Taiwania, **48**(4): 273-281, 2003

Anther and Pollen Wall Development in *Dumasia miaoliensis* Liu and Lu (Fabaceae)

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(Manuscript received 19 September, 2003; accepted 20 October, 2003)

ABSTRACT: This paper is intended for elucidation the anther and pollen wall development of *Dumasia miaoliensis* Liu and Lu (Fabaceae). The undifferentiated anther is ovoid-shaped and tetrasporangiated. The anther wall development is basic type, which is composed of an epidermal layer, an endothecial layer, two middle layers and tapetum. Anther-tapetum is glandular type and its' cells are uniseriate and uninucleate. Pollen grains are 6-porate and 2-celled at the time of shedding. Before protectum development, a glycocalyx layer is deposited on the invaginated plasma membrane, exclusive of the future apertures. Subsequently, the probacules, arising basally from the plasma membrane, elongate under the protectum. The foot layer and endexine formation are concomitant with the callosic wall dissolution. The foot layer is thin and interrupted, but the endexine is thick and continuous. The intine is initiated in the vacuolated stage.

KEY WORDS: Callose, *Dumasia miaoliensis*, Fabaceae, Probacule, Protectum.

INTRODUCTION

Plants of *Dumasia miaoliensis* Liu and Lu (Fabaceae) belong to tribe Phaseoleae of the subfamily Faboideae of Fabaceae (Lackey, 1981). *D. miaoliensis*, endemic and endangered to Taiwan (Lu *et al.*, 2001; Boufford *et al.*, 2003), occurs only in Chingchieng and Erpenshong between 1100 and 1500 m (Lu, 1977; Liu and Huang, 2001). Pollen grains of *D. miaoliensis* were described as 6-porate with reticulate to rugulate exine (Huang, 1972). Four types of anther wall development were described by Davis (1966) based on the secondary parietal layers: Basic type (type I), Dicotyledonous type (type II), Monocotyledonous type (type III) and Reduced type (type IV). In general, one specific type of the anther wall development is found in each families. However, some families possess two types of anther wall development, such as the Commelinaceae having type I and III (Hardy *et al.*, 2000), and the family Solanaceae having type I and II (Carrizo, 2002).

Many papers described exine formation. The exine formation is initiated by deposition of the primexine at the tetrad stage (Heslop-Harrison, 1963; Larson and Levis, 1962; Skvarla and Larson, 1966; Dickinson, 1970), or formation of a glycocalyx (Rowley and Dahl, 1977). However, there is a different view about exine initiation in the reports of Skvarla and Rowley (1987), Takahashi (1989), Takahashi and Skvarla (1991). They suggested that the exine formation is initiated by invagination of the microspore plasma membrane at the early tetrad stage and the protectum is the first to be laid down on the invaginated plasma membrane.

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Because having a few studies on microsporogenesis (Jane, 1995; Liu, 1997; Liu and Huang, 1999), there is not any information about *Dumasia miaoliensis* beyond that of Ikuse (1954) and Huang (1972). Therefore, using a combination of transmission and scanning electron microscopy, the present paper is an attempt to understand the anther wall development and pollen exine formation in *Dumasia miaoliensis*.

MATERIALS AND METHODS

The plants were collected from the field and then cultivated in the greenhouse of the Botany Department, National Taiwan University. Buds and flowers of different stages were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) for 4 hours, after three buffer rinses, then postfixed in 1% OsO_4 for 2 hours. Sampled were dehydrated in a graded ethonal-acetone series and embedded in Spurr resin (Spurr, 1969). Thick sections (1 μm) were stained with 1% toluidine blue in 1% borax at 60℃, and thin sections (70-90 nm) were stained with uranyl acetate and lead citrate. LM and TEM examination were accomplished with Leica Microscope and Hitachi-600 Transmission Electron Microscope.

Fresh pollen grains were dehydrated in an ethanol series and dried in critical point drying, and then coated with gold and examined with Hitachi S 520 Scanning Electron Microscope.

