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Identification of the new allele *ptc1-2* and analysis of the regulatory role of *PTC1* gene in rice anther development

Qing Ye[1,2†], WenXiang Jiang[1,2†], XiaoQing Wang[1,2], XiaFei Hu[1,2], ZeLing Zhang[1,2], Zhen Wu[1,2], Huang Wang[1,2], SiNing Li[1,2], Dandan Guo[1,2], HaoHua He[1,2*] and Li Fang Hu[1,2*]

Abstract

Anther development involves a series of important biological events that are precisely regulated by many genes. Although several important genes involved in rice anther development have been identified, the regulatory network involved in tapetal development and pollen wall formation is still largely unclear. *PERSISTENT TAPETAL CELL 1* (*PTC1*) encodes a PHD-Finger protein, which plays a critical role in the regulation of tapetal cell death and pollen development in rice. Here, we report the isolation and characterization of a new allele *ptc1-2* with 2-base deletion in the third exon, causing the absent of the PHD domain due to the sequence change. Cytological analysis revealed delayed tapetal PCD, defective pollen exine formation and abnormal ubisch bodies development. Transcriptome analysis revealed that genes related to pollen wall formation (secondary metabolism, phenylalanine synthesis, and cutin and wax biosynthesis pathways), cell death (cysteine and methionine metabolism and DNA repair pathways), and carbohydrate synthesis (starch and sucrose metabolism pathways) were significantly altered in *ptc1-2* mutant. A total of 13 reported anther development genes exhibited significant expression changes in the *ptc1-2* mutant. Yeast two-hybrid and BiFC analyses showed that PTC1 could interact with API5, an inhibitor of apoptosis, and the citrin-binding enzyme EDT1. This work is helpful in deepening the understanding of the regulatory network of male reproductive development in rice.

Keywords Anther, Tapetum, *ptc1-2*, API5, EDT1

Introduction

Rice is an important food crop, and its yield is closely related to food security. The discovery of rice male sterile lines has led to the successful use of heterosis in rice, greatly improving the yield per unit area of rice and providing a strong guarantee for solving the problem of food security. As a male reproductive organ, anther development is closely related to the fertility of pollen. Abnormal anther development usually leads to male sterility. It is of great theoretical and practical significance to study the genes related to anther development in rice [1].

The anther of rice starts from the top of the stamen primordium in the flower bud. At first, the archesporial cells

†Qing Ye and WenXiang Jiang contributed equally to this work.

*Correspondence:
HaoHua He
hhhua64@163.com
Li Fang Hu
lfhu_hn337@163.com
[1]College of Agriculture, Jiangxi Agricultural University, Nanchang 330045, China
[2]Key Laboratory of Crop Physiology, Ecology, Genetics and Breeding, Ministry of Education, Jiangxi Agricultural University, Nanchang 330045, China

differentiate from the four corners under the anther protoderm, and then the archesporial cells differentiate into four layers of wall cells and pollen mother cells through a series of development processes. Subsequently, the pollen mother cells undergo one meiosis to form microspores, and the microspores undergo two mitoses to develop into mature pollen grains with three nuclei [2, 3]. The tapetum, as the innermost layer of the anther wall, is the transportation hub for pollen mother cells. It secretes callose-degrading enzymes to release microspores, provides nutrients for microspore development, and synthesizes sporopollenin for the pollen wall and shell, all of which are essential for the normal development of pollen mother cells and microspores at later stages [4, 5].

Several key regulators regulating the development of the rice tapetum have been identified, including the bHLH transcription factors UDT1 [6, 7], Tip2/bHLH142 [8], TDR [9] and EAT1 [10]; the MYB transcription factors GAMYB [8, 11] and OsMYB103/OsMS188/MYB80 [12–15]; the AGO transcription factor OsAGO2 [16]; and the glucose-4-epimerase BP1 [17], mRNA m^6A demethylase OsALKBH9 [18] and nuclear membrane protein ADT1 [19], UDT1 plays a role in the differentiation of secondary wall cells into mature tapetal cells and the meiosis of microspore mother cells, and Tip2/bHLH142 regulates the division and differentiation of the three layers of anther wall cells in the early stage of anther development and the degradation of tapetum in the later stage (PCD). TDR can control the degradation of tapetum layer by directly regulating the expression levels of *OsCP1* (encoding cysteine protease) and *OsC6* (encoding protease inhibitor) [18, 20]. EAT1 controls tapetal degradation by regulating the expression of two aspartic protease genes, *AP25* and *AP37* [21]. Further studies revealed a complex regulatory network among these genes. Tip2 is located upstream of TDR and EAT1, and its expression promotes the early stage of tapetal differentiation. At the late stage of tapetal cell differentiation, Tip2 activates the expression of the downstream transcription factor TDR and forms a complex with TDR to promote the development of tapetal cells. At the tetrad stage, Tip2 regulates the expression of EAT1, and the EAT1 protein forms a new complex with the TDR protein to regulate the PCD process of the tapetum [22, 23]. OsMYB103/OsMYB80/OsMS188 functions downstream of TDR and upstream of EAT1 [12].

Pollen exine, as the outermost structure of pollen, are mainly composed of sporopollenin. As a protective structure of male gametophytes, pollen exine play an essential role in resisting adversity and facilitating information exchange between male and female gametophytes. Many genes are involved in the biosynthesis and transport of sporopollenin precursors in tapetal cells throughout pollen wall development [23]. At present, the genes known to be involved in anther wall development include *CYP703A3* and *CYP704B2*, which encode cytochrome P450 family proteins [24]. *OsABCG3*, *OsABCG15* and *OsABCG26* encode ABC transporters [25–27]. *OsLTP47* [28] and *OsC6* [29] encode lipid transporters. And *OsACOS12* [30], *OsPKS1* [31], *OsPKS2* [32] and *OsTKPR1* [33] encode phenylalanine metabolism-related proteins, *WDA1* [34], *DPW* [35], *OsGPAT3* [36], *OsNP1* [37], *DPW3* [38], *OsEMF2b* [39] and *OsLecRK-S.7* [40], *OsFLA1* [41], *STS1* [42] encode related enzymes, also *PWA1* encodes the coiled-coil domain-containing protein [43]. These genes mainly regulate the synthesis of sporopollenin precursors and intermediate substances (fatty acids, fatty alcohols, phenols, etc.) and the transport of sporopollenin precursors from tapetal cells to pollen walls. The synthesis and assembly of pollen wall in related mutants will be abnormal to varying degrees, ultimately leading to pollen sterility. Although several important genes involved in rice anther development have been identified, it is a complex and precise process that is regulated by many genes, and the complex molecular regulatory network involved needs further study.

PERSISTANT TAPETAL CELL1 (*PTC1*) (also named *OsMS1*) encodes a plant homeodomain (PHD)-finger transcription factor, which is the homologous gene of Arabidopsis *MS1* [44]. PTC1 functions downstream of GAMYB and OsMYB103 [12, 45], in parallel with TDR [9] in regulating anther development. PTC1 also can interacted with known tapetum genes like *Tip2* and *MADS15*, participating in tapetum PCD and pollen exine formation [46]. Recently, the research showed that *OsMS1* natural allele $OsMS1^{wenmin1}$ confers thermosensitive genic male sterility in rice, and OsMS1 interacted with TDR in nuclei to activate its downstream gene expression in a temperature-dependent manner [47].

Here, we isolated a new allele *ptc1-2* of the rice *PTC1* gene. During the *ptc1*-2 anther development, the tapetum cells remained in a delayed degradation state. The pollen wall could not form the typical three-layer structure (Nexine-Bacula-Sexine) due to the abnormal bacula, and the ubisch bodies morphology was abnormal. Transcriptome analysis revealed that the expression of genes involved in anther wall synthesis, cell death and carbohydrate metabolism changed significantly in *ptc1*-2 mutant anthers. Further analysis revealed that PTC1 could interact with the EDT1 and API5 proteins, which can regulate rice anther development. This discovery provides a new avenue for further understanding the molecular mechanism of male sterility in rice.

Materials and methods

Materials and growth conditions

Through the use of ^{60}Co-γ radiation, the rice variety 9522 (*Oryza sativa* L. ssp. *Japonica*) was mutagenized, a stable

hereditary male-sterile mutant *ptc1-2* was identified in its progeny. All rice plants were cultivated and grown in rice paddy of Jiangxi Agricultural University at Nanchang, Jiangxi, and Sanya, Hainan, China.

Characterization of the mutant phenotype

Rice samples were photographed using a smartphone and a stereomicroscope. Following the methods described by Li et al. (2006), mature florets and seeds of 9522 (*Oryza sativa* L. ssp. *Japonica*) and the *ptc1-2* mutant were randomly selected for semithin sectioning and transmission electron microscopy (TEM) observation of anther development. The floral organ structure and iodine staining effect were observed under a stereomicroscope and photographed.

Map-based cloning

The *ptc1-2* mutant (*Oryza sativa* L. ssp. *Japonica*) was crossed with Nipponbare to obtain the F_1 generation, and the F_2 generation was obtained by self-pollination. In the F_2 population, 121 male sterile plants were selected for genetic analysis. Subsequently, candidate genes were selected based on the resequencing results, and primers were designed to amplify the mutant templates. The amplified products were then sequenced and compared with the resequencing results to identify the mutated genes.

Phylogenetic tree analysis

Multi-species PHD domain protein sequences wereobtained from NCBl. ClustalW in MEGA7 (megasoftware.net)) software was used to compare the PHD domain homologous protein sequences of rice, MEGA7 software was used to analyze the phylogenetic tree and derive the constructed evolutionary tree. The protein sequence homology was compared by MEGA7 biology software. The domain of ADTl was analyzed by SMART (embl-heidelberg.de) online software.

Transcriptomic analysis

The cDNA libraries of the *ptc1-2* mutant and 9522 (*Oryza sativa* L. ssp. *Japonica*) anther were constructed and subjected to high-throughput sequencing by BGI Genomics. DESeq2 software was used to analyse gene expression levels, and significantly differentially expressed genes with an FDR < 0.05 and a log2(|fc|) > 1 were identified. Differentially expressed genes were mapped to GO terms using the Gene Ontology database (http://www.geneontology.org/), and enrichment analysis was performed using the hypergeometric test to identify significantly enriched GO terms compared to the background.

