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水稻非花粉型细胞质雄性不育系及其保持系花药发育过程中 Ca^{2+} 的分布变化

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摘 要: 钙在高等植物中被称为第二信使,与植物的有性生殖有关。为了研究水稻(*Oryza sativa* L.)花药中钙的定位与花粉败育的关系,利用焦锑酸钾沉淀法研究了非花粉型细胞质雄性不育系 G37A 及其保持系 G37B 花药的发育过程及其细胞中 Ca^{2+} 的分布变化。研究发现,在2个材料间花药中钙的分布存在大量差异。G37B 的可育花药在花粉母细胞时期及二分体时期,很少看到有 Ca^{2+} 的沉积;而在单核花粉时期,Ca^{2+} 沉积急速地增加,主要定位在绒毡层细胞、花粉外壁外层及乌氏体的表面;随后花药壁上沉积的 Ca^{2+} 减少而花粉的外壁外层仍然有很多 Ca^{2+} 沉积物。相反,G37A 的不育花药在花粉母细胞时期和二分体时期有大量的 Ca^{2+} 沉积在小孢子母细胞和花药壁,中间层和绒毡层特别多。在二分体时期之后,不育花药的 Ca^{2+} 沉积减少,特别是绒毡层内切向质膜附近的 Ca^{2+} 几乎消失。但是同时期的可育花药中,有大量的 Ca^{2+} 沉积在绒毡层。不育花药的 Ca^{2+} 沉积在开花几天后消失。根据研究结果推测在不育花药发育早期中更多的钙离子与花粉败育有一定的关系。

关键词: 水稻; 细胞质雄性不育; 花粉; 焦锑酸钾; Ca^{2+}

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Calcium Distribution in Developing Anther Cells of No-pollen Type CMS and Maintainer Lines of Rice (*Oryza sativa* L.)

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Abstract: Ca^{2+} is a well-known "second messenger" in higher plants and is related to their sexual reproduction. To test the effects of calcium localization on pollen abortion in developing rice anthers, calcium distribution during anther development in no-pollen type cytoplasmic male sterile (CMS) rice G37A and its maintainer line G37B at different stages was examined by potassium antimonate precipitation method. Our study showed that many differences existed in Ca^{2+} distribution in developing anthers between the two studied rice lines. In the fertile anthers of G37B, few Ca^{2+} precipitates were detected at the pollen mother cell stage and the dyad stage. However, Ca^{2+} precipitates dramatically increased in the tapetal cells, on the exine of pollen grains and the surface of Ubisch Bodies at the uninucleate pollen stage. After this, Ca^{2+} precipitates decreased on the anther wall, although many Ca^{2+} precipitates still existed on the exine of pollen grains. In the sterile anthers of G37A, abundant Ca^{2+} precipitates accumulated in the microsporocyte, on the anther wall and especially in the middle layer, the tapetum and the ubisch bodies located at the pollen mother cell stage and the dyad stage. After the tetrad stage, Ca^{2+} precipitates in sterile anthers of G37A decreased generally, and could not be detected at the inner longitudinal plasma membrane of the tapetum. The results proposed that redundant calcium precipitates in sterile anthers may be related with pollen abortion.

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Key words: Rice; CMS; Pollen; Potassium antimonite; Ca^{2+}

Male sterility is the failure of plants to produce functional anthers, pollen, or male gametes during sexual reproduction, and is widespread among flowering plants. There are generally two major types of genetic male sterility in rice: nuclear genetic male sterility (GMS) and cytoplasmic male sterility (CMS). Cytoplasmic male sterility is the most effective genetic tool for developing F_1 rice hybrids [1-3]. The patterns of pollen abortion in cytoplasmic male sterile lines and their morphological features disclose different genetic backgrounds, conducive to hybrid rice production[4]. The patterns of pollen abortion in male sterile rice vary. The most important character is the stage at which pollen abortion occurs. Most of the male sterile material tested can be grouped into four types: pollen-free, uninucleate abortive, binucleate abortive, and trinucleate abortive[4].

