RESEARCH HIGHLIGHT

Producing hybrid seeds like conventional rice

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Production of hybrid rice seed is laborious and costly, because the restorer lines providing pollen need to be removed before seed harvesting to avoid contamination of undesired inbred seeds. In a recent *Cell Research* paper, Li et al. report that a spontaneous mutation in *ARGONAUTE7* could lead to thermo-sensitive female sterility, which enables fully mechanized hybrid seed production in rice.

Owing to hybrid vigor or heterosis, the heterozygous F_1 offspring displays > 20% yield increase over their parental lines in rice.[1,2] However, how to prevent unwanted self-pollination becomes a major challenge for production of hybrid seeds. Thanks to the milestone discovery of rice cytoplasmic male-sterile lines and development of a three-line system in 1970s, hybrid rice seeds can be produced efficiently, and hybrid rice has greatly contributed to the worldwide food security ever since.[3,4] Still, the production of hybrid seeds is laborious and costly, because the restorer lines planted near the male-sterile lines as pollen donors need to be removed before harvesting the hybrid seeds (Fig. 1).

To solve this problem, it has been considered to use female-sterile lines as pollen donors, and thus the female-sterile lines need to be environment sensitive to maintain the restorer lines. Owing to these strict limits, the environment-sensitive female-sterile line is long desired but hardly reported. In a recent paper in *Cell Research*, Li et al. for the first time identified a spontaneous thermo-sensitive female sterility mutant *tfs1*, cloned the *TFS1* gene, which encodes ARGONAUTE7 (AGO7), and revealed that the female sterility was due to the reduction of tasiR-ARF biogenesis caused by defective RNA-Induced Silencing Complex formation in *tfs1*, which could be partially restored under low temperature.[5]

The genetic studies showed that *tfs1* was recessive, and the genetic complementation could rescue the female sterility phenotype. Argonaute proteins functioned in association with Dicer-like proteins to produce small RNAs including microRNAs (miRNAs) and small interfering RNAs (siRNAs) in plants. Specifically, AGO7 loads miR390 to target non-coding *TRANS-ACTING SIRNA3* (*TAS3*), recruits SGS3 and RDR6, and produces phasiRNAs such as tasiR-ARF, one species of the phasiRNAs that targets auxin-responsive factors.[6] In this study, Li et al. further found that the miR390 and miR390* were both accumulated in *tfs1*, but the levels of *TAS3*-derived siRNAs, including tasiR-ARFs and non-tasiR-ARF siRNAs, were both reduced, indicating that tasiR-ARF biogenesis was inhibited in *tfs1*. Furthermore, Li et al. revealed that compared with the wild-type TFS1/AGO7, the mutated TFS1 (mTFS1) was less efficient in binding miR390/miR390*, defective in miR390* ejection, more stable at the protein level, and more effective in cleaving *TAS3b* RNA, but showed a reduced ability to recruit RDR6, which may lead to the decrease in tasiR-ARF biogenesis in the *tfs1* mutant.

Previous reports showed that strong *ago7* mutations in rice could abolish tasiR-ARF biogenesis, and *ago7* mutant plants exhibit strong defects in shoot apical meristem maintenance and are often seedling lethal.[6] Here, the *tfs1* mutant exhibited no obvious phenotype before pollination. The 603 Leu-to-Ile substitution in mTFS1 seems to be a precious rare mutation, and this weak allele could maintain normal vegetative development with low levels of tasiR-ARFs, but low enough to cause female sterility. More interestingly, the authors generated two *ago7* mutants by CRISPR/Cas9, showing that deletion of serine 605 led to female sterility but could not be rescued by low temperature, and 603 Leu-to-Ala mutation had no effect on fertility, indicating that the Leu603Ile natural allele was unique in causing thermo-sensitive female sterility. Consistent with this, under low temperature RDR6 was better recruited by mTFS1, which partially rescued the activity of miR390-mTFS1 and increased tasiR-ARF biogenesis.

To test whether utilizing *tfs1* allele in restorer lines could truly enable mechanized hybrid seed production, Li et al. knocked out the endogenous *TFS1* and introduced *tfs1* in both *indica* and *japonica* backgrounds, and found that introduction of the *tfs1* allele in both backgrounds led to thermos-sensitive female sterility. Furthermore, they performed field trial by transplanting each of four commercial male-sterile lines mixed with their corresponding restorer lines carrying the *tfs1* allele, and obtained a high seed-setting rate of hybrid panicles, fully proving its potential in mechanized hybrid seed production.

