

## Analyzing and overcoming the reproductive barrier between interspecific crosses of rice

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The efficiency of breeding depends on the amount of genetic variations accumulated in a population. However, in general, severe abnormality occurs in/after the hybridization process when two individuals that are genetically diverged from each other are used as parents. Such abnormalities are regarded as the internal reproductive barrier, which maintains biological species and prevents the efficient utilization of relatives of crop in breeding. Therefore, overcoming the reproductive barrier has been a central issue in history of breeding study.

In rice, severe hybrid sterility has frequently been observed when a plant was crossed with the one from closely related species. These observations suggested that hybrid sterility is one of the main reproductive barriers in the genus rice. The African cultivated rice species, *Oryza glaberrima*, which is highly tolerant to biotic and abiotic stresses, is considered as valuable genetic resources for improving the Asian cultivated rice species (*O. sativa*). However, hybrids between these two species show severe pollen and seed sterility as observed in other diverged rice crosses. In 1970's, a locus for hybrid sterility (the *SI* locus) was reported [1]. The *O. glaberrima*-derived allele (denoted  $SI^g$ ) on the *SI* locus causes preferential abortion of gametes with its allelic alternative (denoted  $SI^s$ ) in  $SI^g/SI^s$  heterozygotes [2]. However, its genetic mechanisms were unknown.

Here, I used mutagenesis to disrupt the function of the *SI* gene and found a peptidase-coding gene is involved in this phenomenon [3]. A mutant was obtained by screening fertile hybrids after irradiation of heavy-ion beam. The mutant was considered to have a new allele,  $SI^{mut}$ , which does not confer sterility in the  $SI^{mut}/SI^g$  and  $SI^{mut}/SI^s$  hybrids. By genetic mapping and transformation experiments, I confirmed that the causal mutation of the  $SI^{mut}$  allele was a deletion in the peptidase-coding gene (denoted “*SSP*”) in the *SI* locus of *O. glaberrima*. I also analyzed the evolutionary pathways of *SSP* and found that the gene is present only in the African rice species and some other wild species, not in the Asian one. This signifies the gene was acquired or lost in certain evolutionary pathways to maintain species' boundaries. These studies demonstrated that artificial disruption of a gene for the reproductive barrier creates a “neutral” allele, which facilitates interspecific hybridization for breeding programs. Further research could help improve breeding programs and enhance rice yields to address food shortages in growing populations.

[1] Sano Y., et al.: Genetic studies of speciation in cultivated rice, 1. Genic analysis for the F1 sterility between *O. sativa* L. and *O. glaberrima* Steud. Jpn J Genet 54: 121–132 (1979).

[2] Koide Y., et al.: Sex-independent transmission ratio distortion system responsible for reproductive barriers between Asian and African rice species. New Phytol 179: 888–900 (2008).

[3] Koide Y., et al.: Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. Proc Natl Acad Sci USA 115: E1955–E1962 (2018).