RESULTS

Anther development

The undifferentiated anther is ovoid-shaped and tetrasporangiated in transerves section view, an outer epidermal layer and inner archesporial cells are observed (Fig. 1A). The epidermis only divides anticlinally. Besides anticlinal divisions, the archesporial cells undergo periclinal divisions resulting the primary parietal layer outside and the primary sporogenous layer inside (Fig. 1B). The primary parietal layer undergoes periclinal divisions twice which gives rise to the endothecial layer, two middle layers and the tapetal layer (Fig. 1C). At the same time the sporogenous cells enlarge and undergo periclinal division once generating two rows of microspore mother cells (Fig. 1D). The epidermal, endothecial and middle layer cells have a prominent nucleus, large vacuole, numerous rough endoplasmic reticula and mitochondria (Fig. 2A). The microspore mother cells (Fig. 2B) cytologically resemble to the tapetal cells (Fig. 2A) with an emphatic nucleus, small vacuoles, rough endoplasmic reticula and mitochondria.

Before meiosis, the tapetal cells enlarge and appear as uniseriate and uninucleate (Fig. 1E). The microspore mother cells undergo meiosis and gradually show a tetrahedral arrangement and are enclosed in callosic envelopes (Fig. 1E). The microspore tetrads are still within the callose walls during the late tetrad stage. (Fig. 1F). Callose dissolution originates in the corners and is generally centripetal. When callose was dissolved, the microspores were released to the anther locules and have only small vacuoles in the cytoplasm (Fig. 1G). Mature pollen grains are characterized by containing numerous starch grains and without vacuoles (Fig. 3A). The pollen grains are 6-porate, prolate to oblate in the equatorial view, subangular in the polar view and with a reticulate to rugulate exine (Fig. 3C-H). At the shedding time the pollen grains are 2- celled with a generative cell and a vegetative cell (Fig. 1H).