Calcium (Ca^{2+}), a well-known "second messenger" in plant cells, plays an important role in plant growth and development[5]. It is tightly compartmentalized in specific states of availability and mediates cell physiological processes through multiple independent pathways within plants. Previous studies on calcium function in sexual reproductive systems have mainly focused on pollen germination and pollen tube elongation[6], as well as fertilization[7-10]. To date, however, far less has been done on pollen and anther development. In recent years, Ca^{2+} distribution and its roles in anther development have been an active topic of research, and results have been successively reported in many flowering plants, including tobacco[11], lettuce[12,13], cabbage[14], *Lycium Barbarurn* L.[15] and *Torenia fournieri*[16]. Tian *et al.*[17] observed the difference of calcium distribution in fertile and sterile anthers of a photoperiod-sensitive genetic male-sterile rice and speculated that anomalies in the distribution of calcium correlated with pollen abortion. Soon after, the correlation was confirmed in photoperiod-sensitive cytoplasmic male-sterile wheat[18] and in Honglian-Yuetai cytoplasmic male sterile rice[19]. Xia *et al.*[20,21] studied Ca^{2+} distribution in anther connective tissues and the wall of another photosensitive genetic male-sterile rice Nongken 58S, and presumed that the irregular Ca^{2+} distribution in the anther tissues was probably related with the abortion of rice pollens. Chen *et al.*[22] ascribed abnormalities in the distribution of calcium between tapetum and pollen mother cells in a cytoplasmic male sterile line of Yunnan purple rice. Qiu *et al.*[23] and Xia *et al.*[24] also found the abnormalities of calcium distribution in anther tissues of a thermo-sensitive genetic male-sterile rice Peiai 64S. They uniformly proposed that redundant calcium precipitates in the cytoplasm were one of the most important factors to result in pollen abortion.

Although much research data on calcium distribution in rice anthers has already been accumulated, it is still insufficient for understanding calcium function on pollen and anther development of rice. To better explore the mechanism of male sterility and the function of Ca^{2+} signal in pollen development of rice, Ca^{2+} distribution in developing anther cells of no-pollen type CMS and maintainer lines of rice were investigated using potassium antimonate technique in this study. Under the conditions employed here, we demonstrated that antimonate selectively precipitated cellular Ca^{2+} and many differences of Ca^{2+} distribution in developing anthers existed between two studied rice lines.

1 Materials and methods

1.1 Materials

The no-pollen type CMS rice G37A and its maintainer line G37B were grown in an experimental field of the South China Botanical Gar-

dens, the Chinese Academy of Sciences, under normal growth conditions.

1.2 Methods

Detection of Ca^{2+} precipitates using potassium antimonate was performed as described by Tian *et al.*[17] and Xia *et al.*[20] with slight modification. Anthers from G37A and G37B plants were collected at different developmental stages. At least fifteen anthers from different flowers located at the middle nodes of the inflorescence were fixed and at least six anthers from each treatment were examined. Anthers were fixed overnight at 4℃ with 2% glutaraldehyde in 0.1 mol/L potassium phosphate buffer (PBS, pH 7.8) containing 1% $K_2H_2Sb_2O_7 \cdot 4H_2O$. The fixed anthers were then washed five times with 1% $K_2H_2Sb_2O_7 \cdot 4H_2O$ in 0.1 mol/L PBS buffer (20 min at a time) and post-fixed in 1% OsO_4 for 16 hrs at 4℃ in 0.1 mol/L PBS buffer containing 1% $K_2H_2Sb_2O_7 \cdot 4H_2O$. Anthers were washed five times with 0.1 mol/L PBS buffer without antimonate, and then dehydrated in a graded ethanol series. After displacement by epoxy dimethylmethane the samples were embedded in Epon 812 resin. Semi-thin sections (2–4 μm thick) were cut with glass knives. The sections stained with 0.5% toluidine blue in 0.1 mol/L PBS were examined by light microscope to confirm the developmental stages of the microspores. In addition, 80–90 nm sections were obtained with a diamond knife by Leica-S ultramicrotome, and then serially stained with 2% uranyl acetate for 90 min and 6% lead citrate for 10 min. The stained sections were observed by a Japanese JEM1010 transmission electron microscope. Calcium precipitates in the grids were removed after incubation in a solution of 0.1 mol/L EGTA (pH 8.0) at 60℃ for 60 min. For each sample at least five anthers from each treatment were examined. Controls were treated as above, but $K_2H_2Sb_2O_7 \cdot 4H_2O$ was omitted from solutions during processing.

