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Anther wall development, microsporogenesis and microgametogenesis in male fertile and sterile chrysanthemum (*Chrysanthemum morifolium* Ramat., Asteraceae)

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ABSTRACT

Anther wall development, microsporogenesis and microgametogenesis were compared between a normal male fertile chrysanthemum cultivar 'NJAU04-29-2' and the two male sterile selections 'rm20-11' (anther indehiscent) and 'NJAU05-52-2' (anther aborted). In both of the two male sterile types, the tapetum enlarged abnormally and showed signs of disorganization of walls at the onset of meiosis, the pollen was aborted, the anthers appeared shrunken, and the anther vascular bundle and connective tissue were degenerated by anthesis. In 'rm20-11', the two smaller pollen sacs began to degenerate at the microsporogenesis stage, so that only one or two microsporangia developed, while in 'NJAU05-52-2', only one or two microsporangia were formed following the archesporial cell stage, and most of the microspore mother cells degenerated during the course of meiosis.

1. Introduction

Male sterility is a much sought-after trait in many crop plants as it avoids the need for hand emasculation in the production of hybrid seed (Bino, 1985). A male sterile plant produces no functional pollen, either because the male organs fail to develop normally, microsporogenesis is abnormal, the pollen is not able to fully mature, or the anthers fail to dehisce. Male sterility is a common phenomenon across the whole plant kingdom (Chen et al., 2006). Apart from its utility in the context of hybrid seed production, the trait can also be exploited to gain an understanding of the genetic control of plant sexual reproduction (Tsvetova and Elkonin, 2003). A considerable body of descriptive anatomy relevant to male sterility exists in the literature, including tapetal abnormality in onion (Holford et al., 1991) and rapeseed (Chen et al., 2009), anther indehiscence in thale cress (Dawson et al., 1993; Sanders et al., 1999) and meiotic defects in cotton and soybean (Graybosch and Palmer, 1988; Zhang and Pan, 1991). However, an anatomical characterization of male sterility in the chrysanthemum has not been reported to date.

The chrysanthemum is a prominent cut flower, pot plant and ground-cover crop. Cultivars are generally maintained by vegetative propagation to make sure the homogeneity. Nevertheless, to build a colourful flower border and clusters, ground-cover chrysanthemums are preferred owing to its excellent characteristics such as creeping or low plant architecture, multi-branch, strong resistance, better adaptability, strong coverage ability, dense and colourful flowers and so on (Zhang et al., 2008; Zhao et al., 2009). The high demand for ground-cover cultivars forces producers to produce seeds to supply planting material. To produce F_1 hybrids in chrysanthemum, there is a need to develop methods of hybrid seed production which do not rely on hand emasculation. In this context, the male sterility trait is of substantial value. The flower morphologic anatomy and embryological characteristics in *Chrysanthemum multicaule*, a member of *Chrysanthemum sensu lato*, have been reported (Deng et al., 2010). Here, we report the identification of two independent forms of male sterility in the anemone-type chrysanthemum; one is based on anther indehiscence, and the other on anther abortion. We describe the processes of anther wall development, microsporogenesis and microgametogenesis in both types, and relate these observations to the development of the male sterility trait.

2. Materials and methods

The two male sterile chrysanthemum cultivars *Chrysanthemum morifolium* Ramat. 'rm20-11' and *C. morifolium* Ramat. 'NJAU05-52-2' were compared with the fertile cultivar *C. morifolium* Ramat. 'NJAU04-29-2'. The chrysanthemum disk floret contains five anthers, which in male fertile types appeared golden coloured and full of pollen at maturity (Fig. 1a). In 'rm20-11', the anthers appeared shrunken, brown-coloured and indehiscent,

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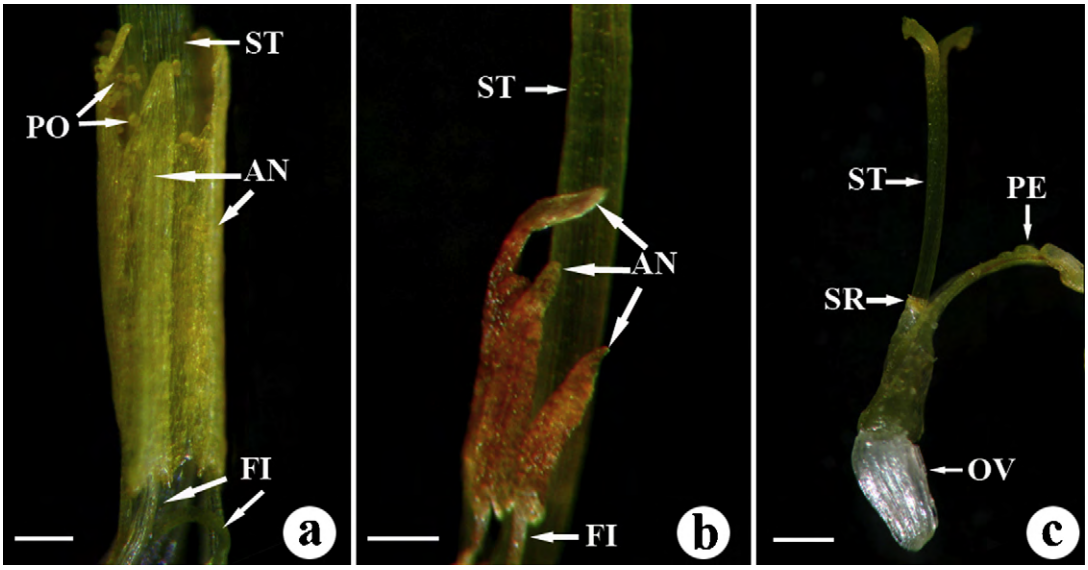