Yeast two-hybrid assay for PTC1

The full-length cDNA sequence of *PTC1*, as well as the PHD and ΔC + PHD sequences, were cloned and inserted into the pGBKT7 vector. After self-activation verification, PHD was confirmed as bait and cotransformed with an pGADT7 plasmid library into yeast cells. Growth was observed on dropout medium plates incubated at 30 °C for 4 days. The AD vectors containing the *API5* and *EDT1* gene CDSs and the pGBKT7 vector containing the *PTC1* gene CDS were separately cotransformed into yeast cells. After incubation at 30 °C for 3 days, single colonies were picked and grown in dropout liquid media and then spotted onto dropout plates for further growth at 30 °C for 2–3 days. Then, 5 μl of bacterial solution was absorbed and tested on media with two or four deficiencies [48].

Bimolecular fluorescence complementation (BiFC) experiment

The full-length CDS of *PTC1* was cloned and inserted into the pSPYCE vector, while API5 and EDT1 were cloned and inserted into the pSPYNE vector. Various constructs were transiently transformed into *Nicotiana benthamiana* using *Agrobacterium tumefaciens* strain EAH105-mediated infiltration [49]. Yellow fluorescence was observed using laser scanning confocal microscopy (ZEISS LSM700) after 72 h of Agrobacterium infection of *N. benthamiana*.

Results

Isolation and genetic analysis of the *ptc1-2* mutant

To explore more genes related to the regulation of rice anther development, we generated a rice mutant library using the japonica subspecies 9522 background (*Oryza sativa* L. ssp. *Japonica*) by treatment with ^{60}Co γ-ray, and found one mutant by its complete male sterility. The male sterility mutant exhibited normal vegetative development and inforescence morphology, except that the plants were slightly shorter (Fig. 1A, B), but the anthers were smaller and yellowish during reproductive growth (Fig. 1C, D). By 1% I_2–KI staining, it was found that the pollen grains of the wild-type anthers were obviously stained dark black, while no pollen grains of the mutant anthers were detected (Fig. 1E, F), and the plants could not bear seeds normally at maturity, indicating complete male sterility (Fig. 1G). When the mutant was crossed with the wild-type, the F_1 generation could produce normal seeds, while in the F_2 generation population, the separation ratio of fertility traits between normal and mutant plants was approximately 3:1 (fertile 369: sterile 121, $X^2 = 1.1774$), indicating that the mutant was controlled by a single recessive nuclear gene.

To map the target gene, the mutant was crossed with Nip to obtain the F_1 population, and the F_2 population was obtained after the F_1 population self-breeding.

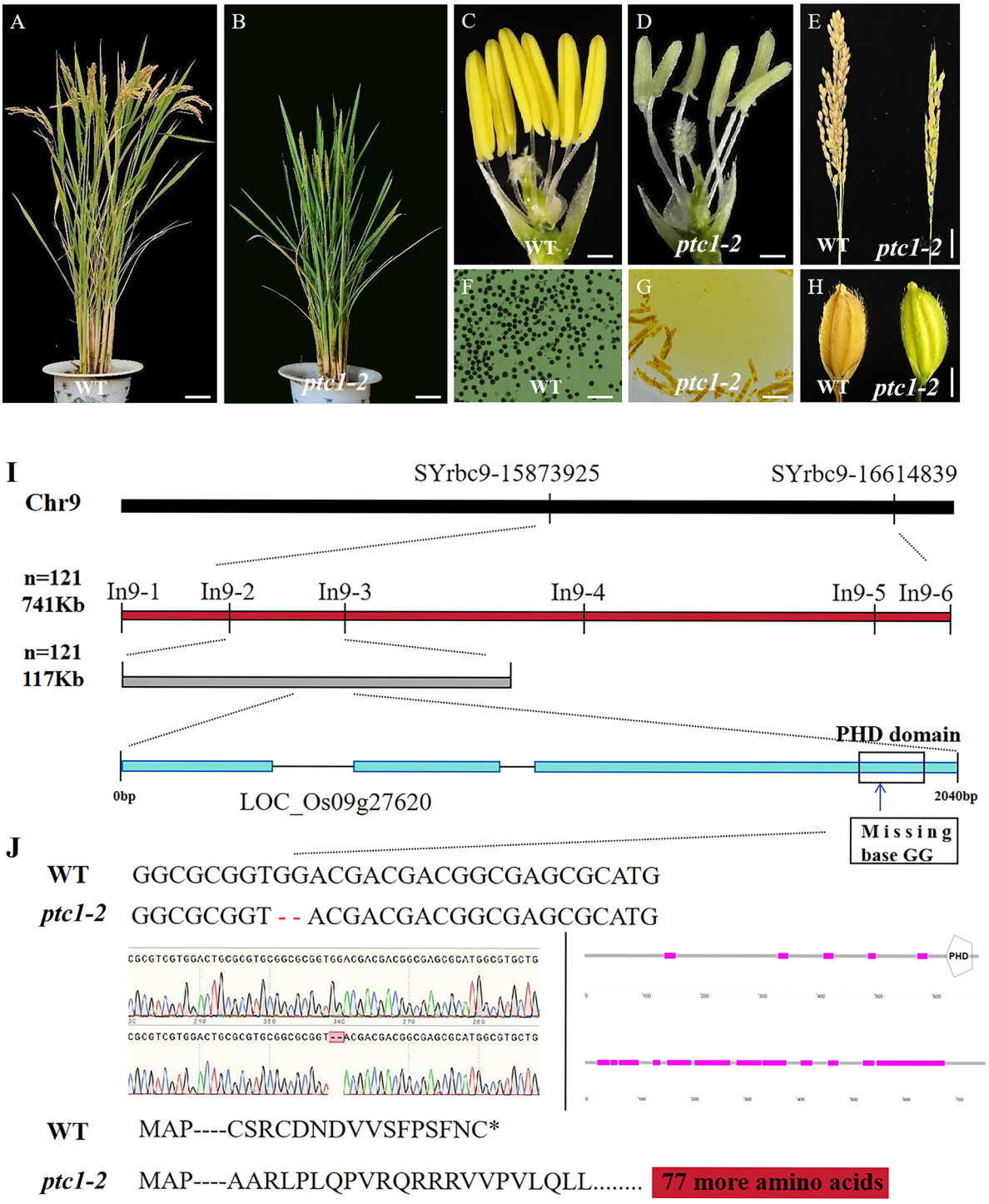


Fig. 1 Comparison of the phenotypes of the wild-type and *ptc1-2* mutant and map-based cloning of *PTC1* gene. (**A**-**B**) Wild-type and *ptc1-2* mutant plants after bolting. (**C**-**D**) The spikelets of the wild-type and *ptc1-2* mutant with the palea and lemma removed. (**E**-**F**) Pollen I_2–KI staining of the wild-type and *ptc1-2* mutant. (**G**) Spikelets of the wild-type (left) and *ptc1-2* mutant (right) strains. Bars = 10 cm in (**A**-**B**), 10 μm in (**C**-**D**), 40 μm in (**E**-**F**), and 1 cm in (**G**). **I**: Mapping cloning of the *PTC1* gene on chromosome 9; **J**: Gene structure and mutation mode of *ptc1-2*

First, the target *g*ene was found to be closely linked to two markers, SYbrc9-15873925 and SYrbc9-16614839, on chromosome 9 by the BSA method. Subsequently, the population was expanded to 121, and 6 polymorphic markers were developed in the vicinity of the linked markers; the target gene was finally mapped to an interval of 117 kb. Changes in the sequence of *LOC_Os09g21020* and *LOC_Os09g27620* were detected in the mutant via

the resequencing of the wild-type and mutant (Fig. 1I). Given that only *LOC_Os09g21020* (*PTC1*) changed in the coding region, more importantly, previous reports have shown that *PTC1* can regulate the development of rice anther [46], which was selected as a candidate gene for further analysis. Expectedly, the following sequence analysis revealed in the mutant, *PTC1* gene was deleted two GG bases in the third exon, resulting in an abnormal PTC1 protein with an additional 77 amino acids. Thus, we named this mutant *ptc1*-2. Bioinformatics analysis revealed the PHD domain was missing due to the sequence changes in the *ptc1*-2 mutant (Fig. 1J).

Morphological analysis of *ptc1-2* mutant anthers

To further understand the defects in anther development caused by the *ptc1-2* mutant, we observed semithin sections of the wild-type and *ptc1-2* mutant at different stages. At stage 8 of anther development, the pollen mother cells of the wild-type formed tetrads through meiosis, and the tapetal cytoplasm was condensed and stained deeply. At this time, the *ptc1-2* mutant did not show significant phenotypic differences (Fig. 2A, E). At stage 9, the anther of the wild-type completed meiosis, the spherical microspores were released from the tetrad, and the tapetum was concentrated and stained deeply. At this time, although the microspores of the *ptc1-2* mutant could also be separated from the tetrad, their shape was irregular with a loose structure, and the tapetum was still at a low concentration, showing severe vacuolation and light staining, which seemed to be maintained in the previous state (Fig. 2B, F). At stage 10, the tapetum of the wild-type became banded due to degradation, the volume of microspores increased, the microspores began to grow showing vacuolated shape, evenly arranged around the anther cavity, and the outline of the microspore exine was clear. At this time, the tapetum of the *ptc1-2* mutant was still vacuolated and lightly stained. Although the microspores could expand, their exine was not clear and seemed to be unable to form normally. Some cells were disrupted and attached to the tapetum in a disordered fashion (Fig. 2C, G). At stage 11, the tapetum of the wild-type was completely degraded, and the microspores gradually became round pollen grains due to starch accumulation; however, an obvious tapetum structure could still be observed in the *ptc1-2* mutant, and only the microspore degradation fragments remained in the anther cavity. (Fig. 2D, H).

The above results indicated that the main phenotypic defects of the *ptc1-2* mutant were delayed tapetum degradation and abnormal microspore development, which finally led to complete male sterility.