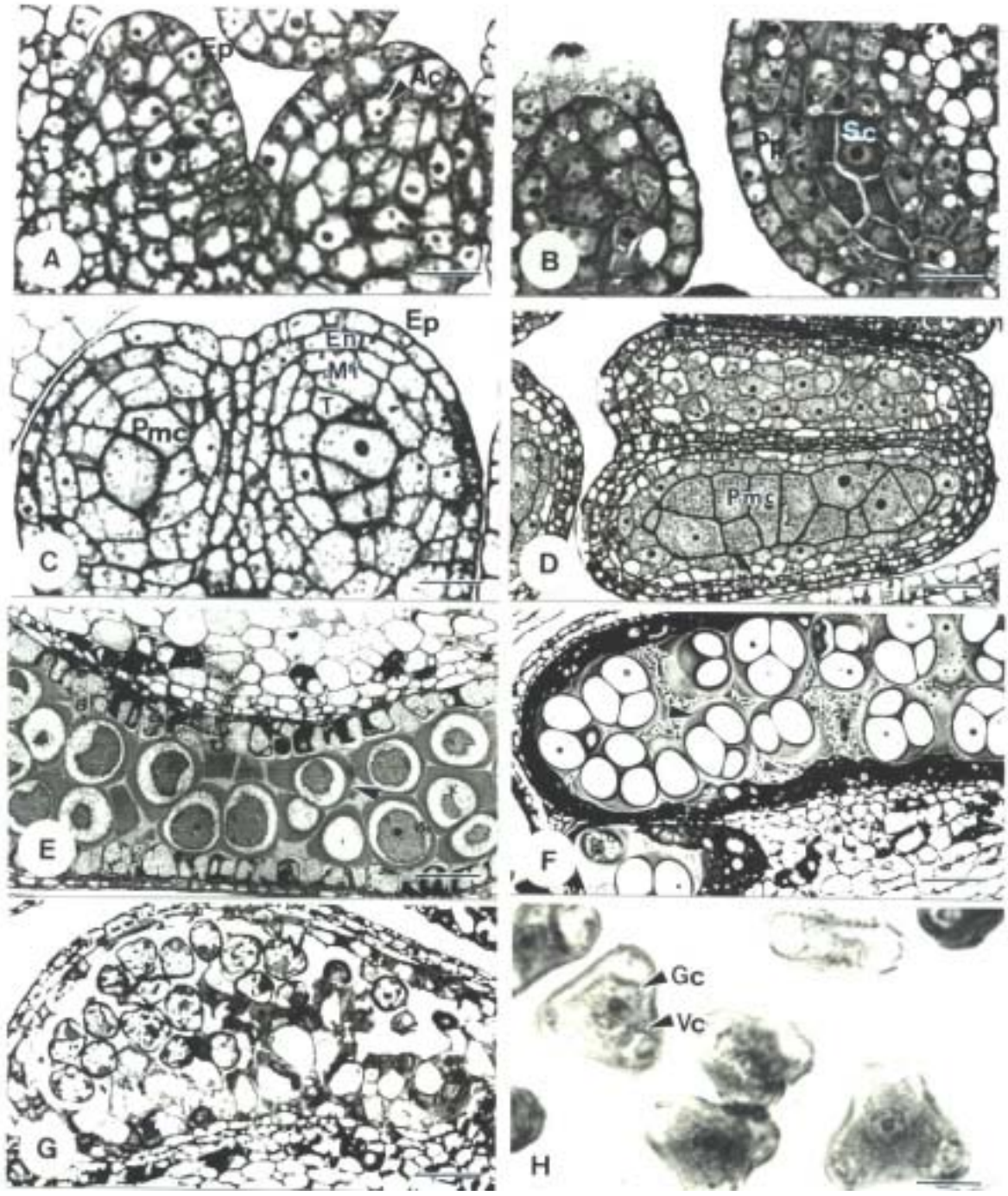

Fig. 1. Anther development. A-C: Transerves sections. A: An ovoid-shaped undifferentiated anther with an epidermal layer and archesporial cells (arrowhead). B: Tetrasporangiated anther composed of the epidermis, the primary parital cell and the sporogenous cells. C: The pollen sac with epidermis, endothecium, two middle layers, tapetum and microspore mother cells. D-H: Longitudinal sections. D: The lower anther lobe showing 2-rows of microspore mother cells. E: The microspore mother cells within callose wall (arrowhead). F: The microspore tetrad still encased in callose wall (arrowhead). G: Free microspores with lots of small vacuoles in vacuolated stage. H: Mature pollen grains with 2- celled, a generative cell and a vegetative cell at the shedding time. (Ac: archesporial cell, E: epidermis, En: endothecium, Gc: generative cell, Ml: middle layers, Pmc: microspore mother cells, Pp: primary parital cell, Sc: sporogenous cells, T: tapetum, V: vacuole, Vc: vegetative cell). A-C, H, Bar=10 μm. D-G, Bar=25 μm.

