Cytological Mechanism of Pollen Abortion in Photoperiod-Temperature Sensitive Genic Male Sterile Line Peiai 64S in Rice (Oryza sativa L.)

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水稻光温敏核雄性不育系培矮 64S 花粉败育的细胞学机理

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摘　要: 采用半薄切片及薄切片技术, 对水稻光温敏核雄性不育系培矮 64S 与正常水稻品种 IR36 的花粉形成发育过程进行了比较研究。结果表明, 小孢子母细胞减数分裂之前, 培矮 64S 与 IR36 的发育过程基本相似。但从减数分裂开始, 培矮 64S 的雄性性细胞出现一些异常变化, 并最终发育至二胞花粉早期败育。这些变化主要发生在两个阶段: (1) 减数分裂前期, 约半数的小孢子母细胞的胞质出现异常, 游离核糖体稀少, 并具有不发育线粒体和大量泡状内质网。这类异常的小孢子母细胞在随后的发育中逐渐液泡化并最终解体。(2) 早期小孢子形成之后, 几乎所有小孢子外壁均发育异常, 表层与里层之间界限不清, 缺少中间透明带, 同时内壁没有形成。但是, 在花粉发育的整个过程中, 培矮 64S 与 IR36 绒毡层的发育和降解过程基本相似。据此认为, 培矮 64S 的花粉败育可能是由小孢子母细胞或花粉外壁的异常发育造成的, 而不是由绒毡层发育引起的。

关键词: 水稻; 光温敏核雄性不育系; 花粉; 发育; 败育; 细胞学

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Abstract: By using the methods of semi-thin and thin section, the process of pollen development was comparatively studied in Peiai 64S, a photoperiod-temperature sensitive genic male sterile line (PTGMS) and IR36, a fertile cultivar in rice. The development of Peiai 64S and IR36 did not differ up to microsporocyte formation stage; but since meiosis stage, male reproductive cells of Peiai 64S underwent several structural changes and ultimately terminated by early bicellular stage. These abnormal changes mainly occurred at two stages: (1) at meiotic prophase, almost half of Peiai 64S microsporocytes exhibited aberration, with sparse free ribosomes, underdeveloped mitochondria and many swollen endoplasmic reticula. These abnormal cells became dramatically vacuolated and hereafter disintegrated completely at later stage. (2) After the early uninucleate stage, nearly all of Peiai 64S microspores possessed malfunctional exine that was deviod of electron transparent region between sexine and nexine, and no intine was established. But, the development and disintegration of tapetum in Peiai 64S resembled those of IR36. The results proposed that the abnormalities of microsporocytic cytoplasm or pollen exine, rather than the tapetal development caused the pollen abortion of Peiai 64S.

Key words: rice; photoperiod-temperature sensitive genic male sterile line (PTGMS); pollen; development; abortion; cytology

A comprehensive understanding of the mechanisms involved in male sterility is essential for the efficient use of various types of male sterile lines in the production of F_1 hybrids. Developmental and structural studies characterizing the differences between normal and sterile anthers help to elucidate the causes leading to male sterility. Photoperiod-temperature sensitive genic male sterile line (PTGMS) is a new rice germplasm with fertility alternating between the sterile and fertile under certain day length and temperature[1] and has been used in production of hybrid rice by two-line system[2]. Therefore, the studies on PTGMS rapidly became one of the most attractive fields in China. Regarding the mechanism of

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pollen abortion, current studies presented some prospectives and mainly focused on original material Nongken 58S. Wang *et al.* [3] suggested that the pollen abortion was ascribed to abnormal vascular bundle and delayed disintegration of tapetum. At transmission electron microscopy(TEM) level, Li *et al.* [4] reported that the main causes of pollen abortion were aggregation of ribosomes, disintegration of mitochondria, endoplasmic reticula and other organelles, and intactness of tapetum. However, Sun *et al.* [5] emphasized that the pollen abortion was related to anther walls, of which tapetum couldn't collapse in time and the middle layer delayed to disintegrate. Since different researchers drawn various conclusions on the same material, it is necessary to further conduct systematic studies on the cytological mechanism of pollen abortion in PTGMS. In this investigation we compared development of microsporogeneous tissue and associated tapetum in the fertile cultivar IR36 and a widely used PTGMS Peiai 64S.