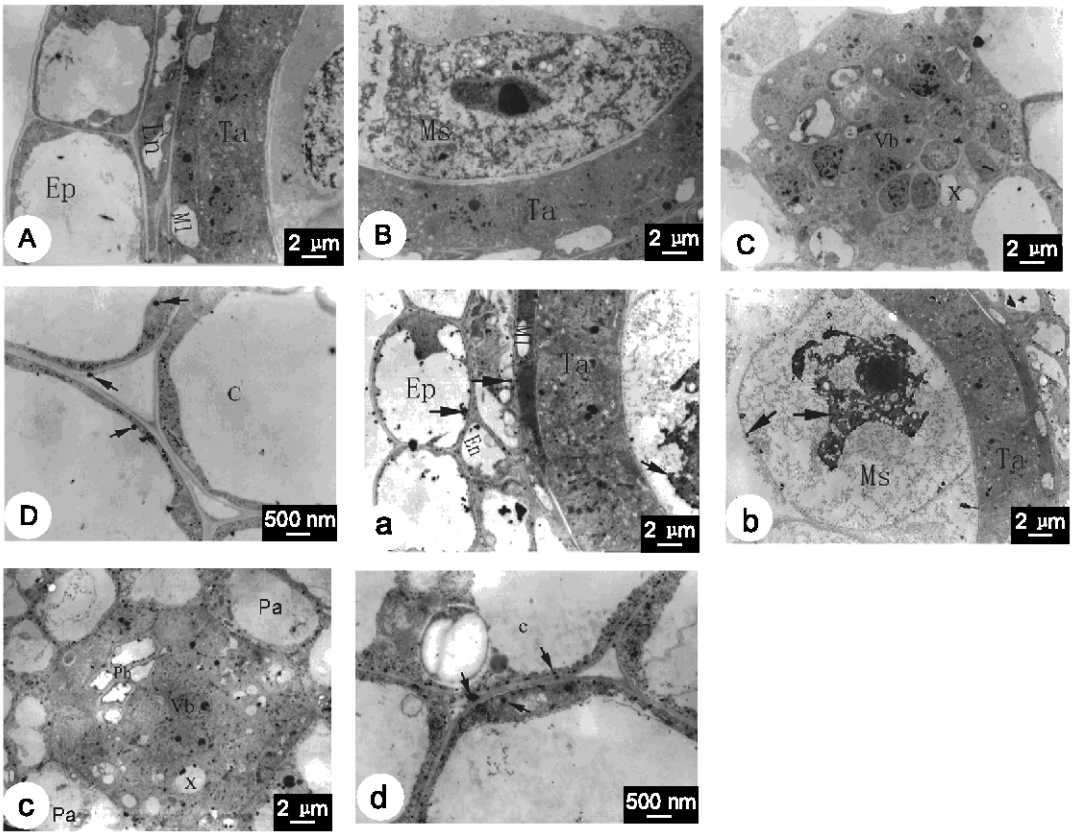
2 Results

2.1 Ca^{2+} distribution in fertile anthers during their development

At the pollen mother cell stage, few Ca^{2+} precipitates was observed in the microsporocyte and locule (Fig. 1: A, B). In the anther wall, Ca^{2+} precipitates in the epidermis and endothecium were less abundant than in tapetal cells (Fig. 1: A). Only a few Ca^{2+} precipitates appeared in vascular bundle and parenchymatous cells of connective tissue (Fig. 1: C, D).

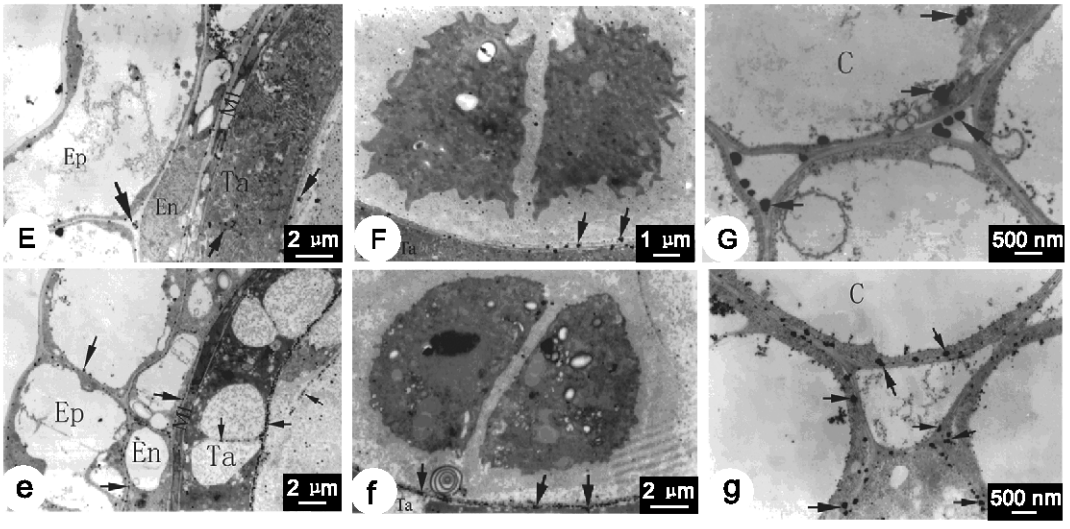
At the dyad stage, some Ca^{2+} precipitates were sparsely distributed in the locule and on the inner surface of the pollen sac (Fig. 2: F). Epidermis cells were highly vacuolated and no Ca^{2+} precipitate was found in the vacuole membrane. However some small Ca^{2+} precipitates were deposited on the plasma membrane. There were abundant Ca^{2+} precipitates in the interstitial space between the epidermis cell and the endothecium cell, but few in the middle layer and endothecium (Fig. 2: E). Many small Ca^{2+} precipitates accumulated on the plasma membrane and in the cytoplasm of the tapetal cells (Fig. 2: E). The Ca^{2+} precipitates in the vascular bundle greatly increased. Numerous Ca^{2+} were deposited on the plasma membrane, the vacuole membrane of parenchymatous cells, and the xylem cells wall. More Ca^{2+} precipitates on the vacuole membrane were detected in the connective tissue than at the pollen mother cell stage. Some large Ca^{2+} precipitates were found in the interstitial space between the parenchymatous cells of connective tissue (Fig. 2: G).