To further explore the defects in anther development in *ptc1-2* mutants, which were analysed by transmission electron microscopy (TEM). At stage 9 of anther development, the tapetum of the wild-type was deeply stained, the cytoplasm was highly condensed and squeezed at the edge with obvious gaps in the middle, and the nucleus was degraded, while the tapetum cytoplasm of the *ptc1-2* mutant was less condensed, with many small vacuoles clearly observed, and the nucleus was basically intact

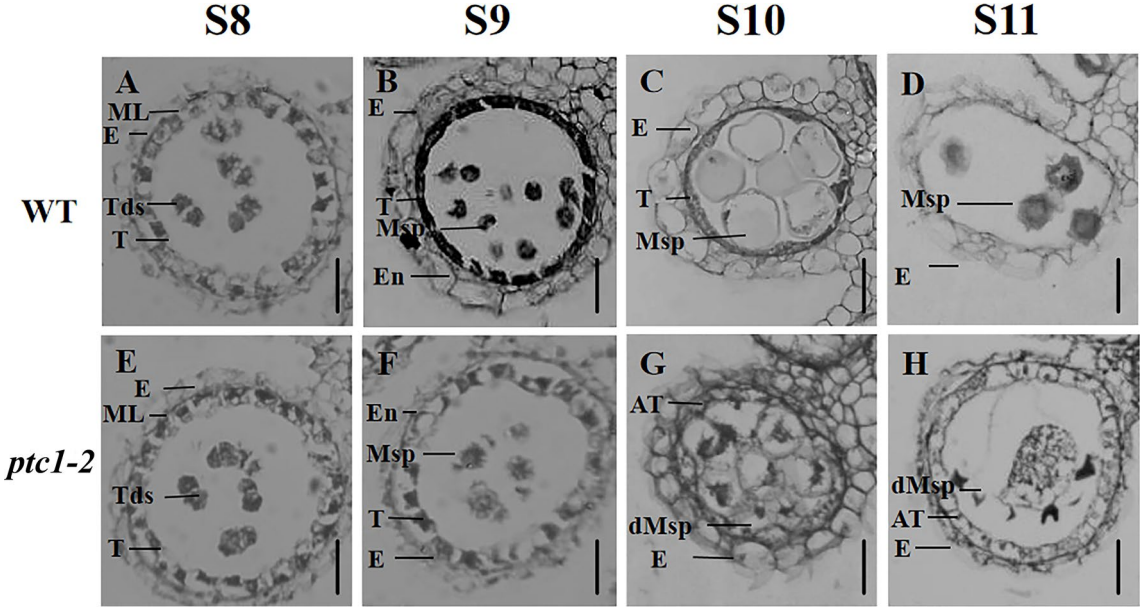


Fig. 2 Histological features of anther development in the wild-type (WT) and *ptc1-2*. Locules from the anther section of wild-type (**A**–**D**) and *ptc1-2* (**E**–**H**) plants from stage 8 to stage 11 are shown. **A**, **E**: Stage 8 of anther development; **B**, **F**: Stage 9 of anther development; **C**, **G**: Stage 10; **D**, **H**: Stage 11. **E**: epidermis; En: endothelium; ML: middle layer; Tds: tetrads; T: tapetum; Msp: microspore; dMsp: degraded microspore; AT: abnormal tapetum; Mp: mature pollen; dMp: degraded mature pollen. Bars = 50 μm, S8-S11: anthers at stages 8 to 11

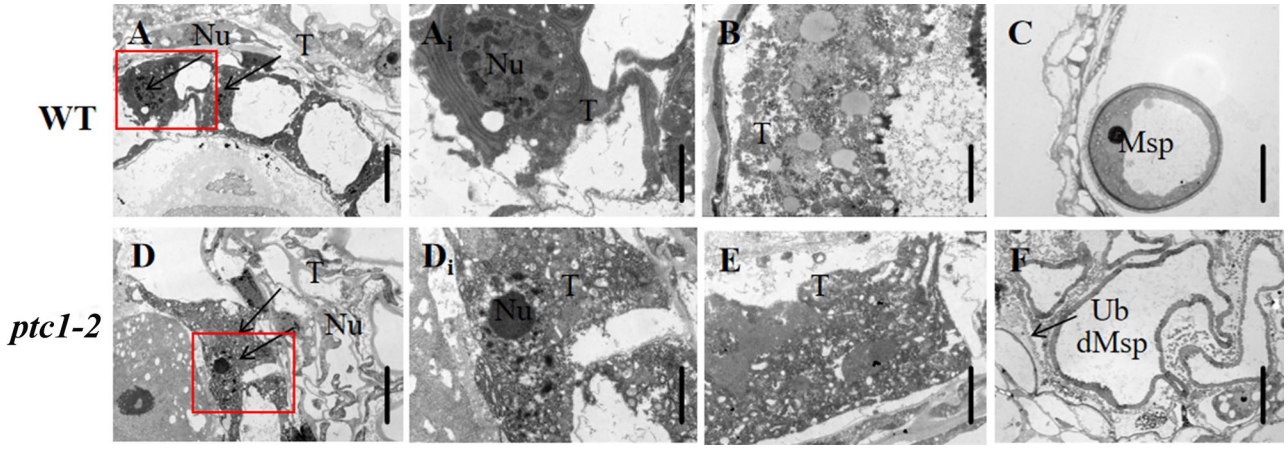


Fig. 3 Transmission electron microscopy images of anthers of the wild-type and *ptc1-2* mutant. **A**-**C**: Transmission electron micrograph of the wild-type; **D**-**F**: Transmission electron micrograph of the *ptc1-2* mutant. A1 is the enlarged view of A, and D1 is the enlarged view of **D**. **A**, **D**: Stage 9; **B**, **E**: Stage 10; **C**, **F**: Stage 11; Bars = 50 μm in (**A**, **C**, **D**, **F**), 20 μm in (Ai, B, Di, E). **E**: epidermis; ML: Middle layer layer; T: tapetum; Tds: tetrads; En: inner epidermis; Msp: microspore; dMsp: degraded microspore; AT, abnormal tapetum

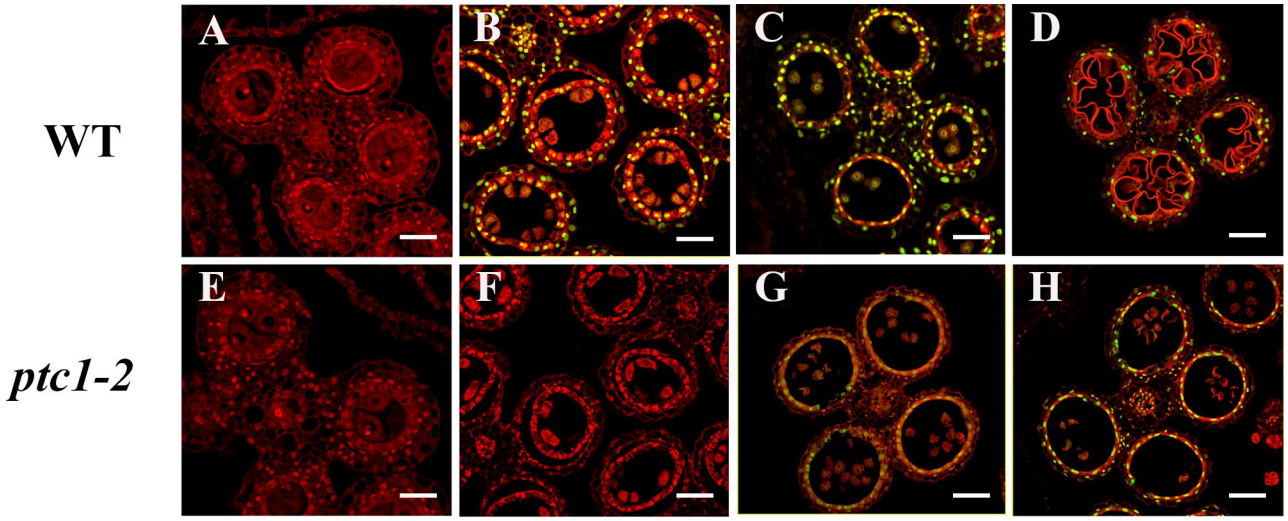


Fig. 4 TUNEL assay of wild type and *ptc1-2*. **A**-**D**: The showed wild-type anthers at stage 7, 8 ,9 and 10, respectively. **E**-**H**: Showed *ptc1-2* anthers at stage 7, 8 ,9 and 10, respectively. Red signal indicates propidium iodide (PI) staining, while yellow and green fluorescence indicates TUNEL positive signal. Bars = 50 μm

(Fig. 3A, D). At stage 10 of anther development, the tapetal cells of the wild-type became loose and lightly stained due to further degradation, the nuclei were completely degraded and disappeared, and a large number of lightly stained spheroids were found in the cells. At this time, the overall morphology of the tapetum of the *ptc1-2* mutant was similar to that of the tapetum of the previous period and seemed to remain in the same state, with no significant degradation observed in the tapetum (Fig. 3B, E). At Stage 11, the tapetum of the wild-type was almost completely degraded, and the microspores were gradually filled with starch. At this time, the tapetum cells of the *ptc1-2* mutant were still clearly visible, and in a degrading state, the microspores were atrophied and randomly distributed in the anther chamber (Fig. 3C, F).

Through the above analysis, we found that the tapetum of *ptc1-2* mutant anthers showed delayed degradation. To explore whether the PCD process in *ptc1-2* mutant tapetum was affected, the Terminal Transferase dUTP Nick End Labeling (TUNEL) assay was performed. In WT tapetum cells, yellow TUNEL signal was not detected at stage 7(Fig. 4A), and began to appear at stage 8 (Fig. 4B), became stronger at stage 9(Fig. 4C), which gradually became weaker at stage 10 (Fig. 4D). However, in the *ptc1-2* mutant, the significantly delayed-weak signals detected till stage 9, becoming stronger at stage 10 (Fig. 4E, F, G, H). The TUNEL assay results confrmed the delay of tapetal PCD in *ptc1-2* tapetum, similar to the previous study [46, 48].

Since the tapetum can provide relevant nutrients for pollen wall synthesis, we also analysed the development of the pollen wall of the *ptc1-2* mutant. At stage 10, the exine of the wild-type anther could form a typical three-layer structure of Nexine-Bacula-Sexine (Fig. 5A, B). Compared with the wild-type, the *ptc1-2* mutant also had a three-layer structure of Nexine-Bacula-Sexine, but the overall Sexine structure was discontinuous, and the Bacula between Sexine and Nexine was disrupted (Fig. 5C). At stage 11, the three-layered structure of the pollen wall of the *ptc1-2* mutant seemed to be abnormally degraded, and its degradation products were clustered together in a bead-like structure in comparison with the fully developed three-layered structure in the wild-type (Fig. 5D).

