Commentary

A new module for tapetum development: the functional exploration of TIP2-UDT1-OsUPEX1/2 module in rice

The tapetum plays a pivotal role in the precise development of anther and pollen by supplying nutrients and signaling molecules to the microspores and controlling their release (Jung *et al.*, 2005; Ariizumi & Toriyama, 2011). Numerous transcription factors have been reported to regulate the timely degradation of the tapetum and pollen development in rice (Jung *et al.*, 2005; Li *et al.*, 2006; Niu *et al.*, 2013; Fu *et al.*, 2014; Ko *et al.*, 2014). However, the specific relationships among proteins in regulating anther development and their direct target are unclear. Recently, a study by Wang *et al.* (2025; doi: 10.1111/nph.20435) published in *New Phytologist* under the title 'TIP2-UDT1-OsUPEX1/2 module regulates tapetum development and function in rice' reported that Tapetum Degeneration Retardation (TDR) INTERACTING PROTEIN (TIP2) can physically interact with Undeveloped Tapetum1 (UDT1). The TIP2–UDT1 complex activates the expression of *OsUPEX1* and *OsUPEX2*, which encodes putative β-(1,3)-galactosyltransferases and is specifically expressed in the tapetum. These findings reveal the important roles of TIP2-UDT1-OsUPEX1/2 in tapetum development and pollen formation, providing significant insights into the molecular mechanism of the male rice reproductive development.

'*This study fills gaps in our understanding of early anther development by elucidating the TIP2-UDT1-OsUPEX1/2 pathway.*'

Precisely controlling anther and pollen development is critical for reproductive success. Microspore mother cells (MMCs) undergo meiosis to produce microspores, which undergo two mitotic divisions to develop into mature pollen in the central cavity of the anther. The tapetum, the innermost layer of the anther wall, is closely linked to the development of the male gametophyte and plays an important role in microspore maturation (Jung *et al.*, 2005; Ariizumi & Toriyama, 2011). Previous studies have found that four bHLH transcription factors regulate programmed cell death (PCD) of the tapetum and pollen development in rice: UDT1, TDR, ETERNAL TAPETUM1 (EAT1), and TIP2 (Jung *et al.*, 2005; Li *et al.*, 2006; Niu *et al.*, 2013; Fu *et al.*, 2014; Ko *et al.*, 2014). These bHLH transcription factors form heterodimers and constitute a feed-forward transcription cascade to regulate rice anther development in sequential developmental stages. Mutants of these factors exhibit defects in tapetum development and degeneration to varying degrees (Jung *et al.*, 2005; Li *et al.*, 2006; Niu *et al.*, 2013; Fu *et al.*, 2014; Ko *et al.*, 2014; Ono *et al.*, 2019). UDT1 and TIP2 are both initially expressed before meiosis and are essential for the development and degeneration of the tapetum (Jung *et al.*, 2005; Fu *et al.*, 2014). TIP2 activates *TDR* and forms heterodimers with TDR to promote tapetum differentiation and trigger tapetal PCD at a later stage (Fu *et al.*, 2014; Ko *et al.*, 2014). The TIP2–TDR complex directly upregulates the expression of *EAT1*, followed by the formation of TDR–EAT1 heterodimer, which is required for activation of tapetal PCD (Niu *et al.*, 2013; Fu *et al.*, 2014; Ko *et al.*, 2014). Both UDT1 and TIP2 are involved in the early stage of anther wall layer development, and their mutants show similar defective phenotypes. Whether TIP2 can form a heterodimer with UDT1, and how these two factors coordinately regulate anther development remain important questions with significant research value.

Wang *et al.* revealed that UDT1 and TIP2 proteins are localized in overlapping but not entirely identical anther cell layers, suggesting that these two factors have both shared and distinct functions. They further demonstrated that TIP2 can physically interact with UDT1, whereas neither TIP2 nor UDT1 alone possesses transcription activation activity; the TIP2–UDT1 complex is capable of activating target gene expression. These findings provide the first evidence that TIP2 and UDT1 can also heterodimerize to regulate anther development in rice (Fig. 1). This discovery enhances our understanding of the protein regulatory network involved in rice anther development.

The transition from mitosis to meiosis in MMCs involves dramatic cell wall remodeling, which is a critical step for male gametogenesis. During this process, the primary cellulosic walls of MMCs are reorganized and replaced by callose (β-1,3-glucan) (Matsuo *et al.*, 2013). After meiosis, the callose wall is degraded by β-1,3-glucanase (callase), which is synthesized and secreted by tapetal cells to facilitate microspore release (Ariizumi & Toriyama, 2011). While the enzymatic basis for wall degradation is partially understood, the molecular regulatory networks controlling the dynamic callose deposition and degeneration remain poorly defined. Previous studies have reported that *udt1*, *tip2*, and *tdr* mutants in rice display a significant delay in callose degradation (Jung *et al.*, 2005; Fu *et al.*, 2014), indicating that these genes may regulate the synthesis or secretion of callase from the tapetum. Key questions that arise from these observations are how do these transcription factors regulate the synthesis and secretion of callase, and what are the direct target genes of these TFs?