1 Materials and Methods

The seeds of IR36, a normal indica cultivar, and Peiai 64S, a PTGMS which was obtained from China National Hybrid Rice Research and Development Center (Changsha, China), were cultivated under natural conditions with long-day and high temperature (May to August, 1997, in Guangzhou, 23°08′N).

Spikelets of 1, 2, 3, 4, 5, 6, 7, 8, 8.5 mm long were collected. They covered all pollen development stages. A hundred spikelets of each length were collected.

1.1 Methods of semi-thin section

Spikelets (cut off the lemma ends and the rachillae) were fixed in 3% glutaraldehyde and 1.5% paraformaldehyde in 0.025 mol/L phosphate buffer (pH 6.9) for 2 hours at room temperature. After rinsing in the same buffer, the samples were dehydrated in a graded ethanol series of 15%, 30%, 50%, 70%, 80%, 90%, and 95% for 30 min each. Spikelets were infiltrated with the mixture solution 1 : 1, 95% ethanol : 7022 histeresin (Leica instruments GmbH, Heidelberg, Germany) for 2 hours until they became slightly translucent and sank down, then settled in absolute 7022 histeresin at room temperature for 2 hours and stored at 4℃.

The samples were embedded in Leica histeresin, and cut to 2-4 μm thick sections with glass knives. The sections were stained with 0.05% toluidine blue O in 1% sodium tetraborate, and examined and photographed under a Leica DMRXA microscope.

1.2 Methods of thin sections

The spikelets were fixed in 3% glutaraldehyde and 1.5% paraformaldehyde in PHEM buffer (60 mmol/L Pipes, 25 mmol/L Hepes, 10 mmol/L EGTA, 2 mmol/L $MgCl_2$, pH 7.0) for 2 hours at room temperature, rinsed in 3 changes of the buffer over a period of 2 hours, and postfixed in 1% osmium tetroxiode in PHEM buffer overnight at 4℃ and rinsed 6 times in PHEM buffer. They were dehydrated through a graded ethanol series, infiltrated with propylene oxide and embedded in TAAB resin. Thin sections across the spikelets were gotten with a diamond knife, and stained with uranyl acetate and lead citrate. The sections were observed in a Phillips EM400 and photographed on Lekai film (100ASA).

2 Results

Based on observed semi-thin sections of 275 spikelets and thin sections of 58 spikelets in Peiai 64S, the main results were as follows:

2.1 Abnormality of microsporocyte cytoplasm during meiosis

The archesporial cells differentiated at the earliest stage of anther development then underwent several divisions to form microsporocyte. Simultaneously, the anther walls differentiated into 4 layers: tapetum, middle layer, endothecium and epidermis in succession, each of which was single cell thick. In these developmental processes, Peiai 64S was similar to IR36. However, obvious differences occurred between Peiai 64S and IR36 as soon as the microsporocytes entered meiosis stage. In early prophase of meiosis (up to and including pachytene), microsporocytes of IR36 possessed dense uniform cytoplasm (Fig. 1-A) with rich ribosomes, a few of undevelopmental mitochondria, endoplasmic reticula and small vacuoles, and a prominent nucleolus and some condensed chromatin (Fig. 1-B, C). But, part of (almost half of) microsporocytes of Peiai 64S exhibited abnormality (Fig. 1-a). Of abnormal cells some had numerous short swollen endoplasmic reticula and abnormal nuclei (Fig. 1-b). The others had many plastids and mitochondria with darker matrixes and obscure cristae (Fig. 1-c). With the further development, these abnormal cells became dramatically vacuolated, and hereafter disintegrated completely. Another part of Peiai 64S microsporocytes appeared normal at these developmental stages.

