

Molecular mechanisms of hybrid sterility in rice

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Hybrid sterility presents a major bottleneck in hybrid crop breeding and causes postzygotic reproductive isolation in speciation. Here, we summarize the current understanding of the genetics of rice hybrid sterility and highlight new advances in deciphering the molecular basis of the major genetic loci for hybrid sterility in rice. We also discuss practical strategies for overcoming reproductive barriers to utilize hybrid vigor in inter-specific and inter-subspecific hybrid rice breeding.

speciation, reproductive isolation, heterosis, hybrid sterility, rice

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Introduction

Hybrids between evolutionarily divergent species or subspecies often show reduced fitness, such as sterility, weakness, and inviability. The syndromes are referred to as hybrid incompatibility. Inter-specific and inter-subspecific hybrid sterility (HS) is a common mechanism for postzygotic reproductive isolation, which reduces gene flow among species/subspecies during speciation; therefore, the causal genes for HS also are called speciation genes. As proposed by the Bateson-Dobzhansky-Muller model, hybrid incompatibility is caused by deleterious interactions between divergent alleles of certain genetic loci in the parental species or subspecies (Bateson, 1909; Dobzhansky, 1937; Muller, 1942).

Cultivated rice consists of two species, Asian rice (*Oryza*

sativa L.) and African rice (*Oryza glaberrima* Steud). Asian rice is further divided into two subspecies, Xian/*indica* rice and Geng/*japonica* rice (Kato et al., 1928; Huang et al., 2012). The development and application of hybrid rice since the 1970s, which increased grain yield by about 20% compared to inbred varieties, has contributed greatly to the food supply in China (Cheng et al., 2007; Yuan, 2014). However, current hybrid rice varieties are mainly based on crosses between lines of the same subspecies (i.e., *indica* or *japonica*), and the yields of these hybrid rice varieties have reached a plateau due to the relatively narrow genetic diversity between the parental lines. Hybrid rice varieties between the different subspecies (*japonica* and *indica*) and the species (*O. sativa* and *O. glaberrima*) have much stronger hybrid vigor (heterosis), thus holding great promise for further increasing yield potential (Chen and Liu, 2014; Qian et al., 2016).

HS occurs widely in hybrids between the Asian and

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African rice species, between the *indica* and *japonica* sub-species, and between the cultivated rice and wild rice species. The distant hybrids exhibit severe sterility and very low seed setting rates (Figure 1A), therefore, HS is a major barrier for utilization of the strong heterosis in distant hybrid rice breeding. Genetic analysis has reported more than 50 HS loci conferring male and/or female sterility in inter-specific and inter-subspecific hybrids in rice (Ouyang and Zhang, 2013; Ouyang et al., 2016), and in recent years a number of HS genes have been cloned. In this mini-review, we update current genetic models for rice hybrid sterility and summarize recent advances in understanding the molecular genetic mechanisms of major HS loci in rice.

Genetic models for hybrid sterility in rice

Two major models have been proposed to explain the genetics of rice HS: the one-locus sporo-gametophytic interaction model (one-locus allelic interaction model) and the duplicate gametophytic lethal model (two-locus model) (Oka, 1957, 1974). Of the ten known HS loci or locus pairs in rice (Table 1), the genetics of seven loci (*Sa*, *S5*, *HSA1*, *S7*, *SI*, *Sc*, and *qHMS7*) follow the one-locus model (Long et al., 2008; Chen et al., 2008; Yang et al., 2012; Kubo et al., 2016; Yu et al., 2016; Xie et al., 2017a; Shen et al., 2017; Koide et al., 2018), and three pairs (*S27/S28*, *DPL1/DPL2*, and *DGS1/DGS2*) follow the two-locus model. Moreover, since multiple HS loci are often present in inter-specific and inter-subspecific hybrids, the cumulative effect of multiple HS loci results in very low pollen and spikelet fertilities, as widely observed in hybrids of most typical *indica-japonica* and Asian-African rice crosses (Figure 1A).

