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Genetic Mapping of the Gamete Eliminator Locus, S_2 , Causing Hybrid Sterility and Transmission Ratio Distortion Found between *Oryza sativa* and *Oryza glaberrima* Cross Combination

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Abstract: Hybrid sterility is a reproductive barrier that prevents gene flow between species. In *Oryza* species, some hybrid sterility loci, which are classified as gamete eliminators, cause pollen and seed sterility and sex-independent transmission ratio distortion (*si*TRD) in hybrids. However, the molecular basis of *si*TRD has not been fully characterized because of lacking information on causative genes. Here, we analyze one of the hybrid sterility loci, S_2 , which was reported more than forty years ago but has not been located on rice chromosomes. Hybrids between African rice (*Oryza glaberrima*) and a near-isogenic line that possesses introgressed chromosomal segments from Asian rice (*Oryza sativa*) showed sterility and *si*TRD, which confirms the presence of the S_2 locus. Genome-wide SNP marker survey revealed that the near-isogenic line has an introgression on chromosome 4. Further substitution mapping located the S_2 locus between 22.60 Mb and 23.54 Mb on this chromosome. Significant TRD in this chromosomal region was also observed in a calli population derived from cultured anther in hybrids of another cross combination of African and Asian rice species. This indicates that the pollen abortion caused by the S_2 locus occurs before callus induction in anther culture. It also suggests the wide existence of the S_2 -mediated *si*TRD in this interspecific cross combination. Chromosomal location of the S_2 locus will be valuable for identifying causative genes and for understanding of the molecular basis of *si*TRD.

Keywords: rice; *Oryza glaberrima*; hybrid sterility; transmission ratio distortion; reproductive barrier

1. Introduction

African cultivated rice species, *O. glaberrima* Steud, are a useful genetic resource for improving Asian cultivated rice species (*O. sativa* L.). However, severe seed and pollen sterility is observed in F_1 hybrids derived from these species [1–3]. Such a severe hybrid sterility prevents the transfer of useful genes in *O. glaberrima* to *O. sativa* during breeding.

In *Oryza* species, numerous loci for hybrid sterility that fit in the “single locus sporogametophytic interaction model” have been reported [4–7]. In this model, loci are classified in to three classes, namely pollen-killer, egg-killer, and gamete-eliminator, based on sex-specificity in their actions [7]. Pollen-killers and egg-killers abort male and female gametes with one of the two alleles, respectively. However, gamete eliminators abort both male and female gametes with one of the two alleles, which causes sex-independent transmission ratio distortion (*si*TRD) in progenies of hybrids. Although the existence of pollen-killers or egg-killers is frequently reported, reports on gamete-eliminators are relatively rare.

In the interspecific cross between *O. glaberrima* and *O. sativa*, more than 11 loci for hybrid sterility have been reported [6,8]. Among them, only three loci, S_1 , S_2 , and $S37(t)$ are

reported as gamete eliminators [5,9–11]. Recent studies identified the causative genes for a gamete eliminator, the S_1 locus [12–14]. Xie et al. [12] and Koide et al. [13] reported the involvement of peptidase-domain containing protein coding genes in S_1 -locus-mediated hybrid sterility. Additionally, Xie et al. [14] recently reported the involvement of an additional gene that constitutes a tripartite system in S_1 locus-mediated hybrid sterility. However, causative genes remain unclear for the other gamete eliminator loci. Therefore, overall mechanisms causing a severe sterility in hybrids between *O. glaberrima* and *O. sativa* remain unknown.