The identification of female-sterile lines by this study enables a new working system for hybrid rice seed production (Fig. 1). By utilizing the female sterility in the restorer line, the restorer line will have no self-pollination and no longer needs to be removed. Moreover, this allows random transplantation of male-sterile line and restorer line. It should be pointed out that this system requires breeding of both parental lines with a similar heading date. For example, Minghui63 and Zhenshan97A, two parents of Xianyou63, a hybrid rice variety with historically largest total cultivating area in China, have nearly one-month difference in heading date, which needs to be adjusted first to use this system.

Besides the female sterility system, other strategies have also been proposed and pioneered. The genetic basis of heterosis in rice has been extensively studied, which has led to the identification of a series of dominance and overdominance genomic loci in the elite parents of hybrid rice varieties, explaining the yield advantage of hybrids.[1] Therefore, it is possible to generate conventional inbred rice varieties with high yield by

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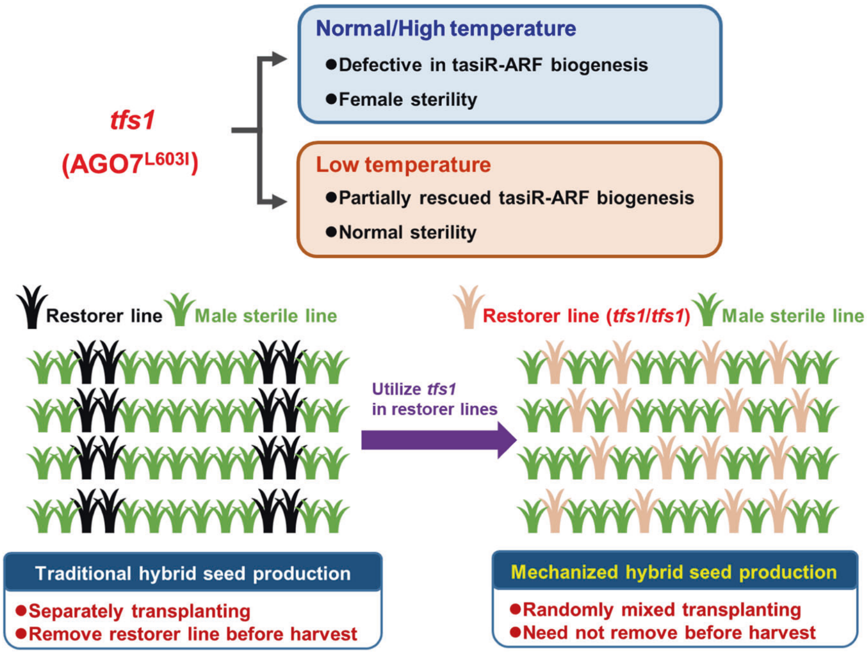

Fig. 1 Fully mechanized hybrid seed production by utilization of tfs1 in restorer lines. A spontaneous mutation of *thermo-sensitive female sterility 1* (*tfs1*) in *AGO7* led to female sterility under normal or high temperature, which can be rescued at low temperature. In hybrid rice seed production, the restorer lines need to be removed before harvest, and they have to be transplanted separately. By utilizing *tfs1* in restorer lines, the restorer lines do not need to be removed and can be transplanted randomly with male sterile lines, enabling a fully mechanized production.

pyramiding the beneficial dominance loci and creating novel alleles of overdominance loci.[7,8] On the other hand, genome editing of key genes could substitute mitosis for meiosis and induce haploid seeds in rice, which could achieve clonal propagation through seeds, enabling the offspring plants to retain the parental heterozygosity.[9,10]

In summary, the work by Li et al., 2022[5] has made a breakthrough by identifying the first thermo-sensitive female sterility gene that does not cause defects in vegetative or male reproductive development, which paves a new path for fully mechanized hybrid seed production in rice. It would be interesting to investigate how low temperature could rescue the defect caused by this unique allele of *TFS1*, which may serve as a temperature sensor for synthetic biology. The findings in this study also demonstrated that creation of novel weak alleles of genes with severe phenotype may get a big surprise.

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ADDITIONAL INFORMATION

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