2.2 Abnormality of pollen exine development

After meiosis, microsporocytes developed to the young microspores. The IR36 microspores just released from the tetrad possessed abundant, uniform cytoplasm and a centrally positioned nucleus, and their exines were not well developed only with small electron dense droplets, which were mainly composed of sporopollenin, deposited on the surface of microspore plasmatic membranes (Fig. 2-A). In the middle microspore stage, significant sporopollenin deposition had occurred, the exine became obvious, with three electron dense lines and regularly spaced dark granules (Fig. 2-B). Up to

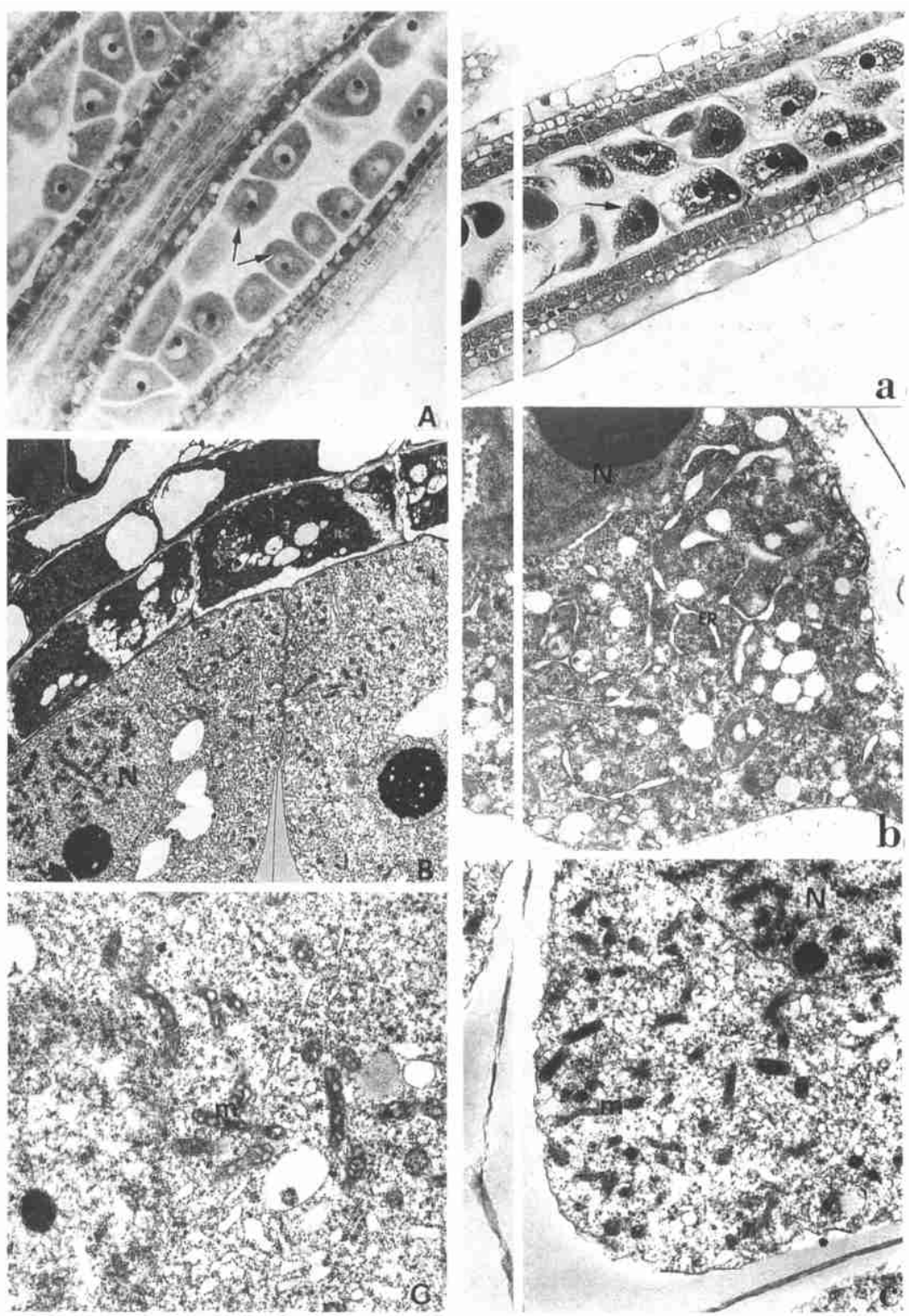


Fig. 1. A- Light micrographs of longitudinal section of IR36 anther at meiosis leptotene. The microsporocytes (arrow) possessed uniform cytoplasm. ×500; B- TEM of cross sections of IR36 anther at meiosis leptotene. The microsporocyte contains uniform cytoplasm with numerous ribosomes and few other organelles. ×4500; C- A part of IR36 microsporocyte with developing mithochrodria. ×9675.

a- Light micrographs of longitudinal section of Peiai 64S anther at meiosis leptotene. The cytoplasm of microsporocytes (arrow) does not evenly distribute. ×500; b- A microsporocyte of Peiai 64S at meiosis leptotene contained a abnormal nucleus and numerous swollen endoplasmic reticula. ×10000; c- A microsporocyte of Peiai 64S at meiosis leptotene has underdeveloped mitochondria with darker matrix. ×10000.