Here, we identify the chromosomal location of the gamete eliminator locus, S_2 . Sano et al. [5] suggested the existence of genetic factor(s) causing both seed and pollen sterility in the F_1 derived from the cross between W025 (a strain of *O. glaberrima*) and a near-isogenic line (NIL) developed by successive backcrossing with W025 to Acc108 (a strain of *O. sativa*). They termed the genetic factor inducing hybrid sterility in this cross as the S_2 locus. The two alleles in the S_2 locus, S_2^g , derived from *O. glaberrima* and S_2^s , derived from *O. sativa*, were assumed (S_2^g and S_2^s were previously denoted S_2 and S_2^a , respectively [5]. To reduce the confusion among the locus name and the allele name, we renamed them as S_2^g and S_2^s). In heterozygotes (S_2^g/S_2^s), male and female gametes carrying the S_2^g allele are preferentially aborted, causing about 50% sterility in pollens and seeds. In addition, only gametes with the S_2^s allele are transmitted to the next generation, which causes *siTRD* in later generations. Although Sano et al. [5] suggested the presence of the S_2 locus by NILs, chromosomal location of the S_2 locus is unknown, which limits molecular studies on this locus. The present study confirms results obtained by Sano et al. [5] and identifies the chromosomal location of the S_2 locus using genome-wide SNP typing and DNA marker surveys.

2. Materials and Methods

2.1. Genetic Stocks

The W025 African rice (*Oryza glaberrima* Steud) strain and the Pehkuh (denoted as Acc108) Asian rice (*O. sativa* L.) strain were used. A NIL, developed by Sano et al. [5], W025S2s, was also used. W025 and Acc108 harbor S_2^g and S_2^s alleles at the S_2 locus, respectively. W025S2s harbors the S_2^s allele at the S_2 locus introduced from Acc108 in the genetic background of W025. For genetic mapping, a total of 150 F_2 plants and two F_3 families derived from the W025 and W025S2s cross were used. To examine the genotype of plants derived from the anther culture, we used 27 calli derived from the anther culture of hybrids developed by crossing Nipponbare (a strain of *O. sativa*) and IRGC 104038 (denoted as WK21, a strain of *O. glaberrima*).

2.2. DNA Marker Survey

For the SNP survey, 1468 KASP markers designed to detect polymorphism between African and Asian rice species were used [15]. The amplification and inflorescence detection were conducted by Kbioscience/LGC. A total of 1182 KASP markers, which can determine the genotypes of SNP in both W025 and W025S2s, were used for analysis. Among the 1182 KASP markers, 26 showed polymorphism between W025 and W025S2s. If two neighboring SNPs are both polymorphic, we considered the region between them to be an SNP cluster region. To confirm the detailed chromosomal region in which the NIL has an introgression from the donor, two SSR markers and seven InDel markers in the SNP cluster region were used (Supplementary Table S1).

2.3. Genetic Mapping Using a Segregating Population

To genetically map the S_2 locus, a total of 150 F_2 plants were used. These F_2 plants were developed by self-pollination of the F_1 plants derived from the W025 and W025S2s cross. The F_2 plants were grown in a greenhouse in the Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan, in 2018 and 2020. The plants were grown in a short-day field (10 h light and 14 h dark) after sowing to prevent delay of heading because of the

plants' photosensitivity. Genomic DNA was isolated from 2-week-old seedlings using a simple method [16]. Genotypes of markers in the SNP cluster region of each F_2 plant were determined using two SSR markers and seven InDel markers described above. To confirm the location of the S_2 locus, two F_3 families were developed using self-pollination of two F_2 plants that have a segment recombined between markers. The genotypes of F_3 families were also determined by markers in the SNP cluster region.

To investigate whether the effect of the S_2 was also observed in calli derived from anther culture, we determined the genotype of anther-culture derived calli. For genotyping, a marker S2_4, which links to the S_2 locus, was used. The procedure of callus induction is based on that of Kanaoka et al. [8].

2.4. Seed and Pollen Fertility

The panicle fertility of each F_2 and F_3 plant was determined by counting fertile and sterile seeds in a panicle. The panicle-fertilities of two or three panicles in a plant were examined and the plant's maximum panicle-fertility was used for the plant's seed fertility. To examine the pollen fertility, spikelets were collected from panicles before flowering. The percentages of stainable and normal size pollen grains were examined with a potassium iodine solution (I_2 -KI). Non-stainable or small size pollen grains were classified as sterile pollen. For a plant, pollen fertility of at least two spikelets was examined and their maximum values were used as the plant's pollen fertility.