In an example of the one-locus allelic interaction model, the hypothetical HS-related locus *S* diverged into genetically incompatible alleles, S^+ in a species (or sub-species) and S^- in another species (or sub-species). In hybrids between these species (or sub-species), an incompatible genetic interaction between S^+ and S^- occurs in sporophytic cells, which results in selective abortion of male or female gametes (or both male and female gametes) carrying a certain allele (S^-) but not the other allele (S^+) (Oka, 1957, 1974). This allele-specific gamete abortion causes semi-sterility (sterility of near 50% pollen grains or spikelets) in hybrids between near isogenic lines with this divergent HS locus (Figure 1B), and exhibits segregation ratio distortion in the hybrid progenies (Figure 2). Because the S^+ allele from a parent can eliminate the S^- allele from another parent in their offspring and shows its transmission advantage over generations, the S^+ type alleles are also called selfish genetic elements.

The duplicate gametophytic lethal model can be explained with a hypothetical original gene *F*, which is essential for fertility (Figure 3). During speciation or sub-speciation,

duplication of *F* on another chromosome produced a new, non-allelic gene copy F' . During evolution, reciprocal loss-of-function mutations of *F* and F' occurred (generating defective *f* and f' alleles, respectively) in divergent populations or different species, which then carry the genotypes $FF/f'f'$ and $ff/F'F'$, respectively. The $Ff/F'f'$ hybrids will segregate 25% sterile ff' -gametes that lack functional *F* and F' genes (Figure 3). This type of HS is reported in hybrids between the wild rice *Oryza glumaepatula* and *japonica* rice for the locus pair *S27/S28* encoding a mitochondrial ribosomal protein (Yamagata et al., 2010), between *indica* and *japonica* rice for *DPL1/DPL2* encoding a plant-specific small protein (Mizuta et al., 2010), and between *japonica* rice and the wild rice *Oryza nivara* for *DGS1/DGS2* encoding a homolog of the DNA-dependent RNA polymerase III subunit C4 (Nguyen et al., 2017).

Molecular genetic mechanisms of hybrid sterility loci in rice

The *Sa* locus was the first characterized *indica-japonica* hybrid male sterility (HMS) locus (Long et al., 2008). Genetic fine-mapping showed that the *Sa* locus consists of two adjacent genes, *SaF* and *SaM*, and *Sa*-HMS involves three divergent genetic components: SaF^+ (the *indica* allele), SaM^+ (the *indica* allele), and SaM^- (the *japonica* allele) (Long et al., 2008; Xie et al., 2017b). Genetic evidence and gene transformation revealed that SaF^+ , SaM^+ , and SaM^- cooperate to determine the selective abortion of male gametes carrying SaM^- in the hybrid plants, thus causing a transmission advantage for the SaF^+ and SaM^+ alleles (Long et al., 2008). Sequencing analysis found that the *Sa* alleles of *indica* and *japonica* rice differed by single nucleotide polymorphisms: a G-to-T mutation in SaM^- produced a premature stop codon, resulting in a small C-terminal truncation of the encoded small ubiquitin-like modifier (SUMO) E3 ligase. In SaF^+ (the *japonica* allele), a T-to-C mutation causes an amino acid substitution (Phe²⁸⁷ to Ser²⁸⁷) in the encoded F-box protein. SaF^+ , SaM^+ , and SaM^- from the divergent alleles interact to form a three-component complex, which triggers sterility in male gametes carrying SaM^- ; the lack of any one of these components results in failure to produce male sterility (Long et al., 2008). This two-gene/three-component interaction model provided an explanation for the longstanding question of how an *indica* allele eliminates a *japonica* allele in the hybrids.

