbush blueberry (*Vaccinium corymbosum*) fruit. PhD diss. Louisiana State Univ., Baton Rouge.
 Lang, G.A. 1993. Southern highbush blueberries: physiological and cultural factors important for optimal cropping of these complex hybrids. *Acta Hort.* 346:72-80.
 Lyrene, P.M. 1984. Late pruning, twig orientation, and flower bud formation in rabbiteye blueberry. *HortScience* 19:98-99.
 Spiers, J.M. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry. *J. Amer. Soc. Hort. Sci.* 103:452-455.
 Vander Kloet, S.P. and P.M. Lyrene. 1987. Self-incompatibility in diploid, tetraploid, and hexaploid *Vaccinium corymbosum*. *Can. J. Bot.* 65:660-665.
 Vander Kloet, S.P. 1983. The relationship between seed number and pollen viability in *Vaccinium corymbosum* L. *HortScience* 18:225-226.

Fig. 2. 'Sharpblue' blueberry floral growth and development during fall and winter. Each data point represents the mean of five individual samples; 1 = formation of sporogenous cells; 2 = formation of microspores; 1 + 2 = microsporogenesis; 3 = microgametogenesis - mature pollen grains; 4 = ovary, ovule and placenta formation; 5 = megasporogenesis; 6 = megagametogenesis.

Fig. 3. Floral differentiation in 'Sharpblue' blueberry during early December. (A) Longitudinal section of proximal floret shows the complete flower structures. (B) Enlarged immature anther shows developing tapetal cells (DTC) and primary sporogenous cells (PSC). (C) Placenta attached with developing ovules, before integument initiation. B and C are the enlargements of boxed areas in A.

Fig. 4. Floral development of 'Sharpblue' blueberry during early, middle, and late January (A–C, respectively). A1, A2, B1, B2, C1, and C2 are the enlargements of the boxed areas in A–C, respectively. (A1) Anthers show slightly more developed sporogenous cells (SC) and tapetal cells (TC). (B1) Early meiotic anther presents well-formed tapetal cells (TC) and developing microspores (MS) within the fully formed anther. (C1) Mature anther shows pollen tetrads and degenerated tapetal cells (DSC). (A2) An ovary locule shows axile placentation of developing ovules. (B2) Well-formed ovules, and (C2) Large and developed ovules show outer integument (OIg) and developing embryo sac (ES).

Fig. 5. Megasporogenesis and megagametogenesis in 'Sharpblue' blueberry in late January. (A) Ovule showing a single large megasporocyte (2n). (B) Ovule at two-cell stage (only one clearly shown here). (C) Ovule showing four megaspores. (D) Ovule with mature embryo sac (only six nuclei shown here). The presented ovules were found in the same floret.