Ubisch bodies are sporopollenin-containing granular materials located at the edge of the tapetum, and their main function is to participate in the material transport from the tapetum to the microspore [29, 46, 48]. Because the microspore exine of the *ptc1-2* mutant could not develop normally, we also analysed the development of the ubisch bodies in the *ptc1-2* mutant anther. At stage 10 of anther development, the wild-type ubisch bodies was in a semicircular hollow state with high electron density at the edge (Fig. 5E), but the *ptc1-2* mutant Ubisch body was smaller and in a closed circular state (Fig. 5G). At stage 11, the number of ubisch bodies in the wild-type was significantly reduced, the amount of high electron density material was reduced, and the Ubisch bodies were lightly stained (Fig. 5F), while a large number of round ubisch bodies were still observed at the edge of the tapetum in the *ptc1-2* mutant (Fig. 5H).

Transcriptome analysis of *ptc1-2* mutant anthers

To further analyse the molecular regulatory network of the *PTC1* gene in rice anther development, transcriptome analysis was performed on wild-type and *ptc1-2* mutant anthers at stage 8. A total of 3292 differentially expressed genes (DEGs) were identified based on the screening criteria of FDR < 0.05 and log2(|fc|) > 1, among which 1308 were upregulated and 1984 were downregulated.

GO analysis of the DEGs revealed that 1339 DEGs were functionally annotated and could be classified into three functional categories: molecular function, cellular component, and biological process. Through P value screening ($q < 0.05$), differentially expressed genes were primarily classified into the biological process category(Fig. 6A). Among the top 20 significantly enriched terms, those related to pollen development, including phenylpropanoid biosynthetic process (GO: 000009699), phenylpropanoid metabolic process (GO:0009698), pollen wall assembly (GO:0010208), and pollen development (GO:0009555), were prominent. Additionally, terms associated with cell death, such as oxidoreductase activity (GO:0016075) and GO: 00016491), were identified.

KEGG annotation analysis revealed that a total of 398 DEGs participated in 124 metabolic pathways. Through P value screening ($q < 0.05$), it was found that the differentially expressed genes were primarily enriched in metabolic pathways (Fig. 6B). The top three significantly enriched pathways were phenylpropanoid biosynthesis, diterpenoid biosynthesis, and biosynthesis of secondary metabolites. Among the top 20 significantly enriched pathways, six were related to cell wall metabolism (phenylpropanoid biosynthesis, diterpenoid biosynthesis, biosynthesis of secondary metabolites, nicotinamide and nicotinamide metabolism, biosynthesis of various secondary metabolites, cutin, suberine, and wax biosynthesis), four were associated with cell death (cysteine and methionine metabolism, DNA replication, mismatch repair, nucleotide excision repair), and three were linked to carbohydrate metabolism (glyoxylate and dicarboxylate metabolism, starch and sucrose metabolism, glycosphingolipid biosynthesis-lacto and neolacto series).

Given the defective anther development in *ptc1-2* mutant, we also analysed the differential expressed genes related to rice anther development in *ptc1-2* mutant. The analysis revealed that a total of 13 anther development genes (9 downregulated, 4 upregulated) exhibited significant expression changes in expression (Fig. 6C). Among the 9 downregulated genes, the *AP37* gene, encoding an aspartic protease, exhibited the most significant decrease (28924.41-fold, log2(|fc|) = 14.82). Additionally, eight other genes were downregulated, including the *DPW* and *DPW2* genes encoding acyl carrier protein reductase and acyltransferase involved in pollen wall synthesis [35, 50], the *OsNP1* gene catalysing the ω-position alcohol dehydrogenation of long-chain fatty acids to regulate tapetum degradation and pollen wall formation [37], the *APX2* gene encoding ascorbate peroxidase involved in active oxygen scavenging processes [51], the *OsABCG15* gene participating in the transport of anther cuticle and sporopollenin precursors (downregulated 2.69-fold, log2(|fc|) = 1.43) [26, 27], the *OsUgp2* gene involved in callose accumulation and seed carbohydrate metabolism [52], and the *EDT1* subunit A gene of ATP-citrate lyase catalysing citrate in the cytoplasm to generate acetyl-CoA and oxaloacetate (downregulated 2.13-14.72-fold, log2(|fc|) = 1.09–3.88) [20].

The four upregulated genes included *MADS32*, involved in the regulation of anther development [53, 54]; *CBSDUF1*, encoding a mitochondrial protein, can regulate ROS homeostasis and anther development [55]; *SUT1*, involved in sucrose transport affecting pollen germination; and *OsCUC1* can regulate the number of stamens [56]. These genes were upregulated by 2.17–3.53

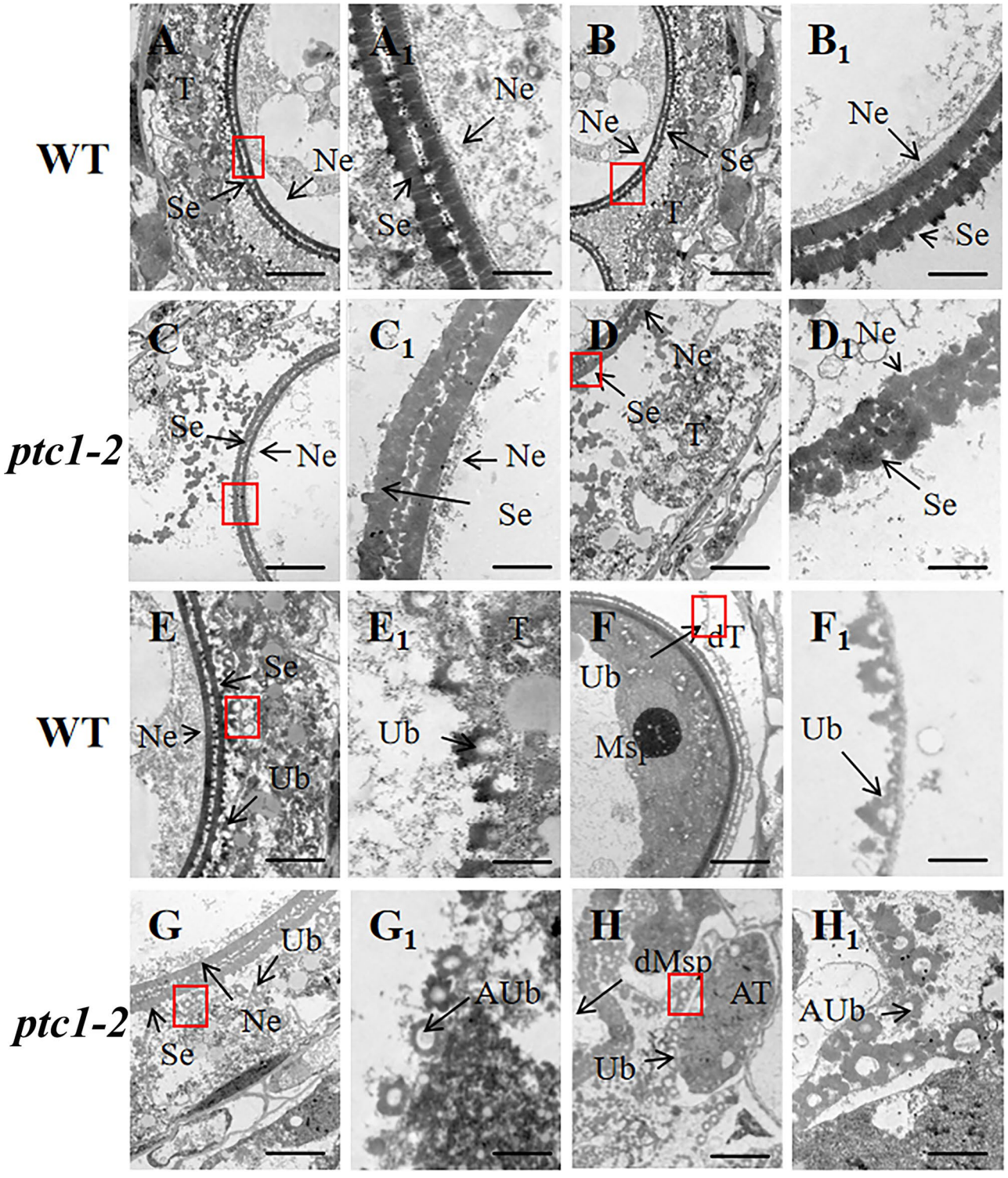
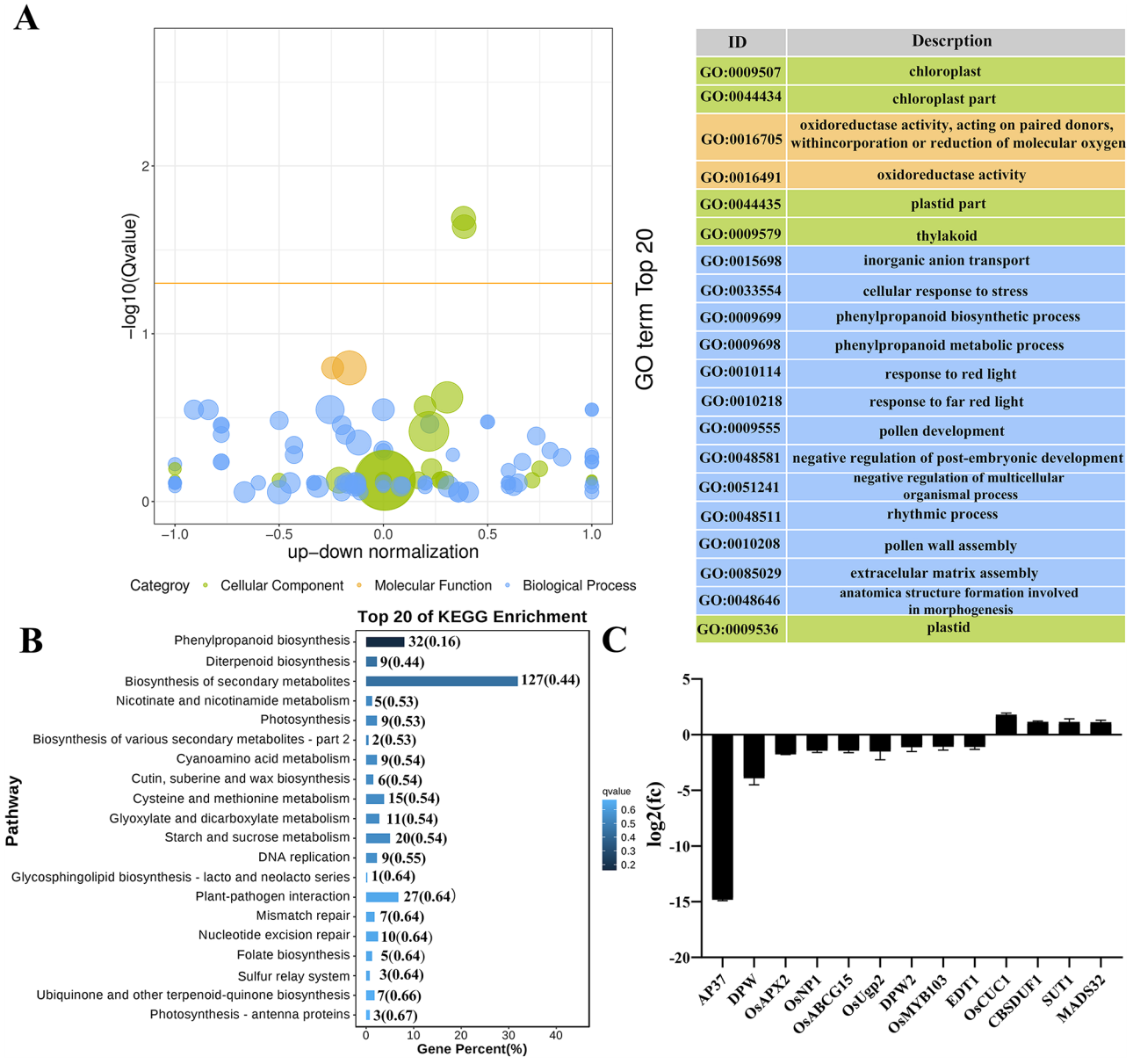


