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Microsporogenesis and male gametogenesis in *Musella* (Musaceae), a monotypic genus from Yunnan, China

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Microsporogenesis and male gametogenesis of *Musella* (Musaceae), a monotypic genus endemic to Yunnan, China, are described for the first time. The anthers are tetrasporangiate. The formation of the anther wall is of the basic type. The mature anther wall consists of an epidermis, an endothecium, many middle layers and a two-layered glandular tapetum with uninucleate cells. The old anther wall consists of an epidermis with annular and helical thickenings and reduced endothecium. Successive cytokinesis follows meiosis of the microspore mother cell thence forming a T-shaped or isobilateral tetrad of microspores. Pollen grains are 2-celled. The generative cell nucleus is clavate in shape. Some special features and relationships between anther structure and pollinator type are discussed.

Key words: development, embryology, male gametogenesis, microsporogenesis, Musaceae, *Musella*

Introduction

The order Zingiberales has long been regarded in the taxonomic literature as a natural monophyletic group within the monocotyledons (Tomlinson 1962, Cronquist 1981, Dahlgren *et al*. 1985, Kress 1990, Rudall *et al*. 1999, Stevenson *et al*. 2000, Kress *et al*. 2001). Two informal groups are often recognized based upon shared morphological and anatomical characters: the "banana families" (Musaceae, Heliconiaceae, Strelitziaceae, Lowiaceae) and the "ginger families" (Zingiberaceae, Costaceae, Cannaceae, Marantaceae) (Dahlgren & Clifford 1982). Musaceae, a conspicuous tropical group of plants with three genera *Musa*, *Ensete* and *Musella* and some 45–48 species, is restricted to the Old World (Cronquist 1981, Andersson 1998). The genus *Musa*, from which the edible bananas are derived, is the largest of the genera, distributed in tropical Asia (with an extension to the subtropics) and limited areas of northeastern Australia. *Ensete* is a smaller group of approximately six species and has a discontinuous distribution from tropical Africa to tropical Asia (Simmond 1960).

Musella is a monotypic genus, restricted to conifer–oak mixed forests between 1500 and 2500 m altitude in southwestern China, primarily in central and western Yunnan (Li 1978,

1979, Wu & Kress 2001). *Musella* is the most easily recognizable genus within Musaceae, distinguished from *Musa* and *Ensete* by its small size, congested pseudo-stems, compact rosette inflorescences with yellow to orange bracts, and short, hirsute fruits. *Musella* has been used by local people for many centuries. It is used as a medicinal plant, for food, for fodder, as weaving material, to alleviate soil erosion, and also as an ornamental plant. As reported earlier, pollination syndromes in the banana families have been shown to include both chiropterophily and ornithophily (Nur 1976, Itino *et al*. 1991, Liu *et al*. 2002b). However, insects such as bumblebees, honeybees and wasps, pollinate *Musella lasiocarpa*. This is the first record of entomophily for the Musaceae (Liu *et al*. 2002a).

Embryological studies are often useful not only in encompassing investigation of virtually all the events relevant to sexual reproduction but also in solving taxonomic and phylogenetic problems at or above generic levels (Davis 1966, Tobe 1989, Johri *et al*. 1992). There are few accounts of the embryology of these families, especially of Musaceae. Microsporogenesis and male gametogenesis in *Musella* has not previously been investigated. The purpose of the present paper is to report a detailed description of microsporogenesis and male gametogenesis in *Musella*.

Material and methods

The material of *Musella lasiocarpa* was collected for embryological study from a cultivated population in Kunming, Yunnan province. The vouchers (Xue Chunying 2001000A) are deposited in the Herbarium of Kunming Institute of Botany (KUN).

Flowers of all ages were fixed in FAA (5 ml formalin: 6ml acetic acid: 89 ml 70% ethanol). After being stained in Ehrfich's hematoxylin, the materials were embedded in paraffin using the conventional method and sections with a thickness of 5–10 μm were cut. Sections were stained with safranin-fast green. Observations and photography were carried out using an Olympus BX15 microscope.