At the uninucleate pollen stage, Ca^{2+} precipitates in fertile anthers increased. A layer of Ca^{2+} precipitates were deposited on the exine of the uninucleate pollen (Fig. 3: H). The Ca^{2+} in the anther wall tended to transport from the outer to the inner layer (Fig. 3: H). The Ca^{2+} precipitates in the epidermis cells decreased and few Ca^{2+}



Abbreviations: Ep: Epidermis; En: Endothecium; Ml: The middle layer; Ta: Tapetum; Ms: The pollen mother cell; Vb: The vascular bundle; X: Xylem; Ph: Phloem; Pa: Parenchyma; C: The connective tissue; →: Indicates Ca^{2+} precipitates.
A – D: Ca^{2+} distribution in fertile anthers, few Ca^{2+} precipitate was observed; a-d: Ca^{2+} distribution in sterile anthers, there were a few Ca^{2+} precipitate on the plasma membrane of anther wall and pollen mother cell.

Fig. 1 Ca^{2+} distribution in anthers at the pollen mother cell stage



Abbreviations: Ep: Epidermis; En: Endothecium; Ml: The middle layer; Ta: Tapetum; C: The connective tissue; →: Indicates Ca^{2+} precipitates.
E – G: Ca^{2+} distribution in fertile anthers, with few Ca^{2+} precipitates in the anther wall and the vascular bundle. e – g: Ca^{2+} distribution in sterile anthers, the Ca^{2+} precipitate increased significantly and much more than in fertile anther wall.

Fig. 2 Ca^{2+} distribution in anthers at the dyad stage

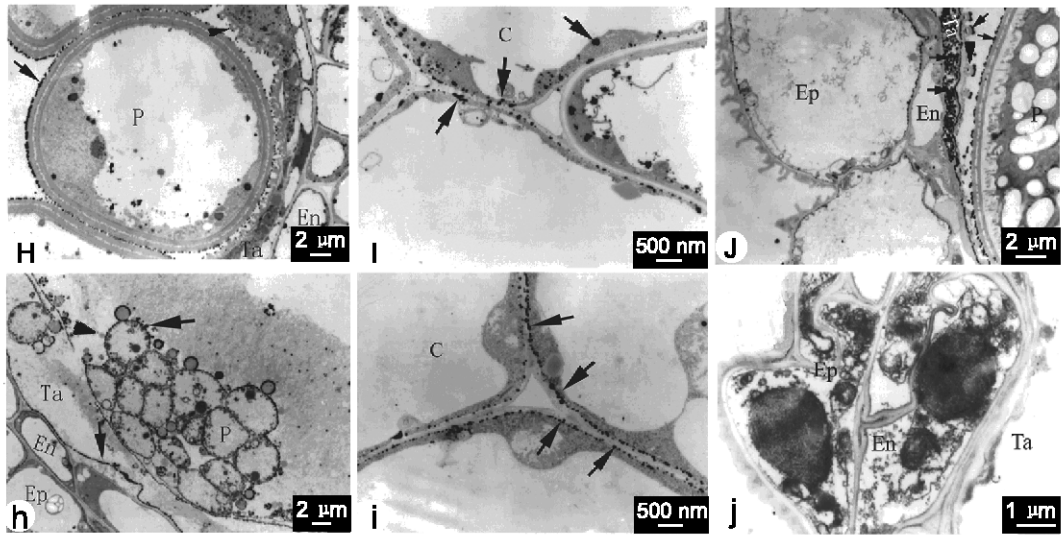
precipitates were deposited in the endothecium cells. But Ca^{2+} precipitates in the tapetum and especially on the Ubisch bodies facing the pollen sacs increased dramatically (Fig.3: H). The Ca^{2+} precipitates increased in the parenchymatous cells of the connective tissue (Fig.3: I).

