Recent progress in overcoming interspecific hybrid sterility in rice

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Inter-specific hybridization enables breeders to transfer valuable genes from one species to another for improving crops. However, reproductive barriers, which are also known as "species barriers," often prevent gene flow between two species. In two cultivated rice species (*Oryza sativa* and *O. glaberrima*), hybrid sterility is considered as the main reproductive barrier. To date, more than 10 loci for hybrid sterility between these species have been found.[1)] Among these loci, the *HYBRID STERILITY 1* (S_1) locus on the short arm of chromosome 6 has been frequently detected,[2)] suggesting that the S_1 locus is the major cause of the sterility barrier. Here, we review the recent progress in overcoming the hybrid sterility caused by the S_1 locus and highlight the usefulness of a forward genetic screening for a mutant with a "neutral" allele of hybrid sterility loci.

In 1990, Sano[3)] showed that the hybrid between a strain of *O. sativa* and near-isogenic lines (NILs) containing a segment of chromosome 6 from *O. glaberrima* in the genetic background of *O. sativa* exhibited partial sterility in pollen and seeds. This phenomenon was explained by the genetic interaction between the S_1^g allele (formerly the S_1 allele of Sano[3)]) and S_1^s allele (formerly the S_1^a allele of Sano[3)]), which were derived from *O. glaberrima* and *O. sativa*, respectively. The S_1^g allele acts as a "gamete eliminator," and both male and female gametes possessing the S_1^s allele are aborted only in the heterozygote (S_1^g/S_1^s).[4)] Although these studies revealed the genetic nature of the S_1 locus, it was still unclear how we can overcome the sterility barrier.

Recently, significant progress has been made by artificial mutagenesis using genome editing[5)] or heavy-ion beam irradiation.[6)] Koide *et al.*[6)] used Acc108 (a variety of *O. sativa*) and NIL, which contain the S_1^s allele and the S_1^g allele at the S_1 locus, respectively. They obtained a total of 2,478 F_1 seeds for heavy-ion beam irradiation via artificial pollination. From 1,817 F_1 hybrids (M_1 generation) irradiated with carbon-ion beam (LET 30 keV/nucleonm, 150 Gy) at the RIKEN RI-beam factory, Wako, Japan, they obtained one plant that had a panicle with >50% seed fertility from the M_1 population (Fig. 1). Then, they developed the M_2 family through self-pollination of the M_1 plant and obtained the mutant Acc108S_1M, which does not induce

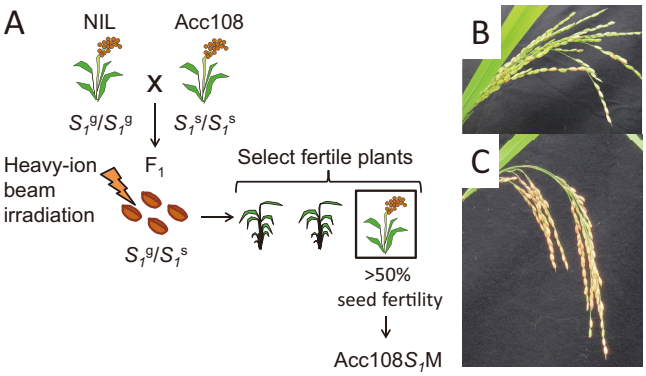

Fig. 1. Forward genetic screening for a mutant with a "neutral" allele of the hybrid sterility locus. (A) The scheme for selecting a mutant with a neutral allele at the hybrid sterility locus, S_1. (B) A sterile rice panicle observed in the M_1 plant. The seed sterility was due to the hybrid sterility locus, S_1. (C) A fertile panicle observed in the M_1 plant. Seed sterility did not occur because of a mutation induced at the S_1 locus.

sterility in either the hybrid with the S_1^g carrier or that with the S_1^s carrier.

The results of the crossing experiments, genetic mapping, and nucleotide sequencing suggested that the causative mutation of Acc108S_1M was a 5-bp deletion in the peptidase-coding gene (denoted by *SSP*) in the S_1 locus. These results indicated that the *SSP* was one of the essential genes for inducing hybrid sterility in heterozygotes (S_1^g/S_1^s) and Acc108S_1M has a neutral allele "S_1^{mut}" at the S_1 locus.[6)]

In summary, recent studies[5,6)] have focused on the S_1 locus and have shown how to obtain a neutral allele through reverse or forward genetic approaches. In general, the number of hybrid sterility loci that have been annotated and characterized in crop gene pools is still limited. In such a case, a forward genetic screening is more practical than approaches in which gene identification is a prerequisite, *e.g.*, genome editing, for creating neutral alleles. Although these two approaches have their own specific advantages, recent studies have demonstrated a technique that allows broader access to desirable traits in distantly related species during crop breeding.

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