Fig. 5 Pollen exine and ubisch bodies analysis of wild-type and *ptc1-2* mutant anthers. **A**-**B**, **E**-**F**: Transmission electron micrographs of wild-type anthers; **C**-**D**, **G**-**H**: Transmission electron micrographs of *ptc1-2* mutant anthers. A_1-H_1 are magnified views of **A**-**H**, respectively. **A**, **C**, **E**, **G**: anther development S10; **B**, **D**, **F**, **H**: anther development S11. Bars = 50 μm (**A**-**H**), 20 μm (A_1-H_1); T: tapetum; AT: abnormal tapetum; Ub: Ubisch bodie; Se: Sexine; Ne: Nexine; Ba: Bacula; Msp: microspore; dMsp: degraded microspore



ID	Descrption
GO:0009507	chloroplast
GO:0044434	chloroplast part
GO:0016705	oxidoreductase activity, acting on paired donors, withincorporation or reduction of molecular oxygen
GO:0016491	oxidoreductase activity
GO:0044435	plastid part
GO:0009579	thylakoid
GO:0015698	inorganic anion transport
GO:0033554	cellular response to stress
GO:0009699	phenylpropanoid biosynthetic process
GO:0009698	phenylpropanoid metabolic process
GO:0010114	response to red light
GO:0010218	response to far red light
GO:0009555	pollen development
GO:0048581	negative regulation of post-embryonic development
GO:0051241	negative regulation of multicellular organismal process
GO:0048511	rhythmic process
GO:0010208	pollen wall assembly
GO:0085029	extracelular matrix assembly
GO:0048646	anatomica structure formation involved in morphogenesis
GO:0009536	plastid

Fig. 6 Transcriptome analysis of wild-type and *ptc1-2* mutant anthers. **A**: Significant enriched GO terms of differentially expressed genes. **B**: Significant enriched pathways of differential KEGG enrichment. C: Differentially expressed genes associated with rice anther development in the *ptc1-2* mutant. fc: fold change

fold (log2(|fc|)=1.12–1.82) in the *ptc1-2* mutant anther (Fig. 6C).

Taken together, these findings indicate that the *PTC1* gene plays an indispensable role in rice anther development. It may regulate the male reproductive development process in rice by potentially influencing the expression of genes associated with pathways such as cell death, phenylpropanoid biosynthesis, cuticle, suberine, and wax biosynthesis pathways related to anther development.

PTC1 interacts with API5 and EDT1

To further investigate the potential mechanisms underlying the role of *PTC1* in anther development, we constructed a rice anther yeast library to screen for proteins that interact with PTC1. Initially, we constructed the bait vector pGBKT7-PTC1 containing the full-length *PTC1* gene for self-activation analysis. It was observed that upon cotransformation with pGADT7-T into Y2H yeast cells, the cells could grow on both two- and four-day-old plates, indicating that pGADT7-T self-activated and could not be used in the next screening library. Subsequently, the truncated bait vectors BD-PHD (Position

AA: 622–668) and BD-ΔC (Position AA: 1–668) were constructed. Yeast transformation results demonstrated that the truncated BD-PHD lacked self-activation activity, which was suitable for further experimentation (Fig. 7A, B).

Using the truncated bait vector BD-PHD for yeast two-hybrid library screening, it was initially used to screen the library on Leu/Trp-deficient medium, and a total of 23 clones were selected. Sequencing analysis revealed there were four nonredundant genes (*GSP1*, *ARG2*, *EDT1*, *and API5*) among the 23 clones. Bioinformatics analysis and relevant references indicated that only EDT1 and API5 are localized in the cell nucleus, similar to PTC1. Given that EDT1 and API5 are involved in regulating rice tapetum degradation and microspore development, sharing functional similarity with PTC1, which were considered the candidate interacting proteins for further analysis. Upon cotransformation of the "prey" protein vectors pGADT7-EDT1 and pGADT7-API5 with the "bait" vector pGBKT7-PTC1 into yeast cells, both strains grew normally on control medium (SD/LT) and selective growth medium (SD/LTHA) (Fig. 7C), indicating the interactions between PTC1 with EDT1 and API5 in yeast.

To further confirm the interaction between PTC1 and the EDT1/API5 proteins, a bimolecular fluorescence complementation (BiFC) assay was conducted in *Nicotiana benthamiana*. The full-length CDS of *PTC1* was inserted into the pSPYCE vector to generate the PTC1-YC construct, while the full-length CDSs of *EDT1* and *API5* were separately inserted into the pSPYNE vector to obtain the YN-EDT1 and YN-API5 constructs, respectively. Significant green fluorescence signals were observed in the experimental combinations coexpressing PTC1-YC/YN-EDT1 and PTC1-YC/YN-API5, which merged completely with the DAPI fluorescence (specifically targeting the nucleus), indicating that these proteins interact within the nucleus. Under the same conditions, coexpressing PTC1-YC with YN (pSPYNE vector), YC with YN-EDT1 (pSPYCE), or YC with YN-API5 did not result in any green fluorescence (Fig. 7D). These results suggest that PTC1 may jointly regulate rice anther development by interacting with EDT1 and API5.

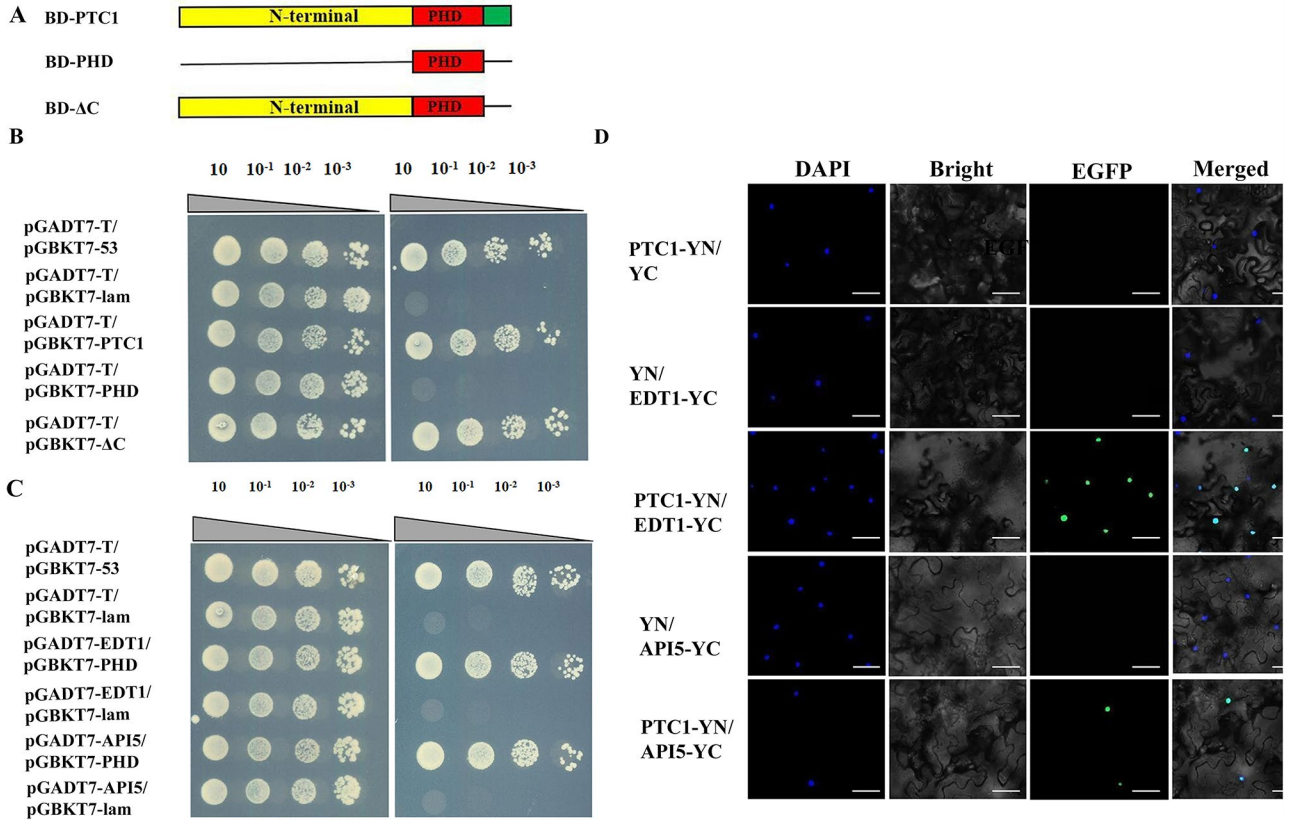


Fig. 7 Interaction between PTC1 protein with EDT1 and API5 proteins. **A**: Schematic representation of the constructed pGBKT7-PTC1, pGBKT7-PHD, and pGBKT7-ΔC truncation vectors for PTC1. **B**: Self-activation validation of the pGBKT7-PTC1, pGBKT7-PHD, and pGBKT7-ΔC vectors. **C**: Screening of yeast on SD/-Leu/-Trp and SD/-Leu/-Trp-His-Ade media. **D**: BiFC analysis of PTC1 with EDT1 and API5 in *Nicotiana benthamiana*. Experimental combinations of PTC1 and YN served as negative controls (first panel). Bars = 50 μm

Discussion

As a male reproductive organ, the anther's primary function is to produce and disseminate pollen, and abnormal anther development typically leads to male infertility. Given the wide application of male sterile lines in production for hybrid breeding to enhance crop yield, research on anther development is receiving increasing amounts of attention. Moreover, anther development is a complex and intricate process in which disruption of any developmental process-related gene may result in pollen abortion. The development of the tapetum and pollen wall is a crucial physiological event during anther development and is especially pivotal for anther formation, making the study of related genes a focus of research on rice anther development [11, 57].