3. Results

3.1. The S_2 Locus Causes Pollen and Seed Sterility in Heterozygotes

To confirm the genetic action of the S_2 locus, we examined pollen and seed fertilities of heterozygous plants (S_2^g/S_2^s). Two parents, W025 and the NIL W025S2s showed 85.6% and 91.2% pollen fertility, respectively. However, the F_1 plants derived from the cross between the parents showed significantly lower pollen fertility (60.2%) than parents (Figure 1). W025 and W025S2s showed 87.8% and 80.7% seed fertility, respectively, while their F_1 plants showed significantly lower seed fertility (60.8%) than parents (Figure 1). These results indicated that genetic factors reduce pollen and seed fertilities in F_1 plants derived from the W025 and W025S2s cross. This result was consistent with one expected from the genetic action of gamete-eliminator. Because we used the same materials as Sano et al. [5], we considered this genetic factor to be the S_2 locus.

3.2. Chromosomal Regions Introgressed into W025S2s

Because W025 has fertile pollens and seeds in self-pollination, the reduction of pollen and seed fertilities in F_1 plants was caused by the S_2 locus located in the chromosomal segment introgressed into the W025S2s. Therefore, we conducted genome-wide SNP marker survey to locate introgressed segments of a donor variety, Acc108, in the genetic background of W025. Genotypes of 1182 SNP loci in W025 and W025S2s were determined using the KASP marker system [15]. Among the 1182 SNP loci, 26 loci showed polymorphism between W025 and W025S2s, which indicates the existence of introgressed segments (Table S2). Seven of the 26 polymorphic SNP loci are located between 21.45 Mb and 22.73 Mb on chromosome 4 as neighboring SNPs (Figure 2A). Additionally, an SNP locus (id4006835) located closely to these (21.12 Mb on chromosome 4) also showed polymorphism between W025 and W025S2s (Figure 2A). Therefore, we considered the chromosomal region (from 21.12 Mb to 22.73 Mb on chromosome 4) to be an SNP cluster. Because there was only one SNP cluster identified by a genome-wide survey, we focused this region for further investigation.

Figure 1. The effect of S_2 . (A) Seed fertility observed in W025, W025S2s and their F1 hybrids. Mean $\pm$ S.D. are shown. (B) Pollen fertility observed in W025, W025S2s and their F1 hybrids. Mean $\pm$ S.D. are shown. (C) Panicles of W025 (left), W025S2s (middle) and their F1 hybrid (right). Bar = 2 cm. (D) Pollen grains of F1 hybrid. Black and orange arrows indicate fertile and sterile pollen grains, respectively. Bar = 100 μ m.

Figure 2. Genetic mapping of the S_2 locus. (A) Chromosome-wide and region-specific views showing location of polymorphic markers. Eight polymorphic markers were found between 20Mb and 24Mb region on chromosome 4. Black and white boxes in the region-specific view indicate the position of polymorphic and non-polymorphic markers, respectively. Positions of two closely linked SNP markers (id4007172 and id4007212) were also shown by asterisk. (B) Graphical genotypes of W025, Acc108 and F2 plants derived from the cross between W025 and W025S2s. White and black bars indicate chromosomes derived from *O. glaberrima* and *O. sativa*, respectively. Pollen and seed fertility of F2 plants divided into four genotypes (W025S2s homozygotes, heterozygotes, and recombinant class 1 and 2) are shown on the right side of graphical genotypes. Number of plants in each genotype was shown in parenthesis. (C) Graphical genotypes of F3 plants obtained by self-pollination of two F2 genotypes (recombinant class 1 and 2). F3 plants were classified by genotype of the marker RM16991. From the recombinant class 1, four and four F3 plants showing heterozygous and homozygous for the *O. glaberrima* type in the marker RM16991 were obtained. From the recombinant class 2, four and three F3 plants showing heterozygous and homozygous for the *O. glaberrima* type in the marker RM16991 were obtained. The average pollen and seed fertilities with the standard error of each genotype class were shown.