Results

Formation of anther wall

The floral morphology of *Musella lasiocarpa* was described in detail by Liu *et al*. (2002a). Male flowers of *Musella* are 30 mm long. The anther has four sporangia and each has a row of archesporia differentiated just beneath the epidermis. Archesporial cells are recognizable by their dense cytoplasm and conspicuous nuclei (Fig. 1a). These cells divide periclinally forming outer primary parietal cells and inner primary sporogenous cells (Fig. 1b). The primary parietal layer divides periclinally and produces two secondary parietal layers (Fig. 1c). Both layers undergo further periclinal divisions. One towards the epidermis differentiates into an endothecium followed by a middle layer (Fig. 1d). The other secondary parietal layer produces another middle layer, and the tapetum (Fig. 1e). The middle layers may undergo further divisions and form four or five layers (Fig. 1f and g). The middle layers have a common histogenetic origin with both the endothecium and the tapetum, microsporangial wall formation therefore conforms to the basic type (Davis 1966). The tapetum, with large nuclei and dense cytoplasm, may be irregularly two-layered (Fig. 1g). The anther wall prior to maturation usually comprises seven to nine cell-layers: an epidermis, an endothecium, four to five middle layers and an irregularly biserial tapetum (Fig. 1g).

Tapetal cells are uninucleate throughout their development. At about the time of microsporocyte meiosis, the walls of the tapetal cells become indistinct and the tapetal cells begin to degenerate (Fig. 2a). The tapetal cells degenerate completely at the mature pollen grain stage. All the tapetal cells degenerate at their original sites (Fig. 2a–c). Therefore, the tapetum of *Musella* is glandular.

During maturation, the epidermal cells enlarge and develop annular and helical thickenings. The endothecium reduces and does not develop fibrous thickenings as in most angiosperms (Fig. 2h). All the middle layers are ephemeral and degenerate during microsporocyte meiosis, when the cells become flattened and disintegrate. Thus, the mature anther wall is composed of the fibrous thickened epidermis and the reduced endothecium (Fig. 2h). The