In this study, we obtained a stably inherited male sterile mutant, *ptc1-2*, from the 60^{Co}-γ radiation-induced mutant library of 9522 (*Oryza sativa* L. ssp. *Japonica*). The anthers of *ptc1-2* plants were slender and pale yellow and lacked pollen grains [58]. Analysis of relevant cytological features indicated that the mutation in *ptc1-2* led to delayed degradation of the tapetum, irregular shapes of microspores, failure to form the typical three-layered structure of the pollen wall, and aberrant hollow spherical structures of the ubisch bodies. These results indicate that *PTC1* is a key gene regulating tapetum degradation, pollen wall formation, and ubisch bodies development in rice anthers [59].

The isolated *PTC1* genes in this study, along with *PTC1* and *OsMS1*, were identified as allelic genes. In the *ptc1* mutant, a single-base substitution occurred in the second exon of the target gene, leading to the absence of the PHD [46]. In the *osms1* mutant, a four-base deletion occurred in the first exon of the target gene, resulting in a PHD domain deficiency [60]. In the *ptc1-2* mutant identified in this study, a two-base insertion in the third

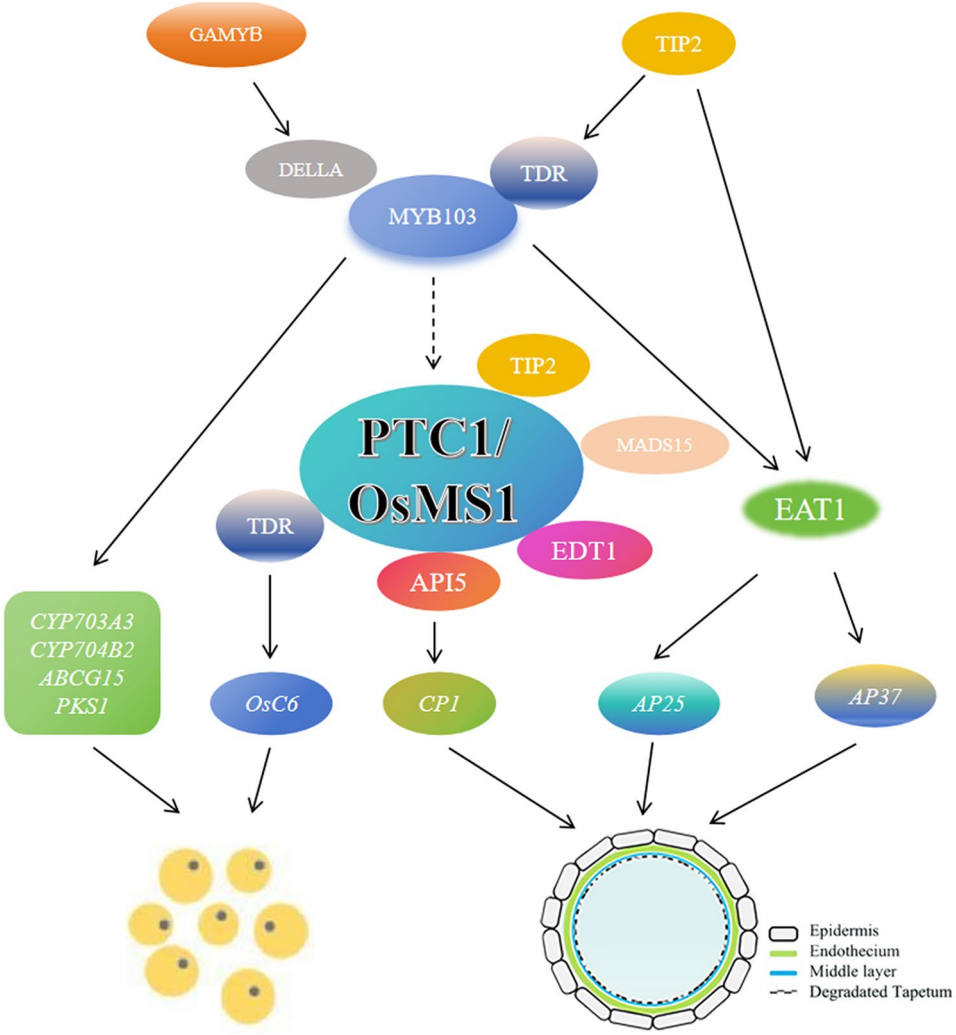


Fig. 8 A model for PTC1 involvement in tapetum degradation and pollen formation. [9, 10, 12, 13, 22, 46, 47, 61]. (OsMS1 associates with the transcription factor TDR to regulate expression of downstream genes in a temperature dependent manner)

exon of the target gene resulted in the absence of the PHD domain. Comparative analysis of the mutation sites revealed that *osms1* is a strong mutant, *ptc1* is a middle mutant, *ptc1-2* is a weak mutant. A comparison of the phenotypic defects of the three allelic mutants *ptc1-2*, *ptc1* and *osms1* revealed certain similarities in tapetum degradation, microspore exine formation, and pollen wall development.

However, the *ptc1* mutant exhibits uncontrolled proliferation followed by subsequent death, while the *osms1* mutant experiences delayed programmed cell death (PCD); in this study, the tapetal cytoplasm of the *ptc1-2* mutant maintained low aggregation since stage 10, and there were many small vacuoles. Concerning pollen wall development control, the *ptc1* mutant displays a thinner pollen wall with fewer rods between the Sexine and Nexine layers, the *osms1* mutant lacks substantial rod-like material support, and the *ptc1-2* mutant presents an overall discontinuous Sexine structure with fragmented rod-like support layers between the Sexine and Nexine layers. In terms of controlling ubisch bodie development, the ubisch bodies of the *ptc1* mutant develop into smaller circular structures compared to those of the wild-type, wheres no obvious ubish bodies were observed on the *osms1* mutant. Interestingly, in the *ptc1-2* mutant, there is an increase in electron density around the ubisch bodies [46, 48]. Certain subtle differences may be due to their difference between their PHD domain. The PHD domain contains a conserved Cys4-His-Cys3 motif, and is mainly involved in eukaryotic transcription regulation, and also were known to recognize a subset of post-translational histone modifiy cations. Thus, We speculate that differences in the PHD domain lead to the discrepancy for their regulated genes, finally affect their phenotype. Recent study showed that *OsMS1* natural allele $OsMS1^{wenmin1}$ confers thermosensitive genic male sterility in rice [47]. In our study, *ptc-1-2* did not exhibited obvious temperature-sensitive phenomenon, which may be due to the different material background and mutation mode, also no encountering the suitable temperature change.

By transcriptome analysis on wild-type and *ptc1-2* mutant anthers at stage 8, pathways related to anther development were identified, including phenylpropanoid biosynthetic process (GO: 000009699), phenylpropanoid metabolic process (GO:0009698), pollen wall assembly (GO:0010208), pollen development (GO:0009555), oxidoreductase activity (GO:0016075, GO:00016491), phenylpropanoid biosynthesis (ko00940), diterpenoid biosynthesis (ko00904), biosynthesis of secondary metabolites (ko01110), nicotinate and nicotinamide metabolism (ko00760), biosynthesis of various secondary metabolites-Part 2 (ko00195), cutin, suberine, and wax biosynthesis (ko00073), cysteine and methionine metabolism (ko00270), DNA replication (ko03030), mismatch repair, nucleotide excision repair (ko03430), glyoxylate and dicarboxylate metabolism (ko03430), starch and sucrose metabolism (ko00500), and glycosphingolipid biosynthesis—lacto and neolacto series (ko00601). Therefore, we hypothesize that it may regulate rice fertility by influencing the expression of genes involved in pathways such as cell death, phenylpropanoid biosynthesis, and cuticle, suberine, and wax biosynthesis during anther development [28, 33]. In the *ptc1-2* mutant, there were significant changes in the expression of 13 genes related to anther development. Notably, genes controlling tapetum degradation, such as *AP37* [10] and *OsMYB103* [12]. exhibited pronounced downregulation. *AP37* encodes an aspartic-acid regulates plant programmed cell death (PCD) regulator [10]. In addition, *OsMYB103*, as a MYB transcription factor, regulates delayed tapetum degradation and defective pollen. Interestingly, in this study, mutations in the *PTC1* gene led to delayed degradation of tapetal cells. This phenotype is similar to that described above. This finding also suggested that the *PTC1* gene may regulate anther development in rice by regulating other genes that control rice fertility. Additionally, genes involved in pollen wall synthesis, including *OsABCG15* [26], *OsNP1* [37], *DPW* [35], and *DPW2* [50], exhibited decreased expression levels. The abnormal pollen wall development observed in our *ptc1-2* mutant may partly result from the downregulation of these genes. These findings further underscore the crucial role of the *PTC1* gene in regulating rice anther development.

Previous studies have shown that PTC1 protein interacts with Tip2 and MADS15 to regulate the development of rice tapetum layer [48]. The classical cascade reaction of Tip2-TDR-OsMYB103-EAT1 indicates that Tip2 is located upstream of TDR and EAT1. TDR is located upstream of OsMYB103 and EAT1, and OsMYB103 is located upstream of EAT1. In addition, TDR can interact with OsMYB103 [9, 12]. Here, we also found that the expression of *OsMYB103* was down-regulated in the *ptc1-2* mutant. These results suggest that OsMYB103 may regulate PTC1. In another classical approach, TDR-OsMs188/MYB80/MYB103-PTC1 [13], PTC1 functions downstream of GAMYB and in parallel with TDR in regulating programmed anther development and pollen formation. Also, TDR directly regulates *OsCP1* and *OsC6*. Meanwhile, it was showed that OsMS188 and OsGAMYB were regulated by SLR protein, which could directly activate the expression of genes related to pollen wall synthesis, like *CYP703A3*, *CYP704B2*, *ABCG15* and *PKS1* [15, 61].