Before the dehiscence of the anthers, Ca^{2+} precipitates in fertile anthers showed a minor decrease compared to those at the binucleate pollen stage. The mature pollen was full of starch. The Ca^{2+} precipitates were rarely observed in the cytoplasm and on the intine. However, some small Ca^{2+} precipitates presented on the vacuole membrane of the epidermis and a generous amount of Ca^{2+} was deposited in the debris of the tapetum (Fig. 3: J). Organelles in the parenchymatous cells degenerated and many small Ca^{2+} precipitates appeared in the cytoplasm and cell wall. Some cell walls, especially on the second cell wall of the xylem cells, had many Ca^{2+} precipitates. The Ca^{2+} precipitates deposited on plasma membrane and vacuole membrane of the connective tissue were more abundant than those at the binucleate pollen stage (Figure not shown).

2.2 Ca^{2+} distribution in sterile anthers during their development

At the pollen mother cell stage, the karyoplasms of the pollen mother cells were unevenly distributed and few Ca^{2+} precipitates were located in the cytoplasm (Fig. 1: b). Compared with fertile anthers at this stage, more abundant Ca^{2+} precipitates occurred in the anther wall, especially on the vacuole membrane and plasma membrane of the epidermis and endothecium (Fig. 1: a). In the connective tissue of sterile anthers, abundant calcium precipitates were located on the plasma membrane of vascular bundle sheath cells and phloem cells, and in the cytoplasm of other parenchyma cells (Fig. 1: c). There were many more Ca^{2+} precipitates in the connective tissue, mainly distributed on the plasma membrane, than those in fertile anthers (Fig. 1: d).

At the dyad stage, many Ca^{2+} precipitates were found around the callose of the dyad and in



Abbreviations: P: Pollen; En: Endothecium; Ep: Epidermis; Ta: Tapetum; C: The connective tissue; →: Indicates Ca^{2+} precipitates; ►: Indicates Ubisch bodies.
H–J: Ca^{2+} distribution in fertile anthers. h–j: Ca^{2+} distribution in sterile anthers, the pollen totally degraded and there were many Ca^{2+} precipitates in the degraded pollen and much less Ca^{2+} precipitates than in fertile anthers.

Fig. 3 Ca^{2+} distribution in anthers at the uninucleate and the tri-nucleate pollen stage, the Ca^{2+} precipitate increased obviously, and was mainly located in the anther wall, on the surface of Ubisch bodies, pollen wall and on the plasma membrane of collective tissues

the pollen sac (Fig. 2: f) than in the fertile anthers at the dyad stage. A layer of large Ca^{2+} precipitates was found on the plasma membrane of the epidermis, endothecium, the middle layer, and the tapetum. But in the fertile anthers, Ca^{2+} was mainly deposited on the vacuole membrane of the middle layer. Large vacuoles with many Ca^{2+} precipitates on its membrane appeared in some of the tapetal cells. Many Ca^{2+} precipitates were deposited on the inner longitudinal cell membrane of the tapetum where Ubisch bodies formed (Fig. 2: e). The Ca^{2+} precipitates in the vascular bundle also greatly increased. At this stage, many Ca^{2+} precipitates were found on the plasma membrane of the phloem cells, in the cytoplasm and on the xylem cell wall. The granules became larger and more abundant than those in the fertile anthers, which was similar to the phenomenon that occurred in the connective tissue (Fig. 2: g).

After the callose degenerated, the tetrads in the sterile anther degenerated rapidly. The uninucleate pollen stopped development, as evaluated by changes in their volume sizes. Pollen cell walls failed to develop normally and pollen grains accumulated (Fig. 3: h). A few Ca^{2+} precipitates were distributed dispersively in and around some of the debris, which came from the degradation of the plasma membrane of the pollen sac cells. Almost no Ca^{2+} precipitates were observed at the inner longitudinal cell wall of the tapetum, in which intact Ubisch bodies failed to form, while a few Ca^{2+} precipitates occurred around the degenerated Ubisch bodies (Fig. 3: h). Many more Ca^{2+} precipitates were deposited on the inner surface of the connective tissue parenchyma cells than in the fertile anthers.