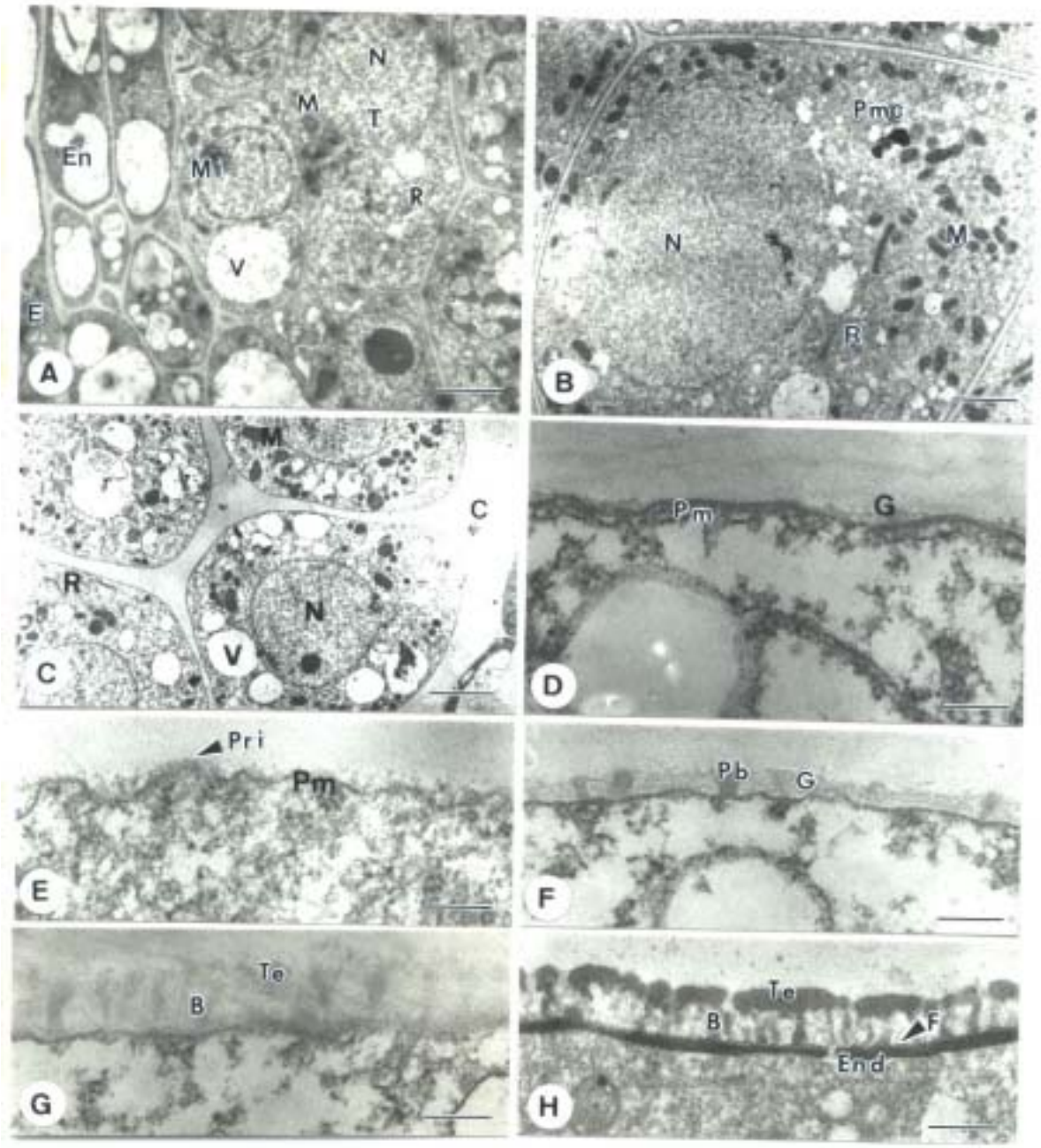

Fig. 2. A-C: Transmission electron micrographs of anther development. A: The anther wall with the epidermis, endothecium, middle layer and tapetum. B: Microspore mother cell with a prominent nucleus, numerous plastids, mitochondria and rough endoplasmic reticula. C: A tetrad enclosed in callose wall with numerous mitochondria, vaculoes, rough endoplasmic reticula and a nucleus. D-H: Transmission electron micrographs of pollen wall development. D: A glococalyx layer deposited on the smooth plasma membrane. E: The protectum deposited on these protuberant sites of invaginated the plasma membrane. F: The glycocalyx layer and probacules arising acropetally from the plasma membrane. G: The protectum and probacules formed within the callose wall. H: The foot layer and endexine established simultaneously with callose wall dissolution. (B: bacule, C: callose, E: epidermis, En: endothecium, End: endexine, F: foot layer, G: glococalyx layer, M: mitochondria, Ml: middle layer, N: nucleus, Pb: probacules, Pm: plasma membrane, Pmc: microspore mother cell, Pri: protectum, R: rough endoplasmic reticulum, T: tapetum, Te: tectum, V: vacuoles). A-C, Bar=1 μm. D-H, Bar=0.2 μm.

