Scanning Electron Microscopy of Floral Development in Freesias

Ludwika Kawa¹

Research Institute of Pomology and Floriculture, 96-100 Skierniewice, Poland

August A. De Hertogh²

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

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Abstract. Shoot apical meristems of *Freesia* × *hybrida* Klatt 'Rossini' reached the reproductive state after 3 weeks of precooling at 9C. Meristems isolated after 6 and 7 weeks of precooling showed the development of the initial four florets of the inflorescence.

The developmental cycle of *Freesia* cultivars is controlled primarily by temperature (De Lint, 1969; Gilbertson-Ferriss, 1985; Smith and Danks, 1985; Vijverberg, 1982). After being harvested, the corms require > 12 weeks of storage at 30C to satisfy internal dormancy. They can subsequently be forced as cut flowers or potted plants.

Currently, freesias being forced as potted plants require 6 to 7 weeks of corm precooling (De Hertogh, 1989). Therefore, this study was undertaken to follow the morphology of floral initiation and development in *Freesia* during this precooling period using the scanning electron microscope (SEM). Floral development of isolated apical meristems was illustrated with light microscopy by Vijverberg (1982). He also provided drawings of meristems. This is the only report illustrating the floral morphology of *Freesia*, but the techniques used did not provide cellular details.

The inflorescence of *Freesia* cultivars is a racemose spike with a central axis on which sessile flowers are borne. Each floret has membranous or dark green bracts, six tepals,

six stamens, and a bifid branched style. The flowering stalk may be branched.

Corms of *Freesia* 'Rossini' were grown in The Netherlands, stored at 30C for at least 3 months, and shipped by air on 29 Sept. 1990 to Raleigh, N.C. After arrival on 4 Oct., corms were stored at 17C, then placed at 9C on 12 Oct. Shoot apices were isolated on this date and at 7-day intervals until 30 Nov. 1990. Meristems were isolated with the use of binocular microscope. The meristems were kept moist at all times by holding them under distilled water during isolation. When necessary, leaf primordia were carefully removed to expose the meristematic mantle. After being isolated, the meristems were fixed in FAA (5 formalin : 5 glacial acetic acid : 45 ethanol : 45 water) for a minimum of 24 h before being dehydrated in a graded ethanol series (30%, 50%, 70%, 95%, 100%, 100%, 100%). Directly after the 100% ethanol step, the meristems were critical-point-dried using liquid CO₂ in a Samdri PVT-3B (Tousimis Research Corp., Rockville, Md.). They were subsequently mounted on SEM stubs with double stick tape (adhesive dots) and sputter-coated with 60 nm gold-palladium. They were viewed and photographed with a Philips 505 T SEM (Philips, Eindhoven, The Netherlands) using a 15-kV accelerating potential.

On arrival, all shoot apical meristems were vegetative and had a single leaf primordium (Fig. 1A). The diameter of the meristems was very small, making them difficult to isolate. Vijverberg (1982) indicated that *freesia* meristems have a diameter < 1 mm. The apical meristem became reproductive after 3 weeks of dry storage at 9C (precooling). The first flower primordium was clearly visible after 4 weeks (Fig. 1B). These observations confirmed those of Vijverberg (1982), who stored corms initially for 24 weeks at 30C, and then at 14C. With continued corm storage at 9C, floret primordia were visible after

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¹Researcher.

²Professor.

Fig. 1. Scanning electron photomicrographs of shoot meristem development of *Freesia* 'Rossini'. (A) On arrival, 0 weeks at 9C, $\times 244$. a = Vegetative shoot apex; LP = leaf primordium. (B) After 4 weeks at 9C, $\times 127$. a = Reproductive shoot apex; Pr-1 = growing primordium of first flower; Br-1 = first flower bearing bract primordium; LP = leaf primordium. (C) After 6 weeks at 9C, $\times 133$. a = Shoot apical meristem; Pr-1, -2, -3 = primordia of first, second, and third flowers; Br-1, -2 = bracts subtending flowers; SS = secondary shoot. (D) After 7 weeks at 9C, $\times 83$. a = Apical meristem; Pr-1, -2, -3, -4 = primordia of second, third, and fourth flowers. The primordium of first flower is not visible. Br-1, -2, -3 = bracts subtending flowers; SS-1, -2, -3 = secondary shoots; LB = scar of removed leaf base.

6 weeks (Fig. 1 C and D); the primordia of first and second flowers were distinctly visible (Fig. 1C) and the third flower primordium was forming. The arrangement was distichous. After 7 weeks, primordia of the first, second, third, and fourth flowers had been initiated (Fig. 1D). In addition, three lateral stem apices were present (Fig. 1D).

Since most *Freesia* cultivars have inflorescences with up to 12 or 13 florets (Smith and Danks, 1985; Vijverberg, 1982), floral development obviously continues for several weeks after planting. Under most commercial forcing conditions, the corms are planted after 7 weeks of precooling (De Her-

togh, 1989). Thus, development of the inflorescence will be affected for several weeks after planting, and the environmental conditions in the greenhouse are important. To observe floral development after 7 weeks of storage at 9C, 64 corms of 'Rossini' (eight corms per 1.6-liter azalea pot) were planted in Sunshine Mix No. 4 on 3 Dec. 1990 and forced at 16 to 18C. The average number of florets produced per inflorescence was 10 and the average number of shoots per corm was 2.6.

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