To confirm and delimit the introgressed segment on chromosome 4 in the W025S2s, we used two SSR markers and nine Indel markers around the SNP cluster. Five of the 11 markers that are located in the region between 21.28 Mb and 23.50 Mb on chromosome 4 showed polymorphism between W025 and W025S2s (Supplementary Figure S1). In these five markers, polymorphisms were not observed between W025S2s and Acc108 (Figure S1). These results indicate that a segment of Acc108 was introgressed into W025S2s in this chromosomal region. However, the other six markers that are located on 20.10 Mb or in the region between 23.54 Mb and 26.99 Mb on chromosome 4 did not show polymorphism between W025 and W025S2s (Supplementary Figure S1). Because there were polymorphisms between W025 and Acc108 in these six markers, these results indicated that W025S2s does not have introgressed segments in these regions. Therefore, we concluded that W025S2s possesses the introgressed segment from Acc108 in the region at most between 20.10 Mb and 23.54 Mb on chromosome 4.

3.3. Transmission Ratio Distortion Observed in the F₂ Population

To examine whether the *S₂* locus is in the introgressed segment on chromosome 4, we developed the F₂ population and determined the genotype of the individual plant in the population (Figure 2B). The *S₂* locus is assumed to be the factor for hybrid male and female sterility induced via preferential abortion of male and female gametes with the *S₂^g* allele in heterozygotes [5]. Therefore, if hybrid sterility induced by the *S₂* locus occurs, TRD in the flanking region of the *S₂* locus is expected in progenies of heterozygotes.

We observed a significant TRD in the F₂ population derived from the W025 and W025S2s cross in 2018 and 2020 (Table 1). In the marker, RM16991, the ratio of homozygotes of the W025S2s-derived allele:heterozygotes:homozygotes of the W025-derived allele was 134:16:0, which shows significant segregation distortion ($p < 0.01$) towards the excess of W025S2s allele. Stronger distortion was also observed in the marker S2_2, S2_4 and S2_5 (Table 1). These results indicated that a genetic factor for TRD exists in the introgressed segment in W025S2s. We also analyzed pollen and seed fertility of the 95 F₂ plants grown in 2020 (Figure 3). All 90 plants homozygotes of the W025S2s-derived allele in the marker S2_4 showed higher pollen and seed fertility than any heterozygote (Figure 3). This result indicated that the factor for hybrid sterility is also located in this region. Because the *S₂* locus is considered to be a factor for hybrid sterility causing TRD, these results strongly indicate that the *S₂* locus is in this chromosomal region (between 20.10 Mb and 23.54 Mb on chromosome 4).

Table 1. Segregation distortion observed in the F₂ population derived from the cross between W025 and W025S2s.

Marker Name	Location (Mb) ¹	Plant No.			<i>P</i> ² (1:2:1)
		s/s	s/g	g/g	
RM16991	21.27	134	16	0	7.16145×10^{-73}
S2_1	22.08	142	9	0	3.69831×10^{-84}
S2_2	22.15	144	7	0	2.34291×10^{-87}
S2_4	22.74	144	7	0	2.34291×10^{-87}
S2_5	23.50	143	8	0	9.49528×10^{-86}

¹ Physical location on the chromosome 4, ² *P* values based on χ^2 test.