Here, we showed that PTC1 could interact with EDT1 and API5 protein through yeast two-hybridization and BiFC experiment. EDT1 encodes an ATP-citrate lyase, which can participate in the regulation of PCD process

in tapetum layer and the formation of anther wall [20]. The knockout of OsAPI5, another interacting protein, leads to defects in male gametophyte formation, resulting in PCD delay in the tapetal layer, similar to *ptc1-2* [53].Meanwhile, EDT as the A subunit of ACL, can form heteropolymers with its B subunit to control the development of tapetum layer [20]. OsAPI5 can interact with two DEAD-box ATP-dependent RNA helicases, AIP1 and AIP2. In addition, AIP1 and AIP2 can form dimers and interact directly with the CP1 promoter so API5-AIP1/2-CP1 acts as a key pathway to regulate the occurrence of PCD in rice tapetum layer. Interestingly, *CP1* in this pathway is regulated by TDR [61]. A recent study also showed that OsMS1 can also regulate rice fertility as a temperature-regulated histone interacting with transcription factor TDR [47].

Based on the previous reports and known pathways about PTC1 (PTC1/OsMS1-TDR-Tip2-MADS15), we have integrated our new research findings (PTC1-API5-EDT1). The summarized and updated the molecular regulatory network of PTC1 in Fig. 8, which showed PTC1 was located in a very complex and important position to regulate rice anther development.

Abbreviations

PHD	Plant homeodomain domian
BiFC	Bimolecular fluorescence complementation
API5	APOPTOSIS INHIBITOR5
EDT1	ATP-Citrate Lyase

Author contributions

HH, LH, QY and WJ designed the study; QY and WJ performed the experiments with assistance from ZW, XH, XW, ZZ, SL, HW and DG; QY and WJ analyzed the data and wrote the manuscript; HH and LH drafted and revised the article. All authors reviewed the manuscript.

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Data availability

All data supporting the conclusions described here are provided in figures and additional files. All gene accession numbers: *PTC1* (LOC_Os09g27620), *API5* (LOC_Os02g20930), *AP37* (LOC_Os04g37570), *DPW* (LOC_Os03g07140), *OsAPX2* (LOC_Os07g49400), *OsNP1* (LOC_Os10g38050), *OsABCG15* (LOC_Os06g40550), *OsUgp2* (LOC_Os02g02560), *DPW2* (LOC_Os01g70025), *OsMYB103* (LOC_Os04g39470), *EDT1* (LOC_Os11g47330), *OsCUC1* (LOC_Os06g23650), *CBSDUF1* (LOC_Os05g32850), *SUT1* (LOC_Os03g07480) and *MADS32* (LOC_Os01g52680) can be obtained in the Rice Genome Database (http://www.ricedata.cn/gene/index.htm).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Pak H, Wang H, Kim Y, Song U, Tu M, Wu D, Jiang L. Creation of male-sterile lines that can be restored to fertility by exogenous methyl jasmonate for the establishment of a two-line system for the hybrid production of rice (Oryza sativa L). Plant Biotechnol J. 2021;19:365–74.
2. Tariq N, Yaseen M, Xu D, Rehman HM, Bibi M, Uzair M, Wicke S, Wicke S. Rice anther tapetum: a vital reproductive cell layer for sporopollenin biosynthesis and pollen exine patterning. Plant Biol (Stuttgart Germany). 2023;25:233–45.
3. Yang DZAL. Specification of tapetum and microsporocyte cells within the anther Dabing Zhang and Li Yang. (2014).
4. Falasca G, D'Angeli S, Biasi R, Fattorini L, Matteucci M, Canini A. Altamura. Tapetum and middle layer control male fertility in Actinidia deliciosa. Ann Bot-London. 2013;112:1045–55.
5. Truskina J, Boeuf S, Renard J, Andersen TG, Geldner N. G. Ingram. Anther development in Arabidopsis thaliana involves symplastic isolation and apoplastic gating of the tapetum-middle layer interface. Development 149 (2022).
6. Jung K, Han M, Lee Y, Kim Y, Hwang I, Kim M, Kim Y, Nahm BH. An. RiceUndeveloped Tapetum1 is a Major Regulator of Early Tapetum Development. Plant Cell. 2005;17:2705–22.
7. Tang J, Tian X, Mei E, He M, Gao J, Yu J, Xu M, Liu J, Song L, Li X, Wang Z, Guan Q, Zhao Z, Wang C, Bu Q. WRKY53 negatively regulates rice cold tolerance at the booting stage by fine-tuning anther gibberellin levels. Plant Cell. 2022;34:4495–515.
8. Ko S, Li M, Ho Y, Yu C, Yang T, Lin Y, Hsing H, Chen T, Jhong C, Li W. Sun-Ben Ku. Rice transcription factor GAMYB modulates bHLH142 and is homeostatically regulated by TDR during anther tapetal and pollen development. J EXP BOT. 2021;72:4888–903.
9. Li N, Zhang D, Liu H, Yin C, Li X, Liang W, Yuan Z, Xu B, Chu H, Wang J, Wen T, Huang H, Luo D, Ma H. Zhang. The RiceTapetum Degeneration Retardation Gene is required for Tapetum Degradation and Anther Development. Plant Cell. 2006;18:2999–3014.
10. Niu N, Liang W, Yang X, Jin W, Wilson ZA, Hu J, Zhang D. EAT1 promotes tapetal cell death by regulating aspartic proteases during male reproductive development in rice. Nat Commun 4 (2013).
11. Li S, Hu Y, Yang H, Tian S, Wei D, Tang Q, Yang Y, Wang Z. The regulatory roles of MYC TFs in plant stamen development. PLANT SCI. 2023;333:111734.
12. T. Lei, L. Zhang, P. Feng, Y. Liu, W. Yin, L. Shang, G. He, N. Wang. OsMYB103 is essential for tapetum degradation in rice. Theor Appl Genet. 135 (2022), 929–945.
13. Jin Y, Song X, Chang H, Zhao Y, Cao C, Qiu X, Zhu J, Wang E, Yang Z. Yu. The Ga–Della–OsMS188 module controls male reproductive development in rice. New Phytol. 2022;233:2629–42.
14. X. Xiang, L. Sun, P. Yu, Z. Yang, P. Zhang, Y. Zhang, W. Wu, D. Chen, X. Zhan, R. Khan, A. Abbas, S. Cheng, L. Cao. The MYB transcription factor Baymax1 plays a critical role in rice male fertility. Theor Appl Genet. 134 (2021), 453–471.
15. Pan X, Yan W, Chang Z, Xu Y, Luo M, Xu C, Chen Z, Wu J, Tang X. OsMYB80 regulates Anther Development and Pollen Fertility by targeting multiple Biological pathways. Plant Cell Physiol. 2020;61:988–1004.
16. Zheng S, Li J, Ma L, Wang H, Zhou H, Ni E, Jiang D, Liu Z, Zhuang C. OsAGO2 controls ROS production and the initiation of tapetal PCD by epigenetically regulatingOsHXK1 expression in rice anthers. Proc Natl Acad Sci. 2019;116:7549–58.
17. Chen H, Zhang S, Li R, Peng G, Chen W, Rautengarten C, Liu M, Zhu L, Xiao Y, Song F, Ni J, Huang J, Wu A, Liu Z, Zhuang C, Heazlewood JL, Xie Y, Chu Z. Zhou. Botryoid Pollen 1 regulates ROS-triggered PCD and pollen wall development by controlling UDP-sugar homeostasis in rice. Plant Cell. 2023;35:3522–43.
18. Tang J, Lei D, Yang J, Chen S, Wang X, Huang X, Zhang S, Cai Z, Zhu S, Wan J. Jia. OsALKBH9-mediated m6A demethylation regulates tapetal PCD and pollen exine accumulation in rice. Plant Biotechnol J. 2024;22:2410–23.
19. J. Liu, Q. Ye, W. Jiang, S. Liu, Z. Wu, X. Hu, X. Wang, Z. Zhang, D. Guo, X. Chen, H. He, L. Hu. Abnormal Degraded Tapetum 1 (ADT1) is required for tapetal cell death and pollen development in rice. Theor Appl Genet. 137 (2024), 170.
20. Bai W, Wang P, Hong J, Kong W, Xiao Y, Yu X, Zheng H, You S, Lu J, Lei D, Wang C, Wang Q, Liu S, Liu X, Tian Y, Chen L, Jiang L, Zhao Z, Wu C. Wan. Earlier