The *Sc* locus is another well-known *indica-japonica* HMS locus conferring selective abortion of the male gamete harboring the *japonica* *Sc* allele (*Sc-j*) in hybrid (*Sc-i Sc-j*) plants also carrying the *indica* allele *Sc-i* (Yang et al., 2004). Our study found that the *Sc-j* allele in *japonica* rice encodes a novel DUF1618 protein (Shen et al., 2017). By contrast, the

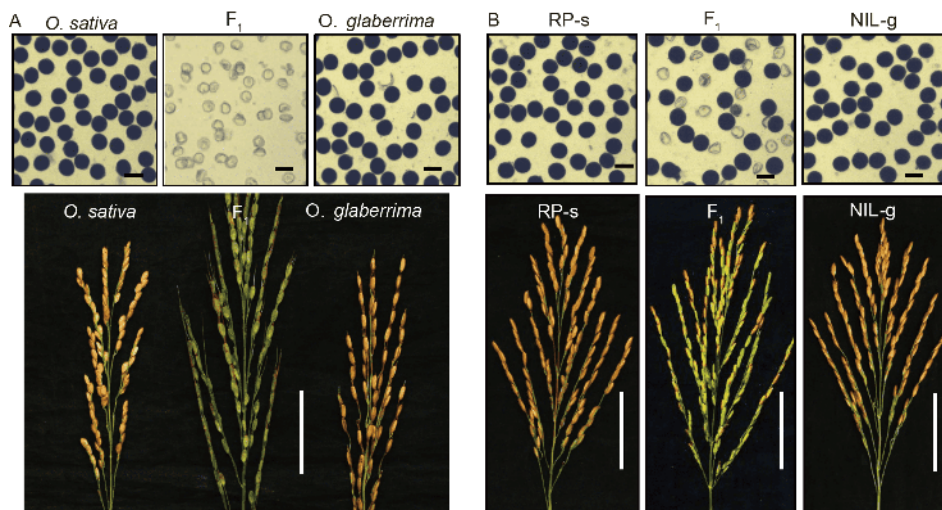


Figure 1 Hybrid sterility in rice. A, Representative pollen and spikelet sterilities of an F_1 hybrid between Asian rice (*japonica*) and African rice cultivars. The presence of multiple HS loci in the interspecific hybrid resulted in cumulative HS effect, thus producing nearly complete pollen and spikelet sterilities (failing to produce filled grains). Sterile pollen grains were empty and sterile spikelets show green color. Scale bars (in A and B), 50 μm for pollen and 10 cm for panicles. B, An example to show typical semi-sterilities (about 50% sterility) of pollen and spikelets conferred by the locus *Sl*, which controls both male and female HS, in an F_1 hybrid between a *japonica* recurrent parent line (RP-s, with the *japonica* allele *Sl-s*) and its near isogenic line (NIL-g, carrying the African rice allele *Sl-g*).

Table 1 Cloned genes for major HS loci in hybrids between species or subspecies in the *Oryza* genus^{a)}