3.4. Substitution Mapping of the *S₂* Locus

Using four individuals that harbor recombination points between markers RM16991 and S2_5, we further narrowed down the candidate region of the *S₂* locus (Figure 2B). High pollen and seed fertilities (92.4% and 89.3%, respectively) were observed in individuals homozygous for W025S2s-derived allele for all five markers (F2_s in Figure 2B). Conversely, low pollen and seed fertilities (53.1% and 48.2%, respectively) were observed in the heterozygote (F2_h in Figure 2B). These results show that hybrid sterility occurred in the heterozygote. In two recombinant classes (recombinant class 1 and 2), pollen and seed

fertilities were higher than those of heterozygotes (94.4% and 90.2% for pollen fertility and 86.4% and 89.5% for seed fertility, respectively), though only one individual was obtained for the recombinant class 2. For further confirmation of these results, we developed two F_3 families derived from the self-pollination of the two recombinant classes. The F_3 plants were further classified by genotype of a marker linking to the S_2 locus (Figure 2C). If the S_2 locus is located between markers RM3643 and S2_1, low pollen and seed fertilities are expected in plants which are heterozygous in this chromosomal region. The result showed that all F_3 plants had high pollen and seed fertilities compared to the heterozygotes. These results confirmed that the S_2 locus is located between S2_1 and S2_10.

Figure 3. Pollen and seed fertility of F_2 plants derived from the cross between W025 and W025S2s observed in 2020. Black and white bars represent *O. glaberrima* homozygotes and heterozygotes, respectively. The genotype of each plant was determined using a DNA marker S2_4. The average pollen and seed fertilities of W025, W025S2s and their F_1 hybrids are shown by arrow heads.

3.5. Transmission Ratio Distortion Observed in Anther-Culture Derived Calli

Kanaoka et al. [8] showed that, in calli derived from the anther culture, TRD is not observed for some loci for hybrid sterility, though it is observed in the F_2 population. This phenomenon was assumed to occur if callus induction begins before pollen abortion; genes for hybrid sterility are unlikely to function in microspores after the initiation of callus induction. Although Kanaoka et al. [8] examined the effect of eleven hybrid sterility loci in calli derived from anther culture in hybrids between *O. sativa* and *O. glaberrima*, the S_2 locus was not included in the analysis. To investigate whether TRD caused by the S_2 locus in the F_2 population is also observed in calli derived from anther culture, we genotyped 27 calli derived from the cultured anther in hybrids between Nipponbare and WK21. Of these, 24 and 3 plants are *O. sativa* homozygote and *O. glaberrima* homozygote for the marker, S2_4, respectively (Table 2). Because a significant distortion was observed in the marker linked to the S_2 locus, we concluded that TRD was induced by the S_2 locus also in the anther-culture derived calli.

Table 2. Segregation distortion observed in calli derived from another culture.

Marker Name	Location (Mb) ¹	No. of Calli		p^2 (1:1)
		s/s	g/g	
S2_4	22.74	24	3	5.31×10^{-5}

¹ Physical location on the chromosome, ² P values based on X^2 test.

4. Discussion

The S_2 locus was first reported by Sano et al. [5], who found that NILs with *O. glaberrima* genetic background showed pollen and seed semi-sterility when they are crossed with a strain of *O. glaberrima*. They also reported restoration of fertility in the progenies of the above cross (Sano et al. [5] described them as BnF2 plants). Based on these observations, they hypothesized that sterility observed in F_1 plants between the NIL and *O. glaberrima* was caused by the locus acting in the mode of “single locus sporo-gametophytic interaction model”. They assumed this locus as the S_2 locus [5].

In this study, we also used the same materials described in Sano et al. [5] and confirmed that pollen and seed sterility occur in the F_1 derived from the NIL (W025S2s) and W025 cross (Figure 1). This result indicated that hybrid sterility observed in this study is caused by the S_2 locus. Genome-wide SNP marker survey and genetic mapping showed that the S_2 locus is in the region between 22.60 Mb and 23.54 Mb on chromosome 4 (Figure 2). In this chromosomal region, no other hybrid sterility loci have been reported in the public databases [Q-TARO (QTL Annotation Rice Online) database (<http://qtaro.abr.affrc.go.jp/> on 19th March 2021) and Oryzabase (<https://shigen.nig.ac.jp/rice/oryzabase/> on 19th March 2021)]. This indicates that there are no mapped loci for hybrid sterility identical to the S_2 locus. Although there are 141 predicted gene loci between 22.60 Mb and 23.54 Mb on chromosome 4, based on the reference genome sequence of *O. sativa* (cv. Nipponbare), it remains unknown whether these loci also exist in the strains used in this study. Further genetic mapping and genome sequencing of W025 are necessary to identify the S_2 locus's causative gene(s).