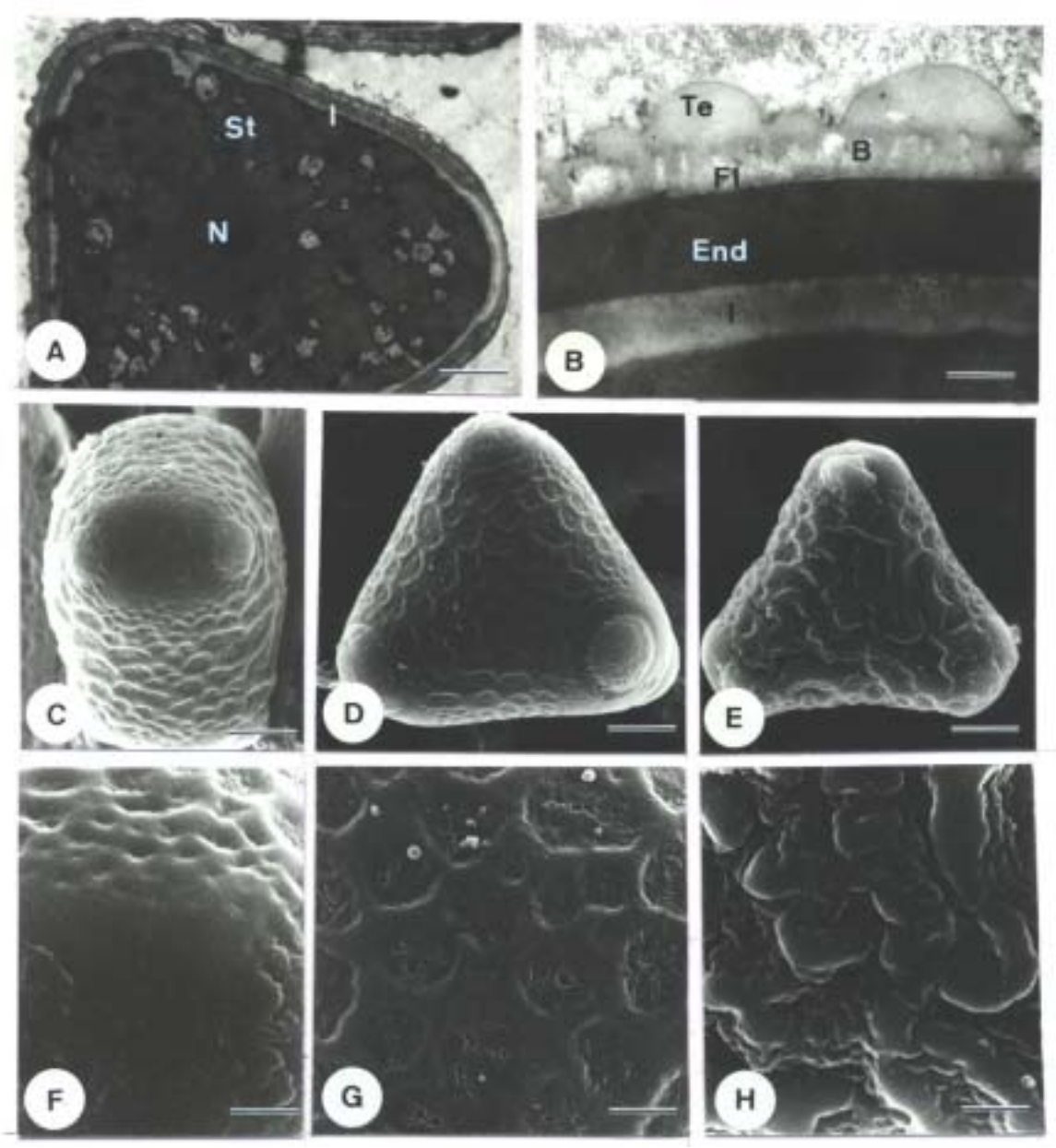

Fig. 3. A, B: Transmission electron micrographs of mature pollen grain. A: Pollen grain with a prominent nucleus and numerous starch grains. B: The exine stratification: thick and discontinuous tectum, solid bacules, thin and discontinued foot layer, thick and uninterupted endexine and intine. C-H: Scanning electron micrographs of mature pollen grains. C: Pollen grain is 6-porate and prolate in equatorial view. D: The reticulate mesocolpi area in equatorial view. E: The rugulate mesocolpi area in equatorial view. F: The detail of the aperture area. G and H: The magnification of mesocolpi in equatorial view. (B: bacule, End: endexine, Fl: footlayer, I: intine, N: nucleus, St: starch grains, Te: tectum) A, C-E, Bar=6 μm. B, Bar=0.6 μm. F-H, Bar=1.5 μm.