Locus	Cross	Sterility	Gene structure	Allelic variation	SGE	Protein	Reference
<i>S27/S28</i>	<i>glu/As</i>	Male	Duplicated genes <i>mtRPL27</i> & <i>mtRPL28</i>	Translocation/reciprocal loss-of-function	–	Mitochondrial ribosomal protein L27	Yamagata et al., 2010
<i>DPL1/DPL2</i>	<i>ij</i>	Male	Duplicated genes <i>DPL1</i> & <i>DPL2</i>	Translocation/reciprocal loss-of-function	–	Plant-specific small protein	Mizuta et al., 2010
<i>DGS1/DGS2</i>	<i>niv/j</i>	Male	Duplicated genes <i>DGS1</i> & <i>DGS2</i>	Translocation/reciprocal loss-of-function	–	RNA polymerase III subunit C4 homolog	Nguyen et al., 2017
<i>Sa</i>	<i>ij</i>	Male	Two adjacent genes <i>SaF</i> , <i>SaM</i>	SNPs in <i>SaF</i> & <i>SaM</i>	<i>Sa-i</i>	F-box protein, SUMO E3 ligase	Long et al., 2008; Xie et al., 2017b
<i>S5</i>	<i>ij</i>	Female	Adjacent genes <i>ORF3</i> , <i>ORF4</i> , & <i>ORF5</i>	SNPs/InDels in <i>ORF3</i> , <i>ORF4</i> & <i>ORF5</i>	<i>S5-i</i>	HSP70, transmembrane protein, aspartic protease	Chen et al., 2008; Yang et al., 2012
<i>HSA1</i>	<i>ij</i>	Female	Two adjacent genes <i>HSA1a</i> & <i>HSA1b</i>	SNPs/InDels in <i>HSA1a</i> & <i>HSA1b</i>	<i>HSA1-j</i>	DUF1618 protein, unknown protein	Kubo et al., 2016
<i>S7</i>	<i>j/Au</i> , <i>i/Au</i>	Female	Single gene <i>ORF3</i>	SNPs in <i>ORF3</i>	<i>S7-ai</i>	TPR protein	Yu et al., 2016
<i>Sl</i>	As/Af	Male & female	Three adjacent genes <i>OgTPR1</i> , <i>SSP</i> & <i>SIA4</i>	SNPs in <i>OgTPR1</i> & <i>OsTP1</i> ; PA in <i>SSP</i> & <i>SIA4</i>	<i>Sl-g</i>	Trypsin-like peptidase, peptidase, unknown protein	Xie et al., 2017a; Koide et al., 2018; Xie et al., 2019
<i>Sc</i>	<i>ij</i>	Male	Single copy <i>Sc-j</i> and duplicated copies of <i>Sc-i</i>	Structure/copy number variations	<i>Sc-i</i>	DUF1618 protein	Shen et al., 2017
<i>qHMS7</i>	<i>mer/As</i>	Male	Two adjacent genes <i>ORF2</i> & <i>ORF3</i>	SNPs in <i>ORF2</i> ; PA in <i>ORF3</i>	<i>qHMS7^d</i>	RIP protein, mitochondrial protein	Yu et al., 2018

a) *ij*, *indica/japonica*; *glu/As*, *O. glumaepatula/Asian rice*; *niv/j*, *O. nivara/japonica rice*; *j/Au*, *japonica/Aus rice*; *i/Au*, *indica/Aus rice*; *As/Af*, Asian rice/African rice; *mer/As*, *O. meridionalis/Asian rice*; SNP, single nucleotide polymorphism; InDel, insertion/deletion; PA, presence/absence polymorphism; SGE, selfish genetic element.

complicated genomic structure of *Sc-i* consists of two or three tandemly-duplicated fragments (28 kb in length), each harboring a DUF1618-coding sequence (*Sc-ia*, *Sc-ib1*, or *Sc-ib2*) homologous to *Sc-j*. However, these *Sc-i* gene copies have an upstream/promoter region (23.9 kb) that differs completely from that of the *Sc-j* gene, indicating that the *Sc-i* genes were derived from recombinant events. These varied

genomic structures resulted in different expression patterns and levels between *Sc-j* and *Sc-i*. The *Sc-j* allele is expressed at low levels specifically in pollen during the early-bicellular stage. However, the *Sc-i* allele is expressed at higher levels in various developmental stages of anthers. The DUF1618 protein encoded by *Sc* is essential for pollen development, and knock-down of *Sc-j* leads to gametophytic sterility.