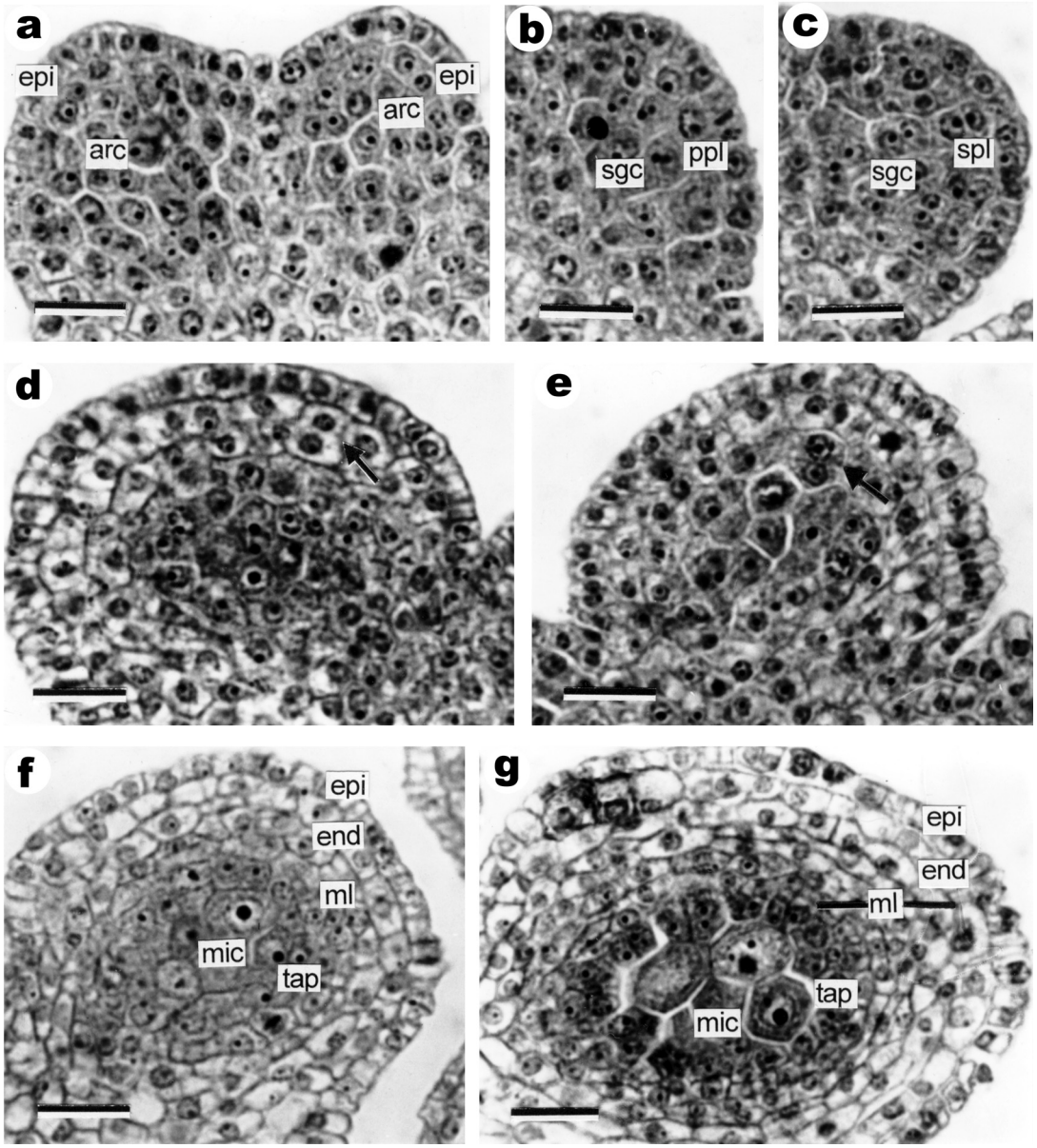

Fig. 1. Anther and microsporogenesis in *Musella lasiocarpa*. — **a**: Archesporial cells (arc) and protoderm (epi). — **b**: Sporogenous cells (sgc) and primary parietal layer (ppl). — **c**: Sporogenous cells (sgc) and secondary parietal layers (spl) beneath protoderm. — **d**: Outer secondary parietal layer divided into endothecium and middle layer (arrowhead). — **e**: Inner secondary parietal layer divided into middle layer and tapetum (arrowhead). — **f**: Microspore mother cells (mic), and an anther wall composed of endothecium (end), middle layers (ml) and tapetum (tap). — **g**: Microspore mother cells (mic), an anther wall composed of an endothecium (end), four or five middle layers (ml) and one or two layers of tapetal cells (tap). Scale bars: 20 µm in Figs. **a**–**d**; 32 µm in Figs. **e**–**f**; 10 µm in **g**.

anther dehisces by two longitudinal slits. Each microsporangium opens by a common slit with the other microsporangium of the same theca as in most other plants. We did not find any thread-like formation in the anther, the epidermal origin of which has been illustrated ultrastructurally by Hesse (1981), Kronestedt and Bystedt (1981), and Kronestedt and Walles (1983).