Pollen wall development

During the tetrad stage, four microspores are separated from each other by a callosic wall. At the early tetrad stage, the microspores contain a nucleus, lots of mitochondria, small vacuoles, rough endoplasmic reticulum and ribosomes (Fig. 2C). Before the initiation of

protectum, a glococalyx layer is deposited on the plasma membrane (Fig. 2D). The protectum is laid down on the protuberant sites of the invaginated plasma membrane (Fig. 2E). Subsequently, the probacules, arising basally from the plasma membrane, are elongated under the protectum (Fig. 2F). The protecta and probacules are deposited during the tetrad stage, and the principal structural features of exine pattern are established within the callose wall (Fig. 2G). The foot layer and endexine formation is concomitant with the callosic envelope dissolution (Fig. 2H). The foot layer is thin and interrupted, but the endexine is thick and continuous (Fig. 2H). The intine starts to develop at the vacuolated stage. In the mature pollen grain, the tectum is reticulate to rugulate; 1-1.5 μm thick; the foot layer is thin and interrupted; the endexine is distinctly thick, about 1.5-2.0 μm thick; the intine is about 1-1.3 μm thick (Figs. 3A, 3B).

DISCUSSION

In general, one specific type of the anther wall development is found in each family. The anther wall development in the Faboideae was reported as Dicotyledonous type (Davis, 1966; Prakash, 1987), including the genera *Trifolium* (Hindmarsh, 1964), *Pisum* and *Lens* (Biddle, 1978), *Indigofera* (Ashrafunnisa and Pullaiah, 1995), *Desmodium* (Buss *et al*., 1969) are also Dicotyledonous type. However, in *Indigofera* and *Rhynchosia* (Oomman, 1971), *Uraria* (Liu and Huang, 1999) and in the present study of *Dumasia miaoliensis*, the anther wall consists of an epidermal layer, an endothecial layer, two middle layers and a tapetal layer, it belongs to *Basic type* (type I).

Invagination of the plasma membrane prior to the exine initiation has been presented in many studies (Larson and Lewis, 1962; Skvarla and Larson, 1966). Rowley and Skvarla (1975)、Rowley (1981) and Gabarayeva *et al.* (1998) suggested the plasma membrane and glycocalyx are part of a system which mediated genetic expression of exine pattern. In the present study, the plasma membrane is invaginated before exine initiation within the callosic envelope, and the protectum is attached to the invaginated site of the plasma membrane. This might indicate that the plasma membrane plays a role in the determination of the future exine pattern.

According to the primexine model (Heslop-Harrison, 1963), the formation of exine components began at the initiation of probacules into the primexine matrix, but Dunbar and Rowley (1984) showed that exine development initiated from the protectum in *Betula*, Skvarla and Rowley (1987), Takahashi and Kouchi (1988) and Takahashi (1989) also found that the protectum are the first to be laid down and they occur directly on the plasma membrane before there is any indication of primexine matrix and probacules. In the present study, the protectum is formed on the protruding site of the invaginated plasma membrane, and after that the probacules are formed under the protectum, which is similar to that of *Betula* (Dunbar and Rowley, 1984).

Larson and Levis (1962) reported that the endexine formation was preceded by foot layer formation in the Fabaceae. Skvarla and Rowley (1987) showed that a foot layer was formed in *Poinciana* just at the end of the tetrad period and an endexine was formed only at the loss of the callosic envelope. Takahashi (1989), and Liu and Huang (1999) showed that the foot layer and endexine began formation simultaneously in *Caesalpinia* and *Uraria*. In the present study, the foot layer and endexine are also formed simultaneously and concomitant with the callose wall dissolution.

ACKNOWLEDGMENTS

The advice of reviewers is greatfully appreciated. This work was supported by the research grant to T. C. Huang from the National Science Council, Republic of China (NSC88-2311-B-020-023).

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苗栗野豇豆 (豆科) 花藥與花粉壁的發育

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(收稿日期：2003 年 9 月 19 日；接受日期：2003 年 10 月 20 日)

摘　　要

未分化的苗栗野豇豆花藥是卵形具有四個小孢子囊，花藥壁是由一層表皮、一層花藥內壁、兩層中間層及一層營養層所構成，是屬於基本型。營養層為分泌型，由單核的單層細胞組成。花粉粒具六孔，釋出時為兩個細胞時期。花粉壁在原蓋頂層發育前，除了未來發芽孔外，細胞膜向內凹陷。接著，位於原蓋頂層下方之臘梅糖和原柱狀層從細胞膜向基開始延長生長。底層及花粉外壁內層形成伴隨著胼祇質瓦解。底層很薄且呈不連續狀，花粉外壁內層很厚且呈連續狀。花粉內層起始於液胞化時期。

關鍵詞：胼祇質、豆科、原柱狀層、原蓋頂層、苗栗野豇豆。

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