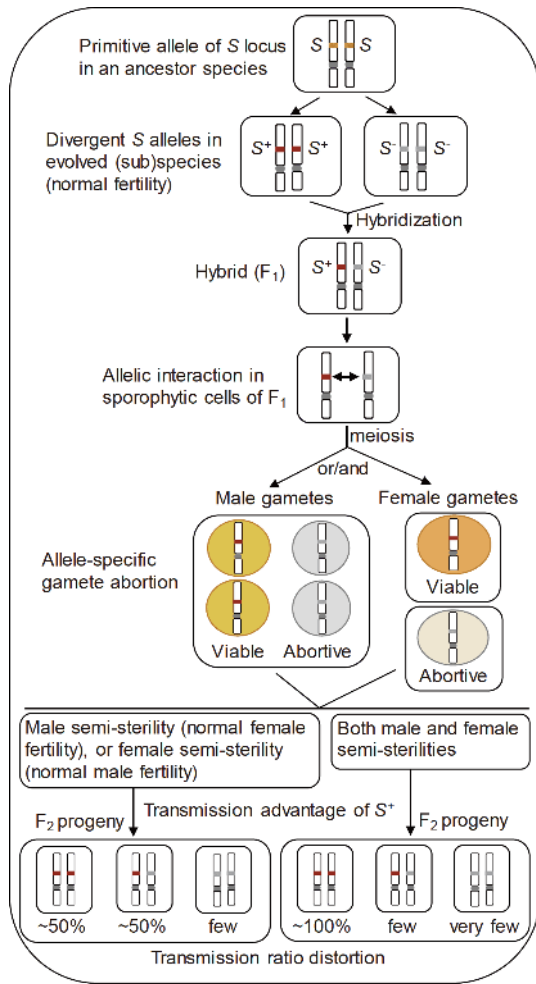


Figure 2 A genetic model for the allelic divergence of a hypothetical HS locus and the sporophytic-gametophytic interaction causing hybrid sterility. The hybrid sterility-related hypothetical locus *S* in the ancestor species differentiated into genetically incompatible alleles, *S*⁺ and *S*⁻, in evolved species/subspecies. In the hybrid with *S*⁺ and *S*⁻, a detrimental interaction between them in sporophytic cells induces a sterility signal to specifically abort most (if not all) of the male or female gametes (or both male and female gametes) carrying *S*⁻, while gametes with *S*⁺ are restored to fertile, thus causing male or female (or both) semi-sterility in hybrids between single-locus near isogenic lines. Therefore, the segregation of the genotypes of the locus in the *F*₂ progenies are distorted from Mendel's law (1:2:1 ratio). In this case, the *S*⁺ allele acts as a selfish genetic element.

Moreover, *Sc-j* expression is selectively suppressed by the strong expression of *Sc-i* in the *Sc-jSc-i* hybrids, thus causing selective abortion of the *Sc-j* pollen. Reduction of the *Sc-i* allele dosage by knocking out one or two of the *Sc-i* copies rescued *Sc-j* expression in the hybrids, thus restoring the fertility of the *Sc-j* pollen.

Based on these findings, an allelic suppression model was proposed for *Sc*-mediated HMS. In this model, repression of *Sc-j* by the high gene dosage of *Sc-i* in sporophytic cells (e.g., pollen mother cells) induces deposition of repressive epigenetic modifications in the *Sc-j* promoter. This putative epigenetic signature may be retained by the male gametophytes through meiosis, silencing of *Sc-j* and causing the