Ta- tapetum; Ms- microsporocyte; m- mitochondrium; p- plastid; ER- endoplasmic reticulum; N- nucleus.

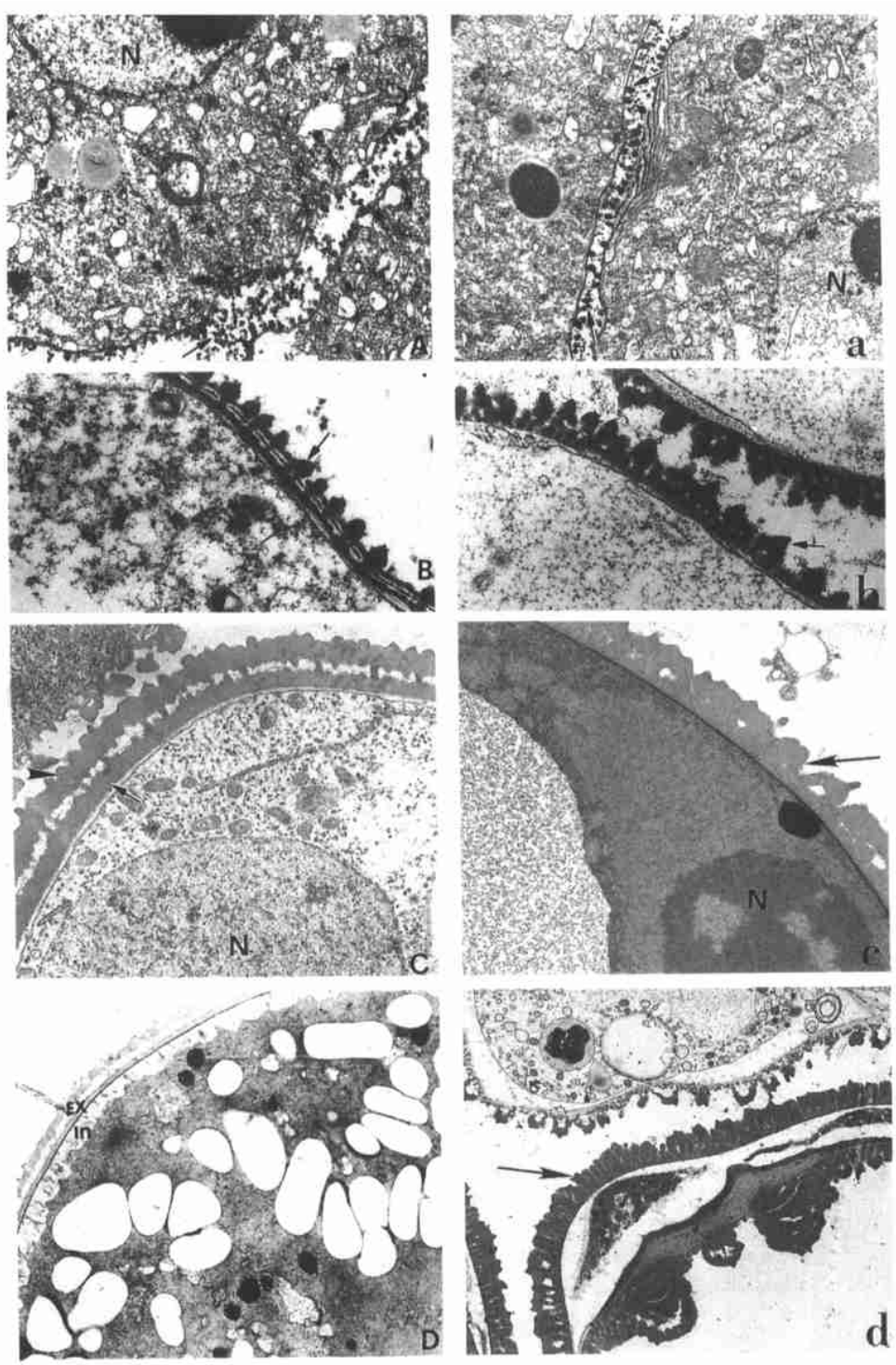


Fig. 2 Development of pollen exine in IR36 (A, B, C, D) and Peiai 64S (a, b, c, d).

