

Understanding a key gene for thermosensitive genic male sterility in rice

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Recently, a joint research team led by Chuxiong Zhuang of South China Agricultural University and Xiaofeng Cao of Institute of Genetics and Development Biology, Chinese Academy of Sciences published their work on the cloning and molecular characterization of the gene *thermosensitive genic male sterile 5 (tms5)* in rice in *Nature Communications* [1]. This is the result of a long-term collaboration representing an important advance in male sterility research in crops.

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. Hybrid rice has significantly increased the yield level compared to inbred varieties. In the past decades, China has been energetically developing and popularizing hybrid rice to meet the food demand of the growing population. The three-line system and two-line system were the two approaches widely used in hybrid rice breeding. The three-line system is composed of a male-sterile line, a restorer line and a maintainer line, while in the two-line system the male-sterile line also serves as the maintainer line for the propagation of male sterile seeds. The sterile male-sterile line used in two-line system becomes fertile under particular growth environmental conditions, which greatly simplifies the process of hybrid breeding and saves tremendous cost in seed production. Photoperiod-sensitive genic male sterile lines (PGMS) and thermo-sensitive genic male sterile lines (TGMS) have been the two main types used in two-line hybrid rice breeding. As the name implies,

the fertility of PGMS and TGMS are sensitive to photoperiod and temperature respectively. Specifically, it is sterile under restrictive conditions (long-day for PGMS and high temperature for TGMS) and fertile under permissive conditions (short-day for PGMS and low temperature for TGMS). Since 1993, two-line hybrid rice has been planted on a gradually increasing scale, which now occupies a large rice area in China.

Recently, the mechanisms of the major types of cytoplasmic male sterility and fertility restoration systems used in the three-line systems in rice have been elucidated [2]. But the genes controlling genic male sterility in the two-line system have not been cloned until the work by Ding et al. [3] who mapped and cloned a long non-coding RNA *LDMAR* (long-day-specific male-fertility-associated RNA) regulating photoperiod-sensitive genic male sterility in rice. The mutation of a single nucleotide between the wild type Nongken 58 and PGMS Nongken 58S increased the methylation level in the promoter region of *LDMAR*, which suppressed the transcription of *LDMAR* under long-day conditions, resulting in male sterility. Soon, Zhou et al. [4] also reported their work on the same locus and found the single nucleotide polymorphism (SNP) located in a 21-nucleotide small RNA *osa-smR5864w* produced by *LDMAR*. They speculated that the SNP may lead to loss-of-function of *osa-smR5864m* (the mutant small RNA corresponding to *osa-smR5864w*), causing PGMS and TGMS in the *japonica* and *indica* lines respectively.

So far, a number of genetic loci for thermo-sensitive

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genic male sterility had been identified in rice, such as *tms1*, *tms2*, *tms3(t)*, *tms4*, *tms5*, *tms6(t)*, *TGMS*, *tms6*, *tms9*, *tms9-1* and *rtms1*. Genetic analysis indicated that the TGMS genes at these loci are all recessive. In the work by Zhou et al. [4], transformation of their identified candidate gene *Os02g0214300* of *TMS5* to TGMS lines AnS-1 and ZhuS1 restored the fertility of the transgenic plants at restrictive temperature. They found that *TMS5* encodes a short form ribonuclease Z (RNase Z^S) referred to as RNase Z^{S1}, and a C-to-A transition at position 71 of *tms5* transcript created a premature stop codon of RNase Z^{S1}. There was no difference in the RNase Z^{S1} expression at the mRNA level and protein level under the permissive and restrictive temperature, which indicated that the RNase Z^{S1} is temperature insensitive and some other factors might be involved in the process of thermo-sensitive genic male sterility mediated by RNase Z^{S1}. Next, they tested the RNase Z^{S1} endonuclease activity and detected that RNase Z^{S1} processed the 3' end of tRNAs *in vitro* but not *in vivo*. Resorting to the analysis of whole genome microarray and RNA-seq, the researchers identified three mRNAs named *Ub_{L40}1*, *Ub_{L40}2* and *Ub_{L40}4*, whose genes belong to the conserved ubiquitin-60S ribosomal protein L40 family (*Ub_{L40}*), accumulating at higher level in the young panicles of *tms5* plants at high temperature than at low temperature. Overexpression of *Ub_{L40}1* and *Ub_{L40}4* in wild type ZH11 reduced the pollen fertility, while the *Ub_{L40}1* and *Ub_{L40}4* RNAi lines in plants containing the *tms5* gene showed increased pollen fertility at restrictive temperature. The mRNAs of the three *Ub_{L40}* genes could be processed into multiple fragments by RNase Z^{S1}. The au-

thors thus proposed a working model for *tms5*-mediated TGMS. In the TGMS line, the *Ub_{L40}* mRNA was expressed at high level at high temperature but could not be processed by dysfunctional RNase Z^{S1}, resulting in excessive accumulation of this mRNA leading to male sterility, while there was a relatively low level of *Ub_{L40}* mRNA expression at low temperature thus increasing male fertility. By contrast, in wild type, *Ub_{L40}* mRNA transcript was kept at low level by RNase Z^{S1} processing regardless of the temperature conditions.

This is the first TGMS gene cloned from a useful germplasm for hybrid rice production. The result may help future application in developing male sterile lines in hybrid rice breeding, in addition to enhanced understanding of male fertility development in plants.

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