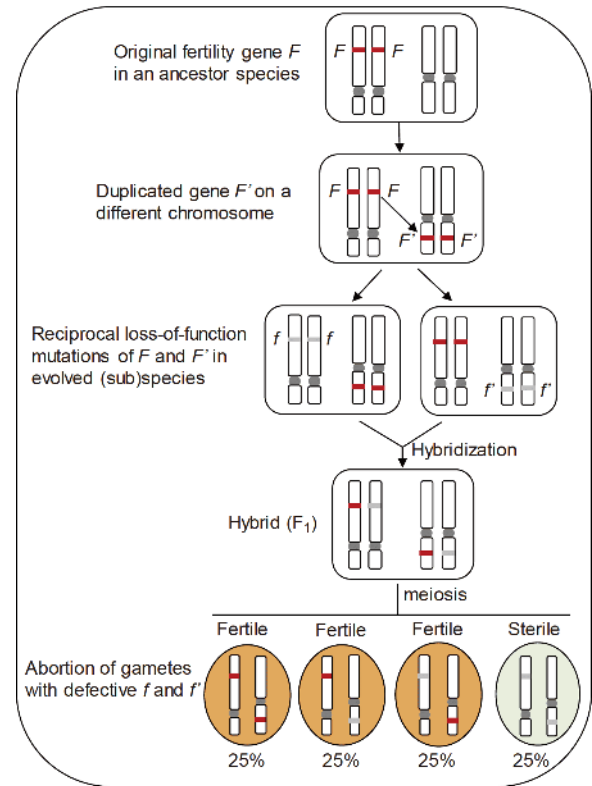


Figure 3 A genetic model for duplication/reciprocal loss-of-function mutations of hypothetical fertility genes that cause hybrid sterility. The hypothetical fertility-essential gene *F* was transferred to a different chromosome, followed by reciprocal loss-of-function mutations (*f* and *f'*) of *F* and *F'* in the evolved different species or subspecies. In the hybrid, meiotic segregation of the chromosomes carrying *F*, *f*, *F'*, or *f'* produces gametes with various genotypes of the alleles; those with *f* and *f'* but lacking either *F* or *F'* (accounting for 25%) are sterile.

selective abortion of *Sc-j* male gametes (Shen et al., 2017). In addition, alteration of the *Sc-i* promoter prevents its epigenetic repression, thus making the *Sc-i* pollen viable in the hybrids. Thus, *Sc-i* acts as both a *Sc-j* pollen killer and a self-protector in this HMS system. Therefore, in this system, genomic structural and copy number variations are important mechanisms for controlling HMS.

The *SI* locus is the most important HS locus in hybrids between *O. sativa* and *O. glaberrima*, in which the *O. glaberrima* allele *SI-g* causes selective abortion of both male and female (embryo-sac) gametes carrying the *O. sativa* allele *SI-s*, thus resulting in a severe transmission advantage to *SI-g* (Koide et al., 2008; Garavito et al., 2010). Our recent study mapped *SI* to a 55-kb region (Xie et al., 2017a). This region has complicated genomic structural variations with several large segmental insertions/deletions between *O. sativa* and *O. glaberrima*. In the *SI-g* region, we identified a candidate gene named *OgTPRI* (*Trypsin-like Peptidase and Ribosome Biogenesis Regulatory Domain Protein 1*). In the *SI-s* region, a C-to-A nucleotide variation in the allelic gene (named *OsTPI*) causes a premature stop codon, thus en-

coding a truncated protein (OsTP1) that contains only a trypsin-like peptidase domain. Knockout of *OgTPR1* at *SI-g* by CRISPR/Cas9 generated *ogtpr1* mutant lines, which had normal male and female fertility, suggesting that *OgTPR1* is not required for gamete development. However, in crosses of the *ogtpr1* lines with a *japonica* line, the hybrids showed normal male and female fertility, indicating that the loss of function of *OgTPR1* could eliminate the *SI*-mediated HS. Another *SI* locus-specific peptidase domain-containing protein gene (*SSP*) is also required for *SI*-mediated HS; a mutation of *SSP* in the *SI* heterozygotes rescued the male and female fertility (Koide et al., 2018). Recently, we found that a third new gene *SIA4* locating nearby *OgTPR1* (renamed as *SITPR*) functions with *SITPR* and *SSP* (renamed as *SIA6*) to form a complex that triggers sterility signal in sporophytic meiotic cells, while *SITPR* alone can rescue gamete fertility in a gametophytic manner, revealing a novel gamete killer-protector system (Xie et al., 2019).

Complex multi-gene loci are common for HS in rice

The finding that the genetically mapped single locus *Sa* is in fact a complex multi-gene locus that contains two interacting genes, *SaF* and *SaM*, for HMS (Long et al., 2008) has important implications for the studies of other HS loci. As shown in Table 1, later studies showed that, of the seven cloned HS loci that follow the one-locus model, six are multi-gene loci. For example, the *S5* locus, which confers female semi-sterility in *indica-japonica* hybrids, consists of three adjacent genes *ORF3*, *ORF4*, and *ORF5* (Yang et al., 2012). The genotypes of the *indica* and *japonica* *S5* alleles are *ORF3+*/*ORF4-*/*ORF5+* and *ORF3-*/*ORF4+*/*ORF5-*, respectively, where “+” indicates a functional form and “-” a defective mutant form. These three genes function together in a killer-protector system. In this system, the putative aspartic protease encoded by *ORF5+* from the *indica* allele, with the aid of the protein encoded by *ORF4+* from the *japonica* allele, functions in the sporophytes of the hybrids as a female gamete killer. The *ORF3+*-encoding product (a HSP70 protein) from the *indica* allele functions as a protector to eliminate the sterility effect in the female gametes containing the *indica* *ORF3+* allele (Yang et al., 2012).