A- an early microspore soon after released from the tetrad began to accumulate electron dense deposits (arrow) on their surface. ×8100; B- The microspore exine at middle uninucleate stage further developed to form three electron dense lines and regularly spaced dark granules (arrow). ×22356; C- The microspore at late microspore stage possessed a well- developed exine with a sexine (arrowhead), a nexine (arrow) and a middle electron transparent region between them. ×8100; D- The wall of mature pollen contained a sporopollenin exine (Ex) and cellulose intine (In). ×4739.

a- Peiai 64S microspores at early microspore stage deposited numerous election dense droplets (arrow) on their surface. ×8100; b- Osmiophilic material (arrow) irregularly piled on the microspore surface, and the microspores of middle stage form an obvious abnormal exine. × 47385; c- The microspore exine (arrow) at late microspore stage was lack of the demarcation between sexine and nexine. ×8100; d- An abortive pollen had only an irregular exine (arrow) and without intine. ×4739.

late microspore stage, the exine of microspores became much thicker with distinct sexine, nexine and middle electron transparent regions(Fig. 2-C). So far, the exine outline was basically established and secretion of the intine by microspore began. As a result, mature pollen wall contained a sporopollenin exine and a cellulose intine. The exine was made up of sexine, nexine and baculum (Fig. 2-D).

Similarly, Peiai 64S microspores at early microspore stage had electron dense droplets deposited on their surfaces (Fig. 2-a). But, up to the middle microspore stage, instead of forming regular electron dense lines as in IR36, the droplets of Peiai 64S irregularly piled on the microspore surfaces (Fig. 2-b). This resulted in no middle election transparant regions, and so the demarcation between sexine and nexine was not distinct (Fig. 2-c). These irregularities lasted till pollen complete abortion. No intine was established (Fig. 2-d).

2.3 Normality of tapetal development

In IR36, when tapetum just formed, the tapetal cells had a prominent nucleus and rich cytoplasm with developed mitochondria and plastids which mostly gathered around nuclei(Fig. 3-A). During meiosis, the tapetal cytoplasm agglomerated, and so blank regions of cytoplasm occurred at the periphery of tapetal cells(Fig. 3- B, arrow), which was usually called "tapetum disintegration" in previous reports[3,5]. Actually, these structural features of tapetum showed its being highly developed. There were some plastids containing electron dense inclusion and abundant mitochondria with dense matrix and some enlarged cristae in these tapetal cells. From dyad to tetrad stages, the prominent character of tapetal cells were presence of extensive layers of rough endoplasmic reticulum and some dilated endoplasmic reticulum vesicles (Fig. 3-C), indicating to be in active metabolic state.

At early microspore stage, the tapetal cells just slightly varied. Up to middle microspore stage, the tapetal wall (facing the locule) began to disintegrate and became loose and discontinuous. Pro-sporopollenin bodies occurred on the inner tangental wall of tapetum. By late microspore stage, tapetal cells obviously disintegrated into mountain-like shape, where cytoplasm remained, some recognizable organelles were lipid bodies, fewer mitochondria and plastids, and occasionally the outline of a nucleus. Sporopollenin bodies formed on the inner tangental wall of tapetum(Fig. 3-D).

As the microspore underwent a mitosis and became early bicellular pollen grains, tapetal cells dramatically disintegrated, contained no cytoplasm, except for some osmiophilic bodies(Fig. 3-E). When the pollen grains were mature, tapeta had completely disintegrated, leaving the secondary tapetal walls(Fig. 3-F, arrow) appressed to the inside surface of the endothecium.