degraded Tapetum1 (EDT1) encodes an ATP-Citrate lyase required for Tapetum programmed cell death. Plant Physiol. 2019;181:1223–38.
21. Kamara N, Lu Z, Jiao Y, Zhu L, Wu J, Chen Z, Wang L, Liu X, Shahid MQ. An uncharacterized protein NY1 targets EAT1 to regulate anther tapetum development in polyploid rice. BMC Plant Biol. 2022;22:582.
22. Ko S, Li M, Sun-Ben Ku M, Ho Y, Lin Y, Chuang M, Hsing H, Lien Y, Yang H, Chang H, Chan M. The bHLH142 transcription factor coordinates with TDR1 to modulate the expression of EAT1 and regulate Pollen Development in Rice. Plant Cell. 2014;26:2486–504.
23. Zhang YF, Li YL, Zhong X, Wang JJ, Zhou L, Han Y, Li DD, Wang N, Huang XH, Zhu J, Yang ZN. Mutation of glucose-methanol-choline oxidoreductase leads to thermosensitive genic male sterility in rice and Arabidopsis. Plant Biotechnol J. 2022;20:2023–35.
24. Yang X, Wu D, Shi J, He Y, Pinot F, Grausem B, Yin C, Zhu L, Chen M, Luo Z, Liang W, Zhang D. Rice CYP703A3, a cytochrome P450 hydroxylase, is essential for development of anther cuticle and pollen exine. J Integr Plant Biol. 2014;56:979–94.
25. Chang Z, Chen Z, Yan W, Xie G, Lu J, Wang N, Lu Q, Yao N, Yang G, Xia J, Tang X. An ABC transporter, OsABCG26, is required for anther cuticle and pollen exine formation and pollen-pistil interactions in rice. Plant SCI. 2016;253:21–30.
26. Zhao G, Shi J, Liang W, Xue F, Luo Q, Zhu L, Qu G, Chen M, Schreiber L, Zhang D. Two ATP binding Cassette G (ABCG) transporters, OsABCG26 and OsABCG15, collaboratively regulate Rice Male Reproduction. Plant Physiol. 2015;262:2015.
27. Qin P, Tu B, Wang Y, Deng L, Quilichini TD, Li T, Wang H, Ma B, Li S. ABCG15 encodes an ABC Transporter Protein, and is essential for post-meiotic Anther and Pollen Exine Development in Rice. Plant Cell Physiol. 2013;54:138–54.
28. Chen L, Ji C, Zhou D, Gou X, Tang J, Jiang Y, Han J, Liu Y, Chen L, Xie Y. OsLTP47 may function in a lipid transfer relay essential for pollen wall development in rice. J Genet Genomics. 2022;49:481–91.
29. Zhang D, Liang W, Yin C, Zong J, Gu F, Zhang D. OsC6, encoding a lipid transfer protein, is required for Postmeiotic Anther Development in Rice. Plant Physiol. 2010;154:149–62.
30. Yang X, Liang W, Chen M, Zhang D, Zhao X, Shi J. Rice fatty acyl-CoA synthetase OsACOS12 is required for tapetum programmed cell death and male fertility. Planta. 2017;246:105–22.
31. Zou T, Xiao Q, Li W, Luo T, Yuan G, He Z, Liu M, Li Q, Xu P, Zhu J, Liang Y, Deng Q, Wang S, Zheng A, Wang L, Li P, Li S. OsLAP6/OsPKS1, an orthologue of Arabidopsis PKSA/LAP6, is critical for proper pollen exine formation. Rice. 10 (2017).
32. Zou T, Liu M, Xiao Q, Wang T, Chen D, Luo T, Yuan G, Li Q, Zhu J, Liang Y, Deng Q, Wang S, Zheng A, Wang L, Li P, Li S. OsPKS2 is required for rice male fertility by participating in pollen wall formation. Plant Cell Rep. 2018;37:759–73.
33. Liu F, Yang H, Tang R, Wang W, Shen H, Xu M, Hao T, Hu Y, Zhang Y, Bao Y. OsTKPR1 proteins with a single amino acid substitution fail the synthesis of a specific sporopollenin precursor and cause abnormal exine and pollen development in rice. Plant Sci. 2023;335:111792.
34. Jung K, Han M, Lee D, Lee Y, Schreiber L, Franke R, Faust A, Yephremov A, Saedler H, Kim Y, Hwang I. An. Wax-deficient anther1 is involved in cuticle and Wax Production in Rice Anther walls and is required for Pollen Development. Plant Cell. 2006;18:3015–32.
35. Shi J, Tan H, Yu X, Liu Y, Liang W, Ranathunge K, Franke RB, Schreiber L, Wang Y, Kai G, Shanklin J, Ma H. Zhang. Defective Pollen Wall is required for Anther and Microspore Development in Rice and encodes a fatty acyl carrier protein reductase. Plant Cell. 2011;23:2225–46.
36. Men X, Shi J, Liang W, Zhang Q, Lian G, Quan S, Zhu L, Luo Z, Chen M, Zhang D. Glycerol-3-Phosphate acyltransferase 3 (OsGPAT3) is required for anther development and male fertility in rice. J Exp Bot (2017), w445.
37. Liu Z, Lin S, Shi J, Yu J, Zhu L, Yang X, Zhang D. Liang. Rice No Pollen 1 (NP1) is required for anther cuticle formation and pollen exine patterning. Plant J. 2017;91:263–77.
38. Mondol PC, Xu D, Duan L, Shi J, Wang C, Chen X, Chen M, Hu J, Liang W. Zhang. Defective Pollen Wall 3 (DPW3), a novel alpha integrin-like protein, is required for pollen wall formation in rice. New Phytol. 2020;225:807–22.
39. Conrad LJ, Khanday I, Johnson C, Guiderdoni E, An G, Vijayraghavan U. Sundaresan. The polycomb group geneEMF2B is essential for maintenance of floral meristem determinacy in rice. Plant J. 2014;80:883–94.
40. Xiaoqun MWYL, Peng. Lectin receptor kinase OsLecRK - S.7 is required for pollen development and male fertility. (2019).
41. Y. Deng, Y. Wan, W. Liu, L. Zhang, K. Zhou, P. Feng, G. He, N. Wang. OsFLA1 encodes a fasciclin-like arabinogalactan protein and affects pollen exine development in rice. Theor Appl Genet. 135 (2022), 1247–1262.
42. Yuan G, Zou T, He Z, Xiao Q, Li G, Liu S, Xiong P, Chen H, Peng K, Zhang X, Luo T, Zhou D, Yang S, Zhou F, Zhang K, Zheng K, Han Y, Zhu J, Liang Y, Deng Q, Wang S, Sun C, Yu X, Liu H, Wang L, Li P, Li S. Swollen tapetum and sterility 1 is required for tapetum degeneration and pollen wall formation in rice. Plant Physiol (Bethesda). 2022;190:352–70.
43. Zhang L, Liu Y, Wei G, Lei T, Wu J, Zheng L, Ma H, He G. Wang. Pollen wall abortion1 is essential for pollen wall development in rice. Plant Physiol. 2022;190:2229–45.
44. Yang C, Vizcay-Barrena G, Conner K, Wilson ZA. Male Sterility1 is required for Tapetal Development and Pollen Wall Biosynthesis. Plant Cell. 2007;19:3530–48.
45. Aya K, Ueguchi-Tanaka M, Kondo M, Hamada K, Yano K, Nishimura M. Matsuoka. Gibberellin modulates Anther Development in Rice via the Transcriptional Regulation of GAMYB. Plant Cell. 2009;21:1453–72.
46. Li H, Yuan Z, Vizcay-Barrena G, Yang C, Liang W, Zong J, Wilson ZA. Zhang. Persistent Tapetal Cell1 encodes a PHD-Finger protein that is required for Tapetal Cell Death and Pollen Development in Rice. Plant Physiol. 2011;156:615–30.
47. Wu L, Jing X, Zhang B, Chen S, Xu R, Duan P, Zou D, Huang S, Zhou T, An C, Luo Y, Li Y. A natural allele of OsMS1 responds to temperature changes and confers thermosensitive genic male sterility. Nat Commun. 2022;13:2055.
48. Yang Z, Liu L, Sun L, Yu P, Zhang P, Abbas A, Xiang X, Wu W, Zhang Y, Cao L. Cheng. OsMS1 functions as a transcriptional activator to regulate programmed tapetum development and pollen exine formation in rice. Plant Mol Biol. 2019;99:175–91.
49. Cao H, Li X, Wang Z, Ding M, Sun Y, Dong F, Chen F, Liu LA, Doughty J, Li Y, Liu Y. Histone H2B monoubiquitination mediated by Histone Monoubiquitination1 and Histone Monoubiquitination2 is involved in Anther Development by regulating Tapetum degradation-related genes in Rice. Plant Physiol. 2015;168:1389–405.
50. Xu D, Shi J, Rautengarten C, Yang L, Qian X, Uzair M, Zhu L, Luo Q, An G, Waßmann F, Schreiber L, Heazlewood JL, Scheller HV, Hu J, Zhang D, Liang W. Defective Pollen Wall 2 (DPW2) Encodes an Acyl Transferase Required for Rice Pollen Development1[OPEN]. Plant physiology (Bethesda). 173 (2016), 240–255.
51. Zhang Z, Zhang Q, Wu J, Zheng X, Zheng S, Sun X, Qiu Q, Lu T, Tran LP. Gene knockout study reveals that cytosolic ascorbate peroxidase 2(OsAPX2) plays a critical role in growth and reproduction in rice under drought, salt and cold stresses. PLoS ONE 8 (2013), e57472.
52. Huang Z, Gan Z, He Y, Li Y, Liu X, Mu H. Functional analysis of a rice late pollen-abundant UDP-glucose pyrophosphorylase (OsUgp2) promoter. Mol Biol Rep. 2011;38:4291–302.
53. Sang X, Li Y, Luo Z, Ren D, Fang L, Wang N, Zhao F, Ling Y, Yang Z, Liu Y, He G. Chimeric Floral Organs1, encoding a monocot-specific MADS Box protein, regulates Floral Organ Identity in Rice. Plant Physiol. 2012;160:788–807.
54. Hu Y, Wang L, Jia R, Liang W, Zhang X, Xu J, Chen X, Lu D, Chen M, Luo Z, Xie J, Cao L, Xu B, Yu Y, Persson S, Zhang D. Yuan. Rice transcription factor MADS32 regulates floral patterning through interactions with multiple floral homeotic genes. J Exp Bot. 2021;72:2434–49.
55. Syed Adeel Zafar SBPM. Degenerated Panicle and Partial Sterility 1 (DPS1) encodes a cystathionine b-synthase domain containing protein required for anther cuticle and panicle development in rice. (2019).
56. Jun JBBZ, Wang. The osa-miR164 target OsCUC1 functions redundantly with OsCUC3 in controlling rice meristem/organ boundary specifica- tion. New Phytoiogist (2020).
57. Xue J, Yao C, Xu Q, Sui C, Jia X, Hu W, Lv Y, Feng Y, Peng Y, Shen S, Yang N, Lou Y, Yang Z. Development of the Middle Layer in the Anther of Arabidopsis. Front Plant Sci; 2021. p. 12.
58. Shen Y, Ye Q, Wu Z, Jiang W, Wang L, Zhang Q, Liu J, Guo D, Hu X, Zhang Z, Wang X, He H, Hu L. Functional characterization of OsCAT2 gene in rice that regulates ROS scavenging and plant growth and development. Plant Growth Regul; 2023.
59. Liu J, Zhou Y, Wang L, Zhang Q, Shen Y, Jiang W, Chen X, He H, Hu L. Cytological Analysis and Fine Mapping of paa1 (post-meiosis abnormal anther 1) mutant with abnormal Tapetum and Microspore Development. Biochem Genet. 2022;60:2268–85.
60. Lu J, Xiong S, Yin W, Teng X, Lou Y, Zhu J, Zhang C, Gu J, Wilson ZA, Yang Z. MS1, a direct target of MS188, regulates the expression of key sporophytic pollen coat protein genes in Arabidopsis. J Exp Bot. 2020;71:4877–89.

61. Han Y, Zhou S, Fan J, Zhou L, Shi Q, Zhang Y, Liu X, Chen X, Zhu J, Yang Z. OsMS188 Is a Key Regulator of Tapetum Development and Sporopollenin Synthesis in Rice. RICE. 14 (2021).

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