Before the dehiscence of the anthers, similar to the phenomenon that occurred in the vascular tissue, anther wall cells in the sterile anthers were turbulent and all Ca^{2+} precipitates disappeared (Fig. 3: j). In fertile anthers, however, many Ca^{2+} precipitates were found in the debris of the tapetal cell cytoplasm and on the exine and the second wall of the xylem cells.

3 Discussion

3.1 Role of Ca^{2+} in fertile anthers

At the pollen mother cell stage, few Ca^{2+} precipitates in fertile anthers were observed. Only a few Ca^{2+} precipitates were located on the vacuole membrane of the epidermis and the connective tissue. The Ca^{2+} quantity in the fertile anthers at the dyad stage increased and was mainly distributed on the plasma membrane of the anther wall and the interstitial space between the epidermis and the endothecium. The majority of Ca^{2+} precipitates in anthers during the uninucleate pollen stage and the binucleate pollen stage were located on the exine, in the tapetal cells and on the Ubisch bodies. Furthermore, Ca^{2+} distribution in the anther wall tended to transport from the outer to the inner layer. The Ca^{2+} precipitates firstly appeared in the epidermis, then decreased at the binucleate pollen stage and disappeared at the mature pollen stage. However, many Ca^{2+} precipitates existed in the tapetal cells and the Ubisch bodies at that time. The Ca^{2+} concentration was at a very low level of 10^{-3} –10^{-7} mol/L in resting cells of plant[25]. When stimulated by environmental factors or during regulation of physiological processes, calcium in the "calcium bank" (vacuole, mitochondria, chloroplast, endoplasmic reticula) was temporally released[26], and it maintained Ca^{2+} equilibrium by Ca^{2+} ion channels and Ca^{2+}-ATPase[25]. Our results showed that temporal high concentration of Ca^{2+} in anthers might be related to the development of anthers. At the earlier development stage, anther development needs some nutrition such as polysaccharid[27], protein[28], RNA[29] and propollenin[30]; and at the later development stage, anther needs protein and plastids from tapetal cells for nutrition. Ubisch bodies play important roles in the trans-

portation of nutrients during these processes[31]. The many Ca^{2+} precipitates found in the tapetal cells and on the Ubisch bodies at the uninucleate pollen stage and the binucleate pollen stage suggested Ca^{2+} might act as a second messenger participating in the development of tapetal cells and the transportation of nutrients from the anther wall to the pollen sac.

3.2 The relationship between Ca^{2+} and male sterility of rice

The pollen abortion of Nongken 58S was mainly found at the late uninucleate pollen stage. The pollen abortion of male-sterile rice Honglian-Yuetai mainly occurred at the binucleate pollen stage. Peiai 64S was a kind of thermo-sensitive sterile line, in which pollen became sterile after the dyad stage when treated by high temperature during the pollen mother cell stage. The different distribution of Ca^{2+} between the male-sterile and fertile materials of Nongken 58S and Honglian-Yuetai was found at the uninucleate and binucleate pollen stages[17,19]. However, in Peiai 64S and Zhenshan 97A, B, there existed minor Ca^{2+} differences during the pollen mother cell stage[24,32]. These studies suggested that the distribution and quantity of Ca^{2+} was closely related to pollen abortion in male sterile rice by the activation of many biological processes at the early development stage with increased concentration of Ca^{2+}. The G37A was a kind of no-pollen type CMS rice, in which pollens degenerated promptly after tetrad and could not form pollen walls. Our study suggested that at the early stage of anther development, many Ca^{2+} precipitates were deposited in the wall of sterile anthers, the microsporocyte and the vascular tissue, but no Ca^{2+} precipitate was found in the wall of fertile anther and the microsporocyte. The time difference of Ca^{2+} distribution observed between our male-sterile and fertile materials was earlier than previous reports for other materials. Our data indicated an earlier pollen abortion in no-pollen type CMS line of rice.

Tapetum located at the inside of the anther wall showed the most affinity to the development of pollen. Rice tapetum was a kind of secretion organ and the pollen sac was its reservoir[33]. The nutrients and ions necessary for pollen development must be transported through the vascular tissue and the connective tissue, and from the anther wall to the pollen sac through the tapetum[34]. Our study suggested that the tapetum of the sterile anther, which degenerated rapidly after the dyad stage, affected the secretion and transportation of nutrients. It seems that many Ca^{2+} precipitates located on the plasma membrane of the middle layer and the tapetum were closely related to the degeneration of the tapetum at the tetrad. Many Ca^{2+} precipitates also congregated on the inner longitudinal wall where Ubisch bodies formed, which may be correlated with the abortion of the pollens.