A recent report on the *qHMS7* locus for HMS between a wild rice (*O. meridionalis*) and Asian cultivated rice shows that this locus, like *Sa*, contains two tightly-linked genes *ORF2* and *ORF3*. *ORF2* encodes a toxic protein that causes pollen to abort in a sporophytic manner, and *ORF3* acts as an antidote that protects pollen in a gametophytic manner (Yu et al., 2018). At the *SI* locus, three closely-linked genes, *OgTPR1*(*SITPR*), *SSP* and *SIA4*, are involved in HS (Xie et al., 2017a; Koide et al., 2018; Xie et al., 2019). Our further study of the *Sc* locus detected additional genes in the du-

licated *Sc-i* allele segments that are also required for *Sc*-mediated HMS (Shen et al., unpublished data). These functionally related genes at the complex HS loci are located in the same regions and act as single genetic loci with minimum recombination between or among the gene alleles. If two or more functionally related HS genes are located on different chromosomes, independent segregation of the alleles in hybrids generates recombined genotypic gametes and may reduce the rate of abortive gametes. Therefore, during speciation, the generation of complex multi-gene HS loci that act as selfish genetic elements might produce advantages such as simple inheritance and maximum genetic effect (due to the minimum recombination of the gene alleles) that allow these HS loci to act in postzygotic reproductive isolation.

The origin and evolution of HS loci

How did selfish genetic elements originate and evolve during speciation, and did the functions of the genes for hybrid incompatibility derive from pre-existing functional genes as by-products, or did they originate primarily as HS genes? HS gene origins and evolution can be classified into three models: the sequential divergence model, parallel divergence model, and parallel-sequential divergence model (Ouyang and Zhang, 2018). For example, sequence analysis of the complex *Sa* locus in *Oryza* species found that the *SaF*⁺/*SaM*⁺ haplotype is a primitive form present in ancient wild rice species and *O. rufipogon*, and that *SaF*⁻/*SaM*⁻ is a diverged form derived from a nucleotide change in *SaF*⁺ followed by a second change in *SaM*⁺. This suggests a step-wise or sequential divergence model for the origin of this complex locus (Long et al., 2008). The intermediate haplotype *SaF*⁻/*SaM*⁺ present in some *O. rufipogon* and *O. sativa* populations is a neutral (hybrid-compatible) allele, which can produce fertility-compatible hybrids when crossing with typical *indica* (carrying *SaF*⁺/*SaM*⁺) and *japonica* rice parents.

The gene products encoded by *S27/S28*, *DPL1/DPL2*, and *DGSI/DGS2*, which originated by parallel divergence, are essential for pollen development (Yamagata et al., 2010; Mizuta et al., 2010; Nguyen et al., 2017). The DUF1618 protein encoded by *Sc* is also required for the development of bicellular pollen (Shen et al., 2017). Therefore, these HS loci were derived from pre-existing reproductive genes and their roles in hybrid incompatibility are by-products of the genes' original functions. However, the genes of the loci *Sa*, *S5*, *SI*, and *qHMS7* are not essential for gametogenesis, because loss-of-function mutations of these genes in parental lines did not affect gamete fertility (Long et al., 2008; Yang et al., 2012; Xie et al., 2017a, 2017b; Yu et al., 2018; Xie et al., 2019). Whether they have other biological functions, and whether these genes originated only for hybrid incompatibility, remain unclear.