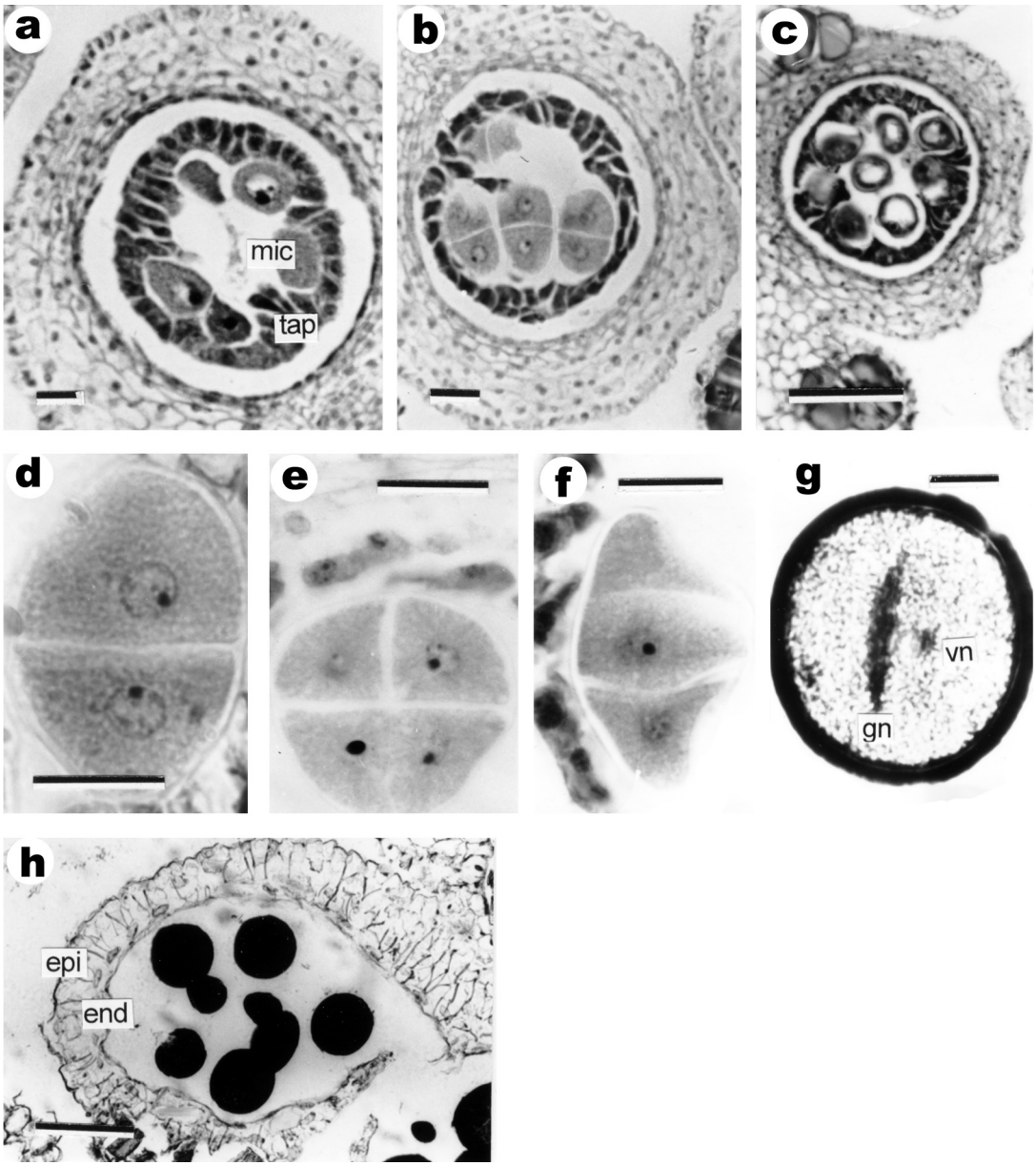

Fig. 2. Anther and microsporogenesis in *Musella lasiocarpa*. — **a**: Microspore mother cell (mic) in prophase and initiation of degeneration in tapetal cells. — **b**: Telophase I of meiosis of microspore mother cells, dyad formed. — **c**: 1-nucleate free microspores and anther wall. — **d**: Dyad. — **e**: Isobilateral tetrad. — **f**: T-shaped tetrad. — **g**: 2-celled pollen grains (gn = generative nucleus, vn = vegetative nucleus). — **h**: Wall of mature anther, showing annular and helical thickenings of the epidermis (epi) and reduced endothecium (end). Scale bars: 10 µm in **a**–**c**, and **h**; 50 µm in **d**–**g**.

Microsporogenesis

A row of sporogenous cells, produced by the archesporia, gives rise to a mass of microspore mother cells by several mitotic divisions (Fig. 1a and b) The microspore mother cells are tightly arranged (Fig. 1f) and, prior to meiosis, separate results from the secretion of a callose wall by the microsporocytes (Fig. 1g).

Successive with changes taking place in the

wall of the microsporangia, the primary sporogenous cells undergo mitosis forming secondary sporogenous cells, from which microsporocytes are derived (Figs. 1f and g, 2a). Meiosis in a microsporocyte is accompanied by successive cytokinesis in which the two cell plates are laid down in a centrifugal manner immediately after the first and second meiotic divisions (Fig. 2b and d–f).

During meiotic divisions a callose wall is secreted around the microspore mother cells. The callose envelops the individual sporocyte (the special cell envelope) and delimits the whole dyad at the end of meiotic division I (Fig. 2d). Meiosis II in each dyad results in a microspore tetrad (Fig. 2e and f). The callosic septation (special cell envelope) between the microspores are of unequal thickness in the tetrads as a result of the successive type of microsporocyte division. The shape of the resultant tetrads is isobilateral (Fig. 2e) or T-shaped (Fig. 2f).

Microgametogenesis

Microspores separate from the tetrad as uninucleate free microspores. Each microspore has a dense cytoplasm with a prominent and centrally placed nucleus. As the central vacuole develops, the nucleus takes a peripheral position (Fig. 2c). The first mitotic division of the microspore nucleus results in the formation of two unequal cells, a large vegetative one and a smaller generative one. The generative cell nucleus becomes clavate during development (Fig. 2g). Pollen grains were 2-celled at the time of anther dehiscence. The pollen grains are inaperturate.

Discussion

Embryological characters

The embryological features of *Musella* are summarized as follows. The anther of each stamen in the flower of *Musella lasiocarpa* is tetrasporangiate. The middle layers have a common histogenetic origin with both the endothecium and the tapetum, so, the formation of the anther wall is of the basic type. The mature anther wall consists of an epidermis, an endothecium, many middle layers and one or two layers of glandular tapetum with uninucleate cells. The older and mature anther wall consists of epidermis with annular and helical thickenings and a reduced endothecium. Successive cytokinesis follows meiosis of the microspore mother cell thence forming T-shaped or isobilateral tetrads of microspores. Pollen grains are 2-celled. The generative cell nucleus is clavate in shape.

