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Journal of Genetics and Genomics 38 (2011) 379–390

JOURNAL OF GENETICS AND GENOMICS

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# Cytological analysis and genetic control of rice anther development

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Received 11 May 2011; revised 29 July 2011; accepted 1 August 2011

**Abstract**

Microsporogenesis and male gametogenesis are essential for the alternating life cycle of flowering plants between diploid sporophyte and haploid gametophyte generations. Rice (*Oryza sativa*) is the world's major staple food, and manipulation of pollen fertility is particularly important for the demands to increase rice grain yield. Towards a better understanding of the mechanisms controlling rice male reproductive development, we describe here the cytological changes of anther development through 14 stages, including cell division, differentiation and degeneration of somatic tissues consisting of four concentric cell layers surrounding and supporting reproductive cells as they form mature pollen grains through meiosis and mitosis. Furthermore, we compare the morphological difference of anthers and pollen grains in both monocot rice and eudicot *Arabidopsis thaliana*. Additionally, we describe the key genes identified to date critical for rice anther development and pollen formation.

*Keywords:* Rice (*Oryza Sativa*); Anther; Developmental stages; Cellular morphology; *Arabidopsis thaliana*

## 1. Introduction

Rice is one of the most important agricultural crops. Hybrid rice exhibits heterosis, or hybrid vigor, which is indicated by more rapid growth and considerablely higher yields than produced by the parental lines. Rice male sterility is frequently caused by environmental effects or genetic mutations, leading to defective anther development and pollen fertility. Since the female reproductive development remains normal in some male sterile lines, these lines can be fertilized by the pollen grains of other rice cultivars, greatly contributing to the production of hybrid seeds (Wilson and Zhang, 2009; Ouyang, et al., 2009, 2010). Furthermore, because of its small genome and high efficiency of transformation, rice also has been used as a model monocot plant for comparative studies with other model plants such as *Arabidopsis thaliana* (IRGSP, 2005; Jung et al., 2008).

Rice and other agriculturally important cereals including barley (*Hordeum vulgare*) and maize (*Zea mays*) belong to the grass family (Poaceae), which is one of the largest families in flowering plants (Linder and Rudall, 2005). As knowledge of its development can be extrapolated to other monocot crops, rice is useful not only as an excellent model plant for biological studies, but also as a model crop for agronomical improvement.

Evolutionary adaptations in the organization and structure of grass inflorescence (or panicle) have resulted in their distinct morphologies from those of core eudicots and non-grass monocots (Grass Phylogeny Working Group, 2001; Zanis, 2007). The rice inflorescence or panicle has a central stem that terminates after the generation of several primary and secondary branches (Fig. 1A). Spikelets are directly formed on primary and secondary branches that are attached on the main axis called the rachis (Itoh et al., 2005). Each rice spikelet contains a flower with one pistil, six stamens and two lodicules subtended by an inner bract or prophyll, called the palea, and an outer bract called the lemma (Fig. 1B) (Yuan et al., 2009b). Each stamen consists of a filament and an

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1673-8527/$ - see front matter 
doi:10.1016/j.jgg.2011.08.001