References:

[1] Lin S C, Yuan L. Hybrid rice breeding in China [C]// Argosino G, Durvasula V S, Smith W H eds. Innovative approaches to rice breeding. Manila, Philippines: International Rice Research Institute, 1980: 35-51.

[2] Virmani S S, Chaudhary R C, Khush G S. Current outlook on hybrid rice[J]. *Oryza*, 1981, 18: 67-84.

[3] Virmani S S, Edwards I B. Current status and future prospects for breeding hybrid rice and wheat[J]. *Adv Agr*, 1983, 36: 145-214.

[4] Rao Y S. Cytohistology of cytoplasmic male sterile lines in hybrid rice[C]// Smith W H, Bostian Lloyd R, Cervantes Emy eds. Hybrid rice. Manila, Philippines: International Rice Research Institute, 1986: 115-128.

[5] Hepler P K. Calcium: A central regulator of plant growth and development[J]. *Plant Cell*, 2005, 17 (8): 2142-2155.

[6] Franklin-Tong V E. Signaling and the modulation of pollen tube growth[J]. *Plant Cell*, 1999, 11 (4): 727-738.

[7] Digonnet C, Aldon D, Leduc N, Dumas, C, Rou-

gier, M. First evidence of a calcium transient in flowering plants at fertilization[J]. *Development*, 1997,124(15): 2867-2874.

[8] Yu F L (余凡立), Liang S P (梁世平), Yang H Y (杨弘远), Wang Y (汪艳). Ultracytochemical localization of calcium in micropyle and embryo sac of Brassica napus before and after pollination[J]. *Acta Bot Sin* (植物学报), 1998,40: 591-597.

[9] Antoine A F, Faure J E, Cordeiro S, Dumas C, Rougier M, Feijo J A. A calcium influx is triggered and propagates in the zygote as a wavefront during in vitro fertilization of flowering plants[J]. *PNAS*, 2000,97(19): 10643-10648.

[10] Yu F L (余凡立), Zhao J (赵洁), Liang S P (梁世平), Yang H Y (杨弘远). Ultracytochemical localization of calcium in the gynoecium and embryo sac of rice[J]. *Acta Bot Sin* (植物学报), 1999,41: 125-129.

[11] Zheng M Z (郑茂钟), Yang Y H (杨延红), Guo J (郭娟), Qiu Y L (邱义兰), Xie C T (谢潮添), Tian H Q (田惠桥). The primary observation of calcium distribution during the anther development of tobacco[J]. *J Xiamen Univ* (厦门大学学报), 2004, 126-132.

[12] Qiu Y L (邱义兰), Liu R S (刘如石), Xie C T (谢潮添), Yang Y H (杨延红), Xu Q (徐青), Tian H Q (田惠桥). The character of calcium distribution in developing anther of lettuce (*Lactuca sativa* L.)[J]. *Acta Biol Exp Sin* (实验生物学报), 2005,38 (5): 377-386.

[13] Qiu Y L, Liu R S, Wei D M, Tian H Q. Calcium distribution in developing anthers of lettuce (*Lactuca sativa*)[J]. *Ann Bot Fennici*, 2009,46: 101-106.

[14] Xie C T, Yang Y H, Qiu Y L, Zhu X Y, Tian H Q. Cytochemical investigation of genic male-sterility in Chinese cabbage[J]. *Sex Plant Repr*, 2005, 18 (2): 75-80.

[15] Yang S J (杨淑娟), Zhang Y (张亚), Ye L (叶律), Song Y X (宋玉霞), Tian H Q (田惠桥). Calcium distribution in the developing anther of *Lycium barbarum* L[J]. *J Mol Cell Biol* (分子细胞生物学报), 2006,39(6): 516-526.

[16] Chen S H, Liao J P, Luo M Z, Kirchoff B K. Calcium distribution and function during anther development of *Torenia fournieri* (Linderniaceae)[J]. *Ann Bot Fennici*, 2008,45: 195-203.

[17] Tian H Q, Kuang A, Musgrave M E, Russell S D. Calcium distribution in fertile and sterile anthers of a photoperiod-sensitive genetic male-sterile rice[J]. *Planta*, 1998,204(2): 183-192.