Some striking features are found in *Musella*. (1) On the basis of the formation of the middle layers, Davis (1966) classified the development of the anther wall into four types: basic, dicotyledonous, monocotyledonous and reduced. The basic type occurs in nine families, e.g. Anacardiaceae, Lecythidaceae, Rhamnaceae, Vitaceae, while the monocotyledonous type includes the majority of monocotyledonous families as well as several dicotyledonous families. However, the formation of the anther wall of *Musella* conforms to the basic type. Because sufficient data on embryology characters of banana families are lacking (Johri *et al*. 1992), the pattern of formation of anther walls of other taxa in Musaceae is unknown. According to Dahlgren *et al*. (1985), Zingiberales have the monocotyledonous type of anther wall formation. (2) The endothecium of *Musella* is reduced and does not develop fibrous thickenings, however, the epidermal layer develops fibrous thickenings. Epidermal thickenings also occur in some members of Zingiberaceae (Johri *et al*. 1992). The endothecium is reported as both developing or not developing fibrous thickenings in *Musa* (Juliano & Alcala 1933, Untawale & Bhasin 1973). Presumably the fibrous, thickened epidermis, instead of the endothecium, is responsible for the dehiscence of the anther in *Musella*. (3) Microsporogenesis of *Musella* is successive. Successive microsporogenesis is predominant in monocotyledons, although the simultaneous type characterizes the "lower" Asparagales (Furness & Rudall 1999). Zingiberales are a relatively well-established and coherent order, and are uniformly successive. The callose walls surrounding the microsporocytes, dyads and tetrads are thin and pollen is mostly exineless and inaperturate (Furness & Rudall 1999). (4) In angiosperms generally, five patterns of the arrangement of microspores in a

tetrad are recognized: tetrahedral, isobilateral, linear, T-shaped and decussate. The tetrad patterns are particularly associated with the type of microsporogenesis. Successive microsporogenesis results in tetrads that are isobilateral (or tetragonal), T-shaped, linear or decussate. Tetrads resulting from simultaneous division are tetrahedral and decussate (Furness & Rudall 1999). The tetrads of *Musella* are T-shaped and isobilateral. Isobilateral, decussate, linear or T-shaped tetrads also occur in Zingiberaceae (Raghavan & Venkatasubban 1941).

Because of a lack of sufficient data from each family of Zingiberales, we have not compared the embryological characters of *Musella* with many other taxa. More intensive studies of individual families are needed to clarify embryological attributes for each family.

Anther structure and pollinator type

As mentioned above, *Musella lasiocarpa*, which is pollinated by insects such as bumblebees, honeybees and wasps, is the only reported entomophilous taxon of Musaceae. However, pollination syndromes in Musaceae have been shown to include both chiropterophily and ornithophily (Nur 1976, Itino *et al*. 1991, Liu *et al*. 2002b). In particular, the large size and visual conspicuousness of the inflorescences, the floral odor, and copious nectar production by flowers in the family are prime examples of characters adapted morphologically for bird (or bat) pollination (Fægri & van der Pijl 1979, Endress 1994). *Strelitzia reginae* (Strelitziaceae) possesses a highly specialized flower presumably evolved as an adaption to ornithophily (Scott-Elliot 1890, Mahanty 1970). In *S. reginae* anthers there are specialized thread-like formations that are derived from specialized epidermal cells in the stomium region. The pollen grains are kept together in aggregates by these threads in the anthers and released in great amounts from the longitudinally opened, long anthers (Kronestedt & Bystedt 1981). *Musella lasiocarpa* shares the broad morphological ornithophily traits: the bright yellow to yellow-orange inflorescence bracts, odorless flowers that product copious nectar, whereas it is entomophilous (Liu *et al*. 2002a). The bright yellow or yellow-orange bract color is a unique character in Musaceae and is one important trait associated with both bird and insect pollination (Fagri & van der Pijl 1979, Waddington 1983, Waser 1983, Barth 1985, Endress 1994). We found no thread-like formations in the anthers of *M. lasiocarpa*, so the pollen grains are not kept together in aggregates and are released individually from the longitudinally opened, long anthers. The different pollinators may have resulted in different pollination mechanisms. Insect pollination in *Musella* may indicate that pollination mechanisms are adapted to entomophily, such as pollen grains that are released individually from the anthers, and anthers that have no thread-like formations. However, we have no unambiguous evidence to indicate that a relationship exists between the thread-like formations and bird pollination in Zingiberales, because the anther development in Zingiberales except for *Strelitzia* and *Musella* has not been studied in sufficient detail.

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