Fig. 1. The appearance of the anthers of three chrysanthemum cultivars contrasting with respect to male fertility. (a) In the male fertile 'NJAU04-29-2', five golden anthers are produced, able to release plenty of pollen. (b) In the male sterile 'rm20-11', the anthers are brown and indehiscent, with no pollen released. (c) In the male sterile 'NJAU05-52-2', the stamens have completely degenerated. AN: anther; FI: filament; OV: ovule; PE: petal; PO: pollen grain; SR: stamen remnant; ST: style; *bars* represent 200 μm, 300 μm and 500 μm, in a, b and c, respectively.

and released no pollen (Fig. 1b). In 'NJAU05-52-2', almost all the anthers had degenerated, although occasionally a thread-like structure remained (Fig. 1c). All three cultivars were cultivated at the Laboratory of Flower Breeding and Genetics, Nanjing Agricultural University, and are maintained at the Chrysanthemum Germplasm Resource Preserving Centre, Nanjing Agricultural University. Seedlings were grown in 8 cm × 10 cm pots, each containing a 2:1 mixture of garden soil and vermiculite with no added fertilizer, in a greenhouse under a 12 h day with a day/night temperature regime of ~25/18 °C and a relative humidity of ~70%. Floral buds at a range of developmental stages were harvested, fixed in FAA (1:1:18 formalin: acetic acid: 70% ethanol), dehydrated through an alcohol series, infiltrated with xylene and embedded in paraffin wax following Teng et al. (2005). Bud sections were cut to a thickness of 8–10 μm using a microtome, stained with Heidenhain's hematoxylin, and observed by light microscopy.

3. Results

3.1. Anther wall development, microsporogenesis and microgametogenesis

3.1.1. 'NJAU04-49-2'

The fertile anther consisted of two thecae bound together by connective tissue containing a central vascular bundle. Each theca included a large and a small pollen sac, within which male gametogenesis occurred (Fig. 2a). Before the stamen developed, the stamen primordium emerged first (Fig. 2b). During early anther development, an archesporial cell arose beneath the epidermis at each corner of the primordium. These cells, recognizable by their dense cytoplasm and conspicuous nuclei (Fig. 2c), divided periclinally to form the primary parietal cell on the outside and the primary sporogenous cell on the inside, respectively (Fig. 2d). The former then differentiated to generate the epidermis, endothecium, middle layer and tapetum, while the latter produced the sporogenous cells (Fig. 2e and f). The four-lobed anther was then initiated, including its two stomium regions and the vascular region. The middle layer originated from the endothecium (Fig. 2f), with the formation of the microsporangial anther wall being of the dicotyledonous type (Davis, 1966; Xue and Li, 2005). The epidermis flattened as the anther expanded. The tapetal cells remained small at the microsporocyte stage (Fig. 2g). At meiotic prophase I, the tapetal cells enlarged rapidly, became vacuolated and multinucleate following their nucleus division (Fig. 2h). The middle layer flattened during meiosis and was still observable by the late uninucleate pollen grain stage (Fig. 2i), but had degenerated by the binucleate stage. At the early and mid microspore stage, the tapetum was parietal but began to lose the wall and intrude into the locular space with the cytoplasm fusing to be periplasmodium around the microspore by the late uninucleate pollen grain stage (Fig. 2j). It was no longer detectable by the binucleate pollen stage (Fig. 2k). At the binucleate pollen grain stage, the endothecium wall thickened, except in the stomium region (Fig. 2k), and the septum cells separating the homolateral pollen sacs began to degenerate and fragmented (Fig. 2k). Following this, the anther became bilocular and dehisced from the stomium region at the trinucleate pollen stage (Fig. 2l). At anther dehiscence, the vascular bundle remained clearly visible, but the connective tissue had degenerated (Fig. 2l). The sporogenous cells developed into microspore mother cells (MMCs) with a prominent nucleus and dense cytoplasm (Fig. 2m). The MMCs underwent the classical meiotic cycle (Fig. 2n), resulting in mostly tetrahedral tetrads (Fig. 2o). A small proportion of decussate tetrads was also produced (Fig. 2p). Two nuclei were formed and no cell wall was detected by the end of meiosis I, indicating that cytokinesis is of the simultaneous type. At the time of their release from the tetrad, the non-vacuolated immature microspores were irregular in shape and had a dense cytoplasm (Fig. 2q) surrounding one nucleus containing two or three nucleoli. The microspores developed into uninucleate microspores. The nucleus of the early uninucleate microspore was located at the centre of the cell (Fig. 2r). As the uninucleate microspore cytoplasm became vacuolated, the nucleus migrated from the centre of the cell towards its periphery (Fig. 2s). Thereafter, the microspore nucleus divided mitotically to form a large vegetative and a small generative cell, thus forming the binucleate pollen grain (Fig. 2t and u). The two nuclei migrated to the centre of the pollen grain as the vacuole disappeared (Fig. 2v). Via one more mitosis, the generative cell divided into two sperm cells, so that the pollen grain was trinucleate at the time of its release (Fig. 2w and x).