In Peiai 64S, although few of tapetal cells presented abnormality, the majority of them were normal. At the time of the microsporocyte formation, the tapetal cells had abundant cytoplasm(Fig. 3-a) and maintained mitotic activity. During meiosis, the tapetal cytoplasm initially agglomerated (Fig. 3-b). At the late stage of meiosis(dyad to tetrad stage), the tapetal cytoplasms were more condensed, and lots of extensive endoplasmic reticula and some mitochondria occurred (Fig. 3-c). With microspore development, the tapetal cells began to rapidly disintegrate, their nuclei were not apparent and their cytoplasms present a amount of osmiphilic remains (Fig. 3-d,e). Finally, when the pollen grains were abortive, the tapetum was completely disintegrated(Fig. 3-f). So, the process of tapetal formation, development and "disintegration" in Peiai 64S was essentially consistent with that in IR36.

3 Discussions

In general, the cytological characteristics of pollen development of PTGMS should be compared with that of the original cultivar of PTGMS rice in the fertile period. But in this investigation, we used IR36 cultivar as control. The main reasons were as follows: 1)The sterility of most PTGMS including Peiai 64S was complete and stable in the sterile period, however, the fertility was rather variable and not complete in the fertile period. Therefore the cytological structure of pollen development under fertile condition could not represent complete normal developmental process. 2) Since the developments of female and male reproductive organs were essential for plant growth, and these developmental processes were highly conservative and similar among different cultivars[3-5]. Furthermore, so far, the reproductive process of IR36 had systematically studied and accumulated detail cytological and anatomical information concerning the pollen development in our laboratory. To avoid repeating, the IR36 was used as control in this study.

The tapetum is a layer of specialized cells, which surrounds the microsporgenous tissue in developing anthers and plays an important role in the nutrition of microspore[6]. Generally, the main cause of male sterility was believed to either persistence or premature breakdown of the tapetum[5,7,8]. Hence, we were especially interested in behavior of the tapetum. Like IR36, most of Peiai 64S tapetal cells normally developed and degraded, despite occasionally fewer hypertrophy or proliferation of tapetum. Thereafter, pollen abortion in Peiai 64S was likely not caused by tapetal development. The developments of pollen are an intricate process involving many tissues and biochemical metabolism,