The present study showed that TRD also occurred in the population derived from anther culture. Kanaoka et al. [8] suggested that if callus induction begins before pollen abortion induced by the gene for hybrid sterility, TRD does not occur in the population derived from anther culture. The present result that a significant distortion was observed in the anther-culture-derived population suggested that pollen abortion induced by the S_2 locus begins before callus induction. Because the timing of callus induction is from uninucleate to two nuclei stages in the development of pollen, these results indicated that the S_2 locus induces pollen abortion before/in the two nuclei stage during pollen development. To confirm the timing of pollen abortion, detailed cytological analysis is necessary. For the anther culture, we used hybrids derived from Nipponbare and WK21. This result suggested that the S_2 locus induces hybrid sterility not only in the specific pairwise-cross combination between Asian and African rice varieties, but also in the broader pairwise-cross combinations between these two species. To confirm this, a survey of allelic distribution is necessary.

Mechanisms causing/maintaining a strong reproductive barrier between species are a long-standing interest in evolutionary biology. In the genus *Oryza*, it is plausible that severe sterility arises from accumulation of hybrid sterility loci. Although the overall effect on sterility would increase with the increasing number of hybrid sterility loci, it is also affected by the phase and strength of linkage between hybrid sterility loci (Figure 4). If preferentially-transmitting alleles in two different hybrid sterility loci (that is, their alternative alleles do not transmit because of the gametes' selective abortion) link in the coupling phase, the overall effect on hybrid sterility decreases with their linkage strength (Figure 4A). Conversely, if these two alleles link in the repulsion phase, the overall effect on hybrid sterility increases with the loci's linkage strength (Figure 4B). Therefore, a difference in the direction of gametic selection in hybrid sterility loci might have a role for arising/maintaining a severe sterility barrier between species. However, a bias in the use of the genetic background species may cause an asymmetric detection of hybrid sterility loci with the specific direction of gametic selection.

Figure 4. The effect of number, linkage and phase of hybrid sterility loci. In the locus X gametes with the allele “x” are preferentially abort, while gametes with the allele “X” survive. In the locus Y, gametes with the allele “y” are preferentially abort, while gametes with the allele “Y” survive. Loci X and Y link with a recombination frequency of “r”. The expected frequency of surviving gametes (no. of surviving gametes/no. of all gametes) against no. of pairs of sterility loci are plotted. The recombination frequency “r” varies from 0.1 to 0.5. (A) Coupling phase. (B) Repulsion phase.

Generally, it is common to use the NILs with the genetic background of *O. sativa*, an Asian cultivated rice species, because this species is easier to handle and maintain than other related species of *Oryza*. In the case of S_2 , preferential abortion of gametes carrying the S_2^g allele derived from *O. glaberrima* occurs. Therefore, in the progeny of heterozygotes (S_2^g/S_2^s), almost all plants are homozygotes for *O. sativa*-derived allele (S_2^s) at the S_2 locus (Table 1; Figure 3). In such a case, it is difficult to develop NILs containing the S_2^g allele at the S_2 locus by backcrossing *O. sativa* to heterozygotes, just as in general cases. The NIL used in the present study was developed by backcrossing *O. glaberrima* to heterozygotes to introduce the S_2^s allele at the S_2 locus in the genetic background of *O. glaberrima*. Such NILs with an “alternative” genetic background enabled detailed genetic analysis of the S_2 locus. Currently, four loci for hybrid sterility in *Oryza* have been reported as gamete eliminator type [7,11,17]. In these four loci, all loci except for the S_2 locus cause preferential abortion of the gametes with the allele derived from *O. sativa*. Further development of NILs with genetic background of species other than *O. sativa* may help us to detect unknown loci for gamete eliminator that potentially contribute to severe sterility barriers between species.