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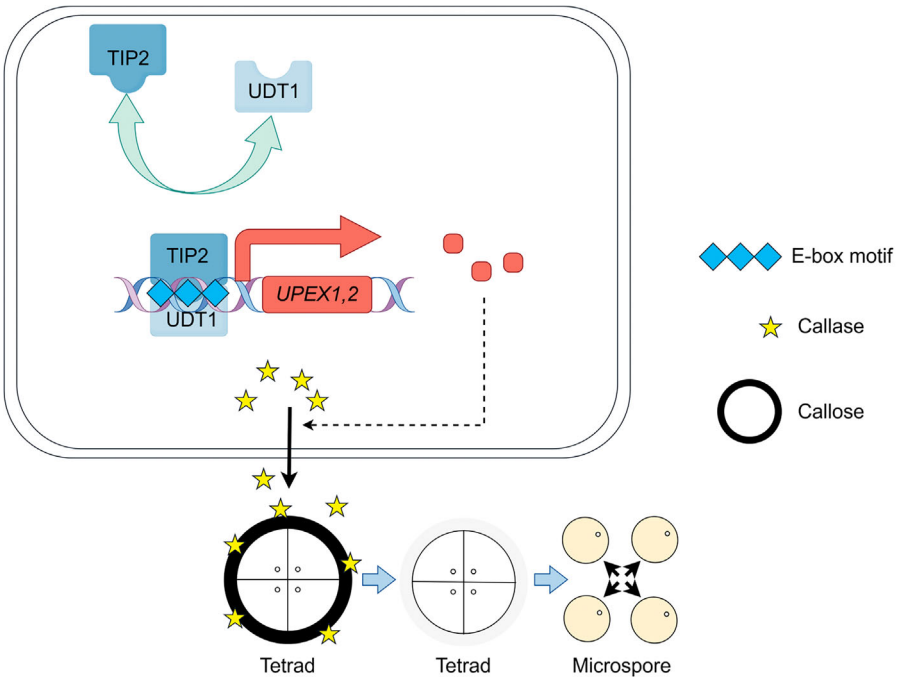

Fig. 1 Schematic representation of Tapetum Degeneration Retardation (TDR) INTERACTING PROTEIN (TIP2)–Undeveloped Tapetum1 (UDT1)–OsUPEX1/2 module in rice tapetum development. TIP2 and UDT1 play critical roles in the early stage of tapetal cell development. TIP2 interacts with UDT1 to form a heterodimer. The heterodimer binds to the E-box motif in the Os*UPEX1* and Os*UPEX2* promoters. *OsUPEX1/2* encodes putative galactosyltransferases and facilitates the secretion of callase from tapetal cells into the locule. By degrading the callose surrounding the tetrads, callase ensures the dissociation of the tetrads, thereby guaranteeing the development of male gametes.

Wang *et al.* identified a large number of differentially expressed genes through transcriptomic analysis of WT, *tip2*, and *udt1-2* spikelets at Stages 6 and 7. Gene Ontology analysis revealed that carbohydrate-active glycosyltransferases and glycosyl hydrolases were enriched among the shared downregulated genes in two mutants. Among these, two genes, designated *OsUPEX1* and *OsUPEX2*, are specifically expressed in the anther and encode putative β-(1,3)-galactosyltransferases. Phylogenetic analysis indicates that they are homologous to *UPEX1/KNS4/RES3* in Arabidopsis and *Male sterile8* (*Ms8*) in maize. In Arabidopsis, *UPEX1/KNS4/RES3*, which encodes an arabinogalactan β-(1,3)-galactosyltransferase, was initially identified as a key player in pollen wall development and later shown to be involved in callose degradation (Dobritsa *et al.*, 2011; Suzuki *et al.*, 2017; Wang *et al.*, 2022). *UPEX1/KNS4/RES3* is regulated by ABORTED MICROSPORE (AMS), the ortholog of TDR in Arabidopsis (Wang *et al.*, 2022). In maize, *Ms8* regulates both callose remodeling and tapetal cell development (Wang *et al.*, 2010, 2013). Wang *et al.* demonstrated that the TIP2–UDT1 complex directly binds to the E-box *cis*-elements in the promoter of *OsUPEX1* and *OsUPEX2* and activates their expression. While no obvious phenotypic differences were observed between WT and *osupex1* or *osupex2* single mutants, the *osupex1osupex2* double mutants exhibited complete male sterility, with anthers ceasing to grow after stage 8 and lacking pollen at maturity. In the *osupex1osupex2* double mutant, meiosis progressed normally with tetrads formation, but microspores collapsed shortly after meiosis. Additionally, tapetal cell structures differed significantly from those in WT from Stage 8. Specifically, the tapetum of the *osupex1-osupex2* double mutants swelled at Stage 8 and precociously degraded by late Stage 9. At Stages 8a and 8b, callose remodeling in *tip2*, *udt1-2*, and *osupex1-1 osupex1-2* mutants was impaired, with callose persisting throughout the locule instead of being confined to areas surrounding dyads and tetrads as in WT. These findings suggest that TIP2, UDT1, and OsUPEX1/OsUPEX2 may regulate the secretion of callase from the tapetum into the locule (Fig. 1). Furthermore, the colocalization of OsUPEX1/2 with Golgi markers and the appearance of OsUPEX1/2-GFP as dot signals in tapetal cells suggest that these proteins may be involved in protein glycosylation within the Golgi apparatus and are essential for the general secretory function of the tapetum.

This study fills gaps in our understanding of early anther development by elucidating the TIP2-UDT1-OsUPEX1/2 pathway. It underscores the critical role of OsUPEX1/2 in tapetum secretion, offering new perspectives on anther and pollen development. Furthermore, this study reveals the conserved role of OsUPEX1/2 and their orthologs in *Arabidopsis* (*AtUPEX1/KNS4*) and maize (*ZmMs8*), while also highlighting species-specific divergences in their roles in tapetal development and regulatory mechanisms. Despite these advances, this study raises several intriguing scientific questions, such as the need to identify the specific proteins modified by the glycosyltransferase OsUPEX1/2 and elucidate the mechanisms by which it regulates callase secretion.

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