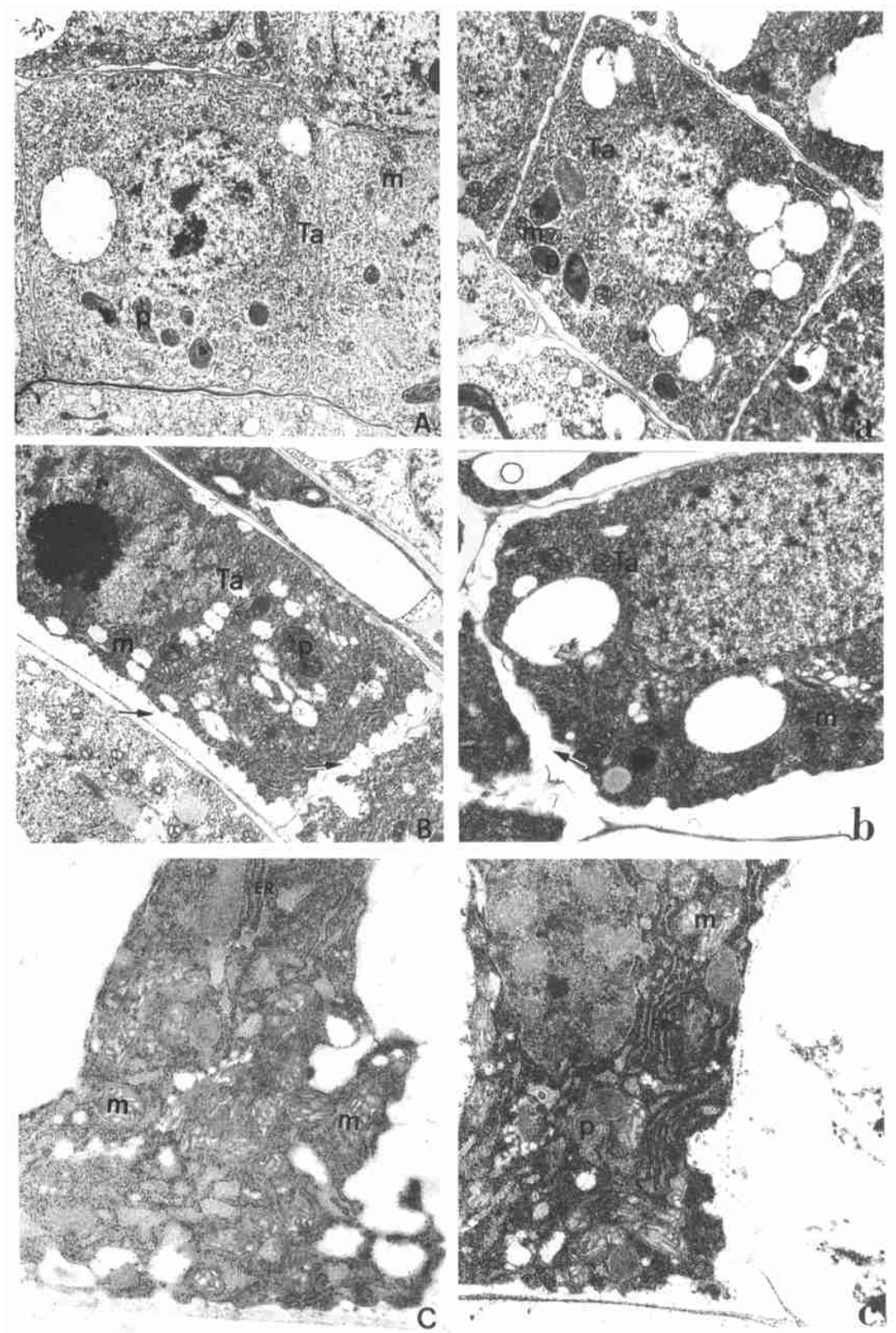
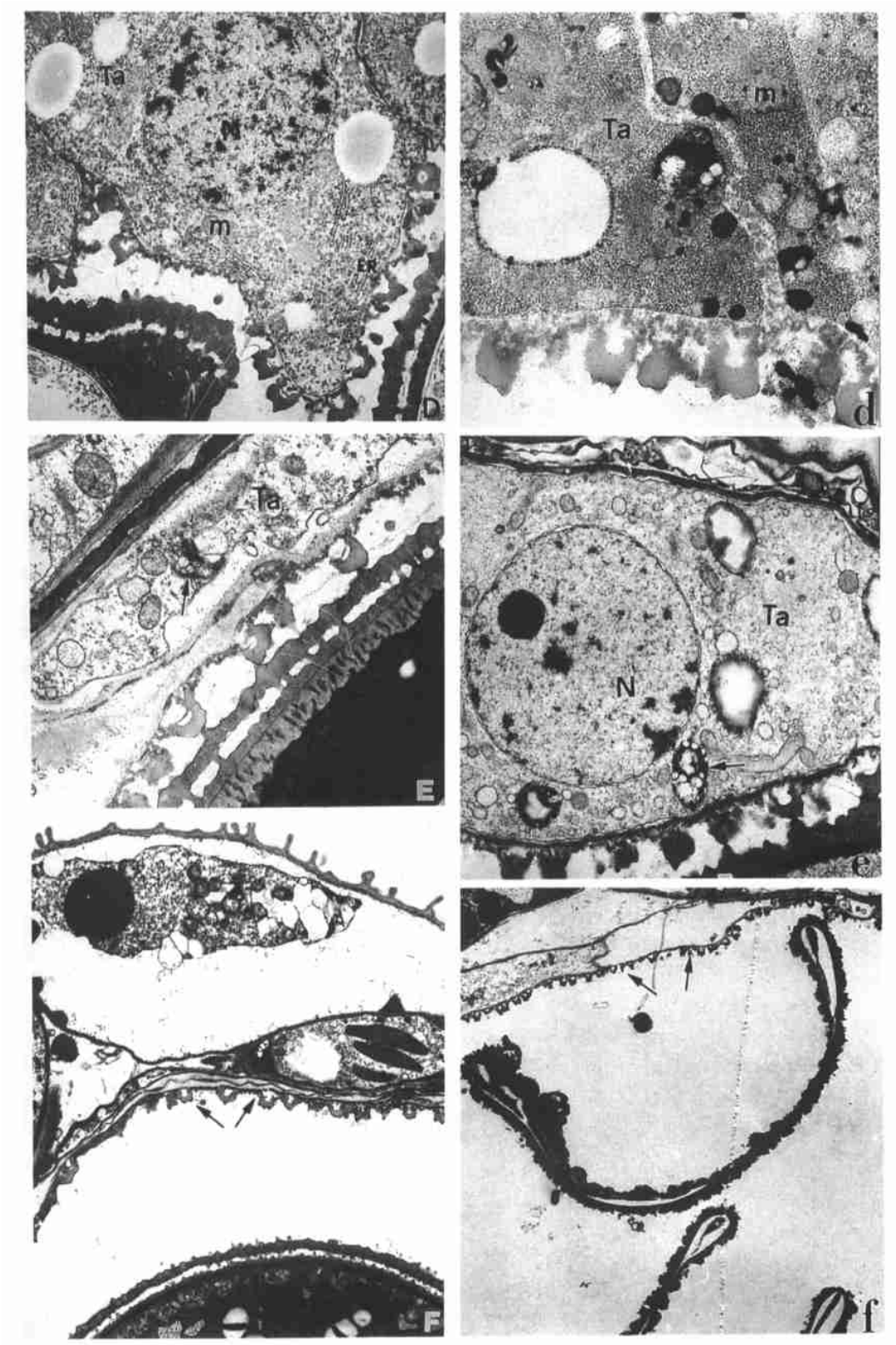