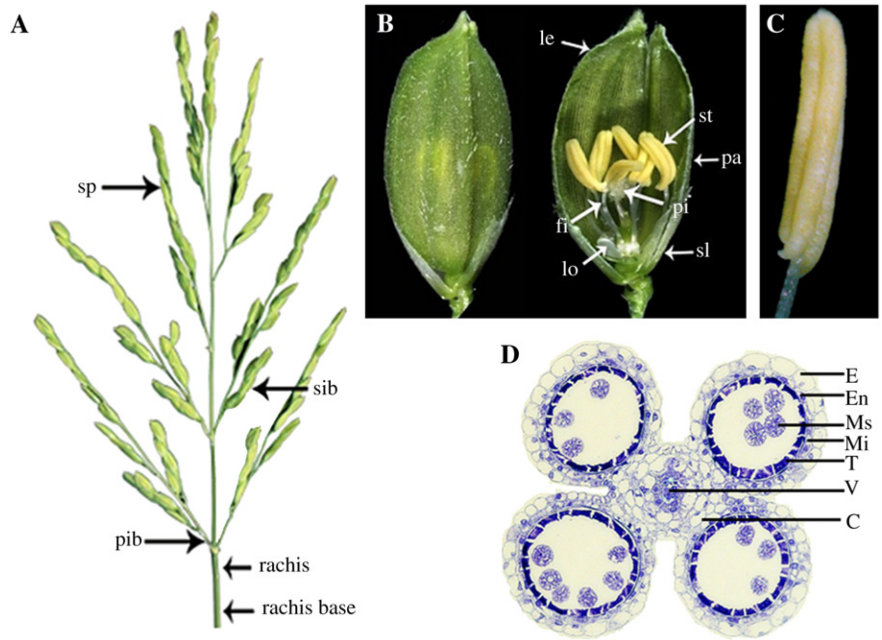


Fig. 1. Morphology of rice panicle, spikelet organs and anther section. **A:** a mature inflorescence of rice at the heading stage. **B:** a mature spikelet of rice with formation of ovule and pollen grains. **C:** a mature anther of rice. **D:** section of anther at stage 9 showing the anther wall layers, microspores in the locule as well as vascular tissues and connective tissues. Rachis, inflorescence axis; pib = primary inflorescence branch; sib = secondary inflorescence branch; sp = spikelet; pi = pistil; st = stamens; sl = sterile lemma; pa = palea; le = lemma; fi = filament; lo = lodicule; E = epeidermis; En = endothecium; T = tapetum; C = cavity for dehiscence; V = vascular bundle.

anther with four lobes linked to the filament by connective tissues (Fig. 1B). The rice anther includes two thecae linked by the connective tissue, and each theca contains two locules, one is longer at the base and the other is shorter. The two locules are connected by a septum and stomium (consisting of small epidermal cells), which are crucial for anther dehiscence (Fig. 1C) (Matsui et al., 1999).

Successful male reproductive development within the male organ, the anther, includes a number of critical developmental events such as meristem specification, cell differentiation, cell-to-cell communication, meiosis and mitosis (Scott et al., 2004, McCormick, 2004, Ma, 2005; Wilson and Zhang, 2009). The number of total protein-coding genes in rice has been estimated at 52,214 [RAP2 (Rice Annotation Project 2008) http://rapdb.dna.affrc.go.jp/] or 56,797 [MSU release 6.1 (Ouyang et al., 2007) http://rice.plantbiology.msu.edu/]. Transcriptome analyses in rice using staged anthers and pollen grains (or dissected tapetal cells/microspores) identified approximately 29,000 unique transcripts in the anther and male gametophyte (Hobo et al., 2008; Suwabe et al., 2008; Huang et al., 2009; Jiao et al., 2009; Fujita et al., 2010; Tang et al., 2010; Wang et al., 2010; Wei et al., 2010). This observation clearly indicates that variations in gene expression occur during anther development and pollen formation.

Several reports published on the developmental staging of rice anther contribute to our understanding of the whole male reproductive developmental process (Feng et al., 2001; Itoh et al., 2005; Zhang and Wilson, 2009). Feng et al. (2001) observed anther morphology in semi-thin sections and divided the anther developmental process into eight stages: microspore mother cell formation stage, microspore mother cell meiosis stage, early microspore stage, middle microspore stage, late microspore stage, early bicellular pollen stage, late bicellular pollen stage and mature pollen stage. Itoh et al. (2005) also described the rice anther development in eight stages, from An1 of archesporial cells (ACs) differentiation to An8 of mature pollen grain production, and meiosis in 12 stages. In *Arabidopsis*, anther development has been divided into 14 stages based on morphological features (Sanders et al., 1999; Ma, 2005). Recently, we analyzed the cellular changes of the rice anther (*O. sativa* ssp. *japonica* cv. 9522 and Zhonghua 11) using light microscopic observations of transverse sections and divided the rice anther developmental course into 14 stages (Zhang and Wilson, 2009), which was meant to be consistent with that of *Arabidopsis* (Sanders et al., 1999; Ma, 2005). Here, we further characterize the cellular changes during the 14 anther developmental stages by detailed analysis of semi-thin sections of *O. sativa* ssp. *japonica* cv. 9522, according to methods described by Li et al. (2006a). The recently reported key genes for rice anther development identified by mutant characterization are also discussed. In addition, we compare the divergent morphology of rice anther development with that of *Arabidopsis*.

## 2. Cytological analysis of rice anther development

Table 1 summarizes the key events that occur at all 14 stages of anther development. The key genes expressed in rice are listed as well as their homologous genes in *Arabidopsis*.

*Stage 1*: The stamen primordium is formed after cell divisions and differentiation of floral meristem. The anther primordium contains three layers, L1, L2, and L3 (Fig. 2, stage 1).