The present study confirmed the existence of the S_2 locus causing the abortion of male and female gametes with the allele derived from *O. glaberrima*. In addition to the S_2 locus, the presence of the S_1 locus causing preferential abortion of the gametes with the allele derived from *O. sativa* has been confirmed by using the same materials [5,9,13,18]. Koide et al. [18] showed that a gamete eliminator locus S_1 is a compound locus of pollen-killer and its modifier affecting the abortion of female gametes, though such a compound effect might depend on the genetic background [18]. These studies have raised the question of how such a (compound) locus with a strong sterility effect appears during/after speciation in *Oryza*. The information of causal genes of the S_2 locus might also shed light on the evolutionary process of gamete eliminators in *Oryza*.

5. Conclusions

The S_2 locus induced both pollen and seed sterility in hybrids of *O. sativa* and *O. glaberrima*. We have mapped this locus between 22.60 Mb and 23.54 Mb on rice chromosome 4 using the segregating population. Although the causative gene(s) remains unidentified,

this study is a first step towards fully understanding the molecular mechanisms of gamete eliminators and their significance to the evolution of reproductive barriers.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2077-0472/11/3/268/s1>, Figure S1: Electrophoresis images of markers used for the mapping of the S_2 locus. The amplified products were electrophoretically resolved on 1% agarose gel in TAE buffer at 100 V for 40 min and DNA fragments were detected by staining with Midori Green Advance (Nippon Genetics CO., Ltd.). FastGene 100bp DNA ladder (Nippon Genetics CO., Ltd.) was applied in the lane no. 1. PCR amplicons of W025, Acc108 and W025S2s were applied in lane nos. 1, 2 and 3, respectively. (A) RM3643, (B) RM16991, (C) S2_1, (D) S2_2, (E) S2_4, (F) S2_5, (G) S2_10, (H) S2_9, (I) S2_8, (J) S2_7, (K) S2_6, Table S1: Primers used in this study, Table S2: SNP genotypes.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chu, Y.E.; Morishima, H.; Oka, H.I. Reproductive barriers distributed in cultivated rice species and their wild relatives. *Jpn. J. Genet.* **1969**, *4*, 207–223. [\[CrossRef\]](#)
2. Bouharmont, J.; Oliver, M.; Dumont de Chassart, M. Cytological observation in some hybrids between the rice species *Oryza sativa* L. and *O. glaberrima* Steud. *Euphytica* **1985**, *34*, 75–81. [\[CrossRef\]](#)
3. Jones, M.P.; Mande, S.; Aluko, K. Diversity and potential of *Oryza glaberrima* Steud in upland rice breeding. *Breed. Sci.* **1997**, *47*, 395–398. [\[CrossRef\]](#)
4. Oka, H.I. Considerations on the genetic basis of intervarietal sterility in *Oryza sativa*. In *Rice Genetics and Cytogenetics*; Chandler, R.F., Ed.; Elsevier: Amsterdam, The Netherlands, 1964; pp. 158–174.
5. Sano, Y.; Chu, Y.E.; Oka, H.I. Genetic studies of speciation in cultivated rice, 1. Genic analysis for the F1 sterility between *O. sativa* L. and *O. glaberrima* Steud. *J. Genet.* **1979**, *54*, 121–132.
6. Doi, K.; Yasui, H.; Yoshimura, A. Genetic variation in rice. *Curr. Opin. Plant Biol.* **2008**, *11*, 144–148. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Koide, Y.; Onishi, K.; Kanazawa, A.; Sano, Y. Genetics of speciation in rice. In *Rice Biology in the Genomics Era*; Hirano, H.Y., Hirai, A., Sano, Y., Sasaki, T., Eds.; Springer: Berlin, Germany, 2008; pp. 247–259.
8. Kanaoka, Y.; Kuniyoshi, D.; Inada, E.; Koide, Y.; Okamoto, Y.; Yasui, H.; Kishima, Y. Anther culture in rice proportionally rescues microspores according to gametophytic gene effect and enhances genetic study of hybrid sterility. *Plant Methods* **2018**, *14*, 102. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Sano, Y. The genic nature of gamete eliminator in rice. *Genetics* **1990**, *125*, 183–191. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Xu, P.; Zhou, J.W.; Li, J.; Hu, F.Y.; Deng, X.N.; Feng, S.F.; Ren, G.Y.; Zhang, Z.; Deng, W.; Tao, D.Y. Mapping three new interspecific hybrid sterile loci between *Oryza sativa* and *O. glaberrima*. *Breed. Sci.* **2014**, *63*, 476–482. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Shen, Y.M.; Zhao, Z.G.; Ma, H.Y.; Bian, X.F.; Yu, Y.; Yu, X.W.; Chen, H.Y.; Liu, L.L.; Zhang, W.W.; Jiang, L.; et al. Fine mapping of S37, a locus responsible for pollen and embryo sac sterility in hybrids between *Oryza sativa* L. and *O. glaberrima* Steud. *Plant Cell Rep.* **2015**, *34*, 1885–1897. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Xie, Y.; Xu, P.; Huang, J.; Ma, S.; Xie, X.; Tao, D.; Chen, L.; Liu, Y.G. Interspecific hybrid sterility in rice is mediated by OgTPR1 at the S1 locus encoding a peptidase-like protein. *Mol. Plants* **2017**, *10*, 1137–1140. [\[CrossRef\]](#) [\[PubMed\]](#)