Fig. 3. The process of tapetal development and disintegration. A- F were of IR36 and a- f were of Peiai 64S.

A, a- The tapetal cell just formed at microsporocyte formation stage had dense cytoplasm with large mitochondria and plastids. A ×9675; a ×10000; B, b- The tapetal cytoplasm gradually agglomerated at meiosis leptotene and some interval (arrow) occurring at the periphery of tapetal protoplast. B ×10000; b ×10000; C, c- A tapetum at tetrad stage presented abundant endoplasmic reticulum, mitochondria and plastid. C ×21000; c ×15000.



D, d- The tapetum disintegrated into mountain-like shape by late uninucleate microspore stage, and retained some recognizable organelles, i. e. mitochondria and endoplasmic reticulum. D×9675; d×21000; E, e- The tapetal cell at bicellular stage dramatically disintegrated and contained no cytoplasm, except for some osmiophilic remains a (arrow). E×12525; e×12500; F, f- The tapetal cell completely disintegrated both in the IR36 mature pollen and the Peiai 64S abortive pollen, leaving the secondary tapetal wall (arrows) appressed to inside surface of the endothecium. F×5850; f×2700.

and not just up to tapetal development. Some previous reports have showed that tapetal development was normal in some sterile lines[9,10]. So the exact relationship between pollen abortion and tapetum is not clear. In many cases, It is likely that tapetal abnormality was one of pleiotropic consequence rather than the primary cause of pollen abortion[11].

Abnormalities take many forms and operate at different time in different male sterile lines[7]. The observation reported here demonstrated two main types of abnormalities in Peiai 64S. The first one was breakdown of numerous microsporocytes during meiosis. Similar abnormality had also been briefly described in other species[9,12]. Generally, it was believed that during meiosis, the microsporocytes were normally very active, and ready for the sporophyte-gametophyte transition[13], and hence they will be more sensitive to surrounding conditions than other tissues. Furthermore, because of the genetic defection in sterile line itself, they became much more sensitive to photoperiod and temperature. The second one was the abnormal development of pollen wall. Sun *et al.* [5] and Loukides *et al.* [8] also observed similar abnormal exine, respectively, but did not describe in details. It was considered that the pollen exine precursors were secreted from the tapetal cells, and the intine material from microspore itself[14,15]. In our investigation, the tapetum of sterile anthers possessed regular process of development and disintegration, and secreted numerous wall materials on the young microspore surface. However, because of abnormality of exine development, the microspore formed disorganized pollen wall. The abnormal pollen wall probably causes a block in the synthesis or the transport of cell material, and eventually results in pollen breakdown at the bicellular stage. So, attention should be paid to general abnormality of pollen exine at late microspore stage.

In conclusion, this comparative study dealt with the microstructural and ultrastructural changes of pollen development in IR36 and the male sterile line Peiai 64S, and in paticular of tapetal cells. These changes help us to decode some of the mechanisms on pollen abortion.

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