[18] Meng X H (孟祥红), Wang J B (王建波), Li R Q (利容千). Effect of photoperiod on calcium distribution in photoperiod-sensitive cytoplasmic male-sterile wheat during anther development[J]. *Acta Bot Sin* (植物学报), 2000,42(1): 15-22.

[19] Li R Q (利容千), Zhu Y G (朱英国), Meng X H (孟祥红), Wang J B (王建波). The distribution of calcium in the pollen and connective tissue of Honglian-Yuetai cytoplasmic male sterile rice[J]. *Acta Agr Sin* (作物学报), 2001,27(2): 230-235.

[20] Xia K F (夏快飞), Liang C Y (梁承邺), Ye X L (叶秀舜), Xu X L (徐信兰). Ca^{2+} Distributions in the developing connectives of different sterile lines of rice[J]. *Acta Bot Boreali-Occident Sin* (西北植物学报), 2005,25(8): 1558-1565.

[21] Xia K F (夏快飞), Duan Z D (段中岗), Liang C Y (梁承邺), Ye X L ((叶秀舜). Ultrastructure and Ca^{2+} distribution in anther wall during development of photosensitive genetic male-sterile line of rice Nongken 58S[J]. *Subtr Plant Sci* (亚热带植物科学), 2005,34(3): 1-4.

[22] Chen X J (陈小军), Liu S N (刘树楠), Wei L (魏磊), Hu Y J (胡耀军), Yu J H (余金洪), Zhao J (赵洁), Ding Y (丁毅). The calcium distribution in the anther of cytoplasmic male sterile line of yunnan purple rice during anther development[J]. *J Wuhan Bot Res* (武汉植物学研究), 2005, 23: 101-106.

[23] Qiu Y L (邱义兰), Li H (李红), Chen S (陈松), Liu R S (刘如石), Chen L B (陈良碧). Calcium distribution in fertile and sterile anthers of a thermo-sensitive genetic male-sterile rice[J]. *Life Sci Res* (生命科学研究), 2007,11(2): 167-171.

[24] Xia K F (夏快飞), Liang C Y (梁承邺), Ye X L (叶秀舜), Zhang M Y (张明永). Calcium precipitate in the anthers of thermo-sensitive genetic male-sterile rice Peiai 64S[J]. *J Trop Subtrop Bot* (热带亚热带植物学报), 2009,17(3): 211-217.

[25] Bush D S. Calcium regulation in plant cells and its role in signaling[J]. *Ann Rev Plant Biol*, 1995, 46 (1): 95-122.

[26] Rudd J J, Franklin-Tong V E. Calcium signal in

plants[J]. *Cell Mol Life Sci*,1999,55：214-232.

[27] Hess M W, Hesse M. Ultrastructural observations on anther tapetum development of freeze-fixed *Ledebouria socialis* Roth (Hyacinthaceae)[J]. *Planta*,1994,192(3)：421-430.

[28] Hihara Y, Hara C, Uchimiya H. Isolation and characterization of two cDNA clones for mRNAs that are abundantly expressed in immature anthers of rice (*Oryza sativa* L.)[J]. *Plant Mol Biol*,1996,30(6)：1181-1193.

[29] Block M, Debrouwer D. Engineered fertility control in transgenic Brassica napus L.：Histochemical analysis of anther development[J]. *Planta*, 1993, 189(2)：218-225.

[30] Jungfermann C, Ahlers F, Grote M, Gubatz S, Steuernagel S, Thom I, Wetzels G, Wiermann R. Solution of sporopollenin and reaggregation of a sporopollenin-like material：a new approach in the sporopollenin research[J]. *J Plant Physiol*,1997, 151(5)：513-519.

[31] Hu S Y (胡适宜). Embryology of Angiosperms (被子植物胚胎学)[M]. Beijing：Higher Education Press,1983：22-30.

[32] Xia K F (夏快飞), Wang Y Q (王亚琴), Ye X L (叶秀舞). Ca^{2+} distribution in the Tapetum in a genetic-cytoplasmic male sterile line of rice, Zhenshan 97A and its maintainer line Zhenshan 97B [J]. *Acta Bot Yunnan*, 2005,27(4)：413-418.

[33] Pacini E. Tapetum and microspore functions[M]// Blackmore S, Knox R B eds. Microspores：Evolution and Ontogeny. London：Academic Press, 1990：213-237.

[34] Murgia M, Charzynska M, Rougier M, Cresti M. Secretory tapetum of Brassica oleracea L.：polarity and ultrastructural features[J]. *Sex Plant Reprod*, 1991,4(1)：28-35.

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