13. Koide, Y.; Ogino, A.; Yoshikawa, T.; Kitashima, Y.; Saito, N.; Kanaoka, Y.; Onishi, K.; Yoshitake, Y.; Tsukiyama, T.; Saito, H.; et al. Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E1955–E1962. [[CrossRef](#)] [[PubMed](#)]
14. Xie, Y.; Tang, J.; Xie, X.; Li, X.; Huang, J.; Fei, Y.; Han, J.; Chen, S.; Tang, H.; Zhao, X.D.; et al. Chen. An asymmetric allelic interaction drives allele transmission bias in interspecific rice hybrids. *Nat. Commun.* **2019**, *10*, 2501. [[CrossRef](#)] [[PubMed](#)]
15. Pariaska-Tanaka, J.; Lorieux, M.; He, C.; McCouch, S.; Thomson, M.J.; Wissuwa, M. Development of a SNP genotyping panel for detecting polymorphisms in *Oryza glaberrima*/*O. sativa* interspecific crosses. *Euphytica* **2015**, *201*, 67–78. [[CrossRef](#)]
16. Yasuda, K.; Ito, M.; Sugita, T.; Tsukiyama, T.; Saito, H.; Naito, K.; Teraishi, M.; Tanisaka, T.; Okumoto, Y. Utilization of transposable element mPing as a novel genetic tool for modification of the stress response in rice. *Mol. Breed.* **2013**, *32*, 505–516. [[CrossRef](#)] [[PubMed](#)]
17. Koide, Y.; Ikenaga, M.; Sawamura, N.; Nishimoto, D.; Matsubara, K.; Onishi, K.; Kanazawa, A.; Sano, Y. The evolution of sex-independent transmission ratio distortion involving multiple allelic interactions at a single locus in rice. *Genetics* **2008**, *180*, 409–420. [[CrossRef](#)] [[PubMed](#)]
18. Koide, Y.; Onishi, K.; Nishimoto, D.; Baruah, A.R.; Kanazawa, A.; Sano, Y. Sex-independent transmission ratio distortion system responsible for reproductive barriers between Asian and African rice species. *New Phytol.* **2008**, *179*, 888–900. [[CrossRef](#)] [[PubMed](#)]