Strategies for overcoming HS in hybrid rice breeding programs

Utilization of hybrid vigor in hybrid rice production has brought a huge boost in grain yield since the 1970s. Current hybrid rice varieties have been mainly derived from intra-subspecific crosses, mostly between *indica* lines. Due to the limited genetic diversity, at present intra-subspecific hybrid vigor only gives limited yield increases (Tang et al., 2017). Due to their greater genetic diversity, distant hybrids derived from inter-(sub)specific crosses demonstrate stronger heterosis, but they have low seed-setting rates due to HS. Therefore, HS studies also aim to break down the reproductive barrier and enable breeders to harness the strong hybrid vigor of these hybrids.

So far, three strategies have been developed to overcome HS in hybrid rice breeding programs. The first strategy is the introgression of neutral alleles of HS loci into parental rice cultivars to generate wide-compatibility lines for inter-subspecific hybrid breeding (Kitamura, 1962; Ikehashi and Araki, 1987). Sequence analysis of the *SaF/SaM* haplotypes in *Oryza* species found three types of *Sa* alleles, *SaF⁺/SaM⁺* (*Sa-i*), *SaF⁻/SaM⁺* (intermediate *Sa-n* allele), and *SaF⁻/SaM⁻* (*Sa-j*). The *Sa-n* allele is neutral or hybrid-compatible, and thus can be used for *indica-japonica* hybrid breeding (Xie et al., 2017b). The wide-compatible varieties carry the *S5-n* neutral alleles (*ORF3+/ORF4+/ORF5n* or *ORF3-/ORF4-/ORF5n*) of the *S5* locus and the *f5-n* allele of the *f5* locus; these varieties are valuable for *indica-japonica* hybrid breeding (Yang et al., 2012; Ouyang et al., 2016; Mi et al., 2016).

The second strategy is to create *indica*-compatible *japonica* lines by introgression of *indica* alleles of HS loci into the *japonica* genetic background. In practice, a series of *indica*-compatible *japonica* lines with multiple *indica* HS alleles created through successive backcrosses have been developed for *indica-japonica* hybrid breeding (Chen et al., 2011; Guo et al., 2016).

The third strategy is to engineer artificial hybrid-compatible lines by molecular approaches, such as CRISPR/Cas-based genome editing and RNA interference technologies. For the HS-related genes that are not required for gamete development (such as those at the complex loci of *Sa*, *S5*, and *SI*), functional knockout or suppression of these genes enables the rapid creation of artificial neutral alleles of these HS loci (Xie et al., 2017a; Xie et al., 2017b; Xie et al., 2019). For the *Sc* locus, although retaining at least one of the *Sc-i* copies is essential for pollen development, knocking out two of the three *Sc-i* copies to reduce the gene dosage created artificial neutral *Sc-n* alleles, which could increase male fertility of the hybrids (Shen et al., 2017). Importantly, the CRISPR/Cas transgenic constructs could be eliminated in the

obtained breeding lines by genetic segregation (Xie et al., 2017b). Therefore, the genetic engineering methods are the most efficient approach to overcome the reproductive barrier for distant hybrid breeding.

Concluding remarks

Emerging molecular evidence for several HS loci indicates that most of the one-locus HS loci contain multiple, closely-linked genes. The genes in the complex loci cooperate to play different roles as gamete killers or protectors, forming specific hybrid incompatibility systems. The utilization of hybrid breeding is a superb strategy for improving grain yield. In the past decades, hybrid rice has mainly benefited from intra-subspecific crosses. Stronger hybrid vigor has been demonstrated in *indica-japonica* crosses and *O. sativa-O. glaberrima* crosses. However, these crosses suffer from severe HS. Therefore, it is necessary to identify and clone more plant HS genes, elucidate their molecular mechanisms, and identify strategies to break down the reproductive barrier. Studies of these HS loci will broaden our knowledge about the molecular basis of hybrid incompatibility and generate new molecular approaches based on genome-editing technologies to solve hybrid incompatibility in rice and other crops.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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