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Mapping three new interspecific hybrid sterile loci between *Oryza sativa* and *O. glaberrima*

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Hybrid sterility hinders the transfer of useful traits between *Oryza sativa* and *O. glaberrima*. In order to further understand the nature of interspecific hybrid sterility between these two species, a strategy of multi-donors was used to elucidate the range of interspecific hybrid sterility in this study. Fifty-nine accessions of *O. glaberrima* were used as female parents for hybridization with *japonica* cultivar Dianjingyou 1, after several backcrossings using Dianjingyou 1 as the recurrent parent and 135 BC_6F_1 sterile plants were selected for genotyping and deducing hybrid sterility QTLs. BC_6F_1 plants containing heterozygous target markers were selected and used to raise BC_7F_1 mapping populations for QTL confirmation and as a result, one locus for gamete elimination on chromosome 1 and two loci for pollen sterility on chromosome 4 and 12, which were distinguished from previous reports, were confirmed and designated as *S37*(t), *S38*(t) and *S39*(t), respectively. These results will be valuable for understanding the range of interspecific hybrid sterility, cloning these genes and improving rice breeding through gene introgression.

Key Words: *Oryza sativa*, *Oryza glaberrima*, interspecific hybrid, sterility.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop for more than half of the world's population. Narrow genetic diversity has been considered as a bottleneck for further yield increase (Tanksley and McCouch 1997); thus, mining and introgression of favorable alleles from relatives of *O. sativa* to enhance its genetic diversities have been attracting increasing attention.

O. glaberrima, an African cultivated rice species with the same AA genome, is closely related to *O. sativa*. It contains various valuable traits for resistance to biotic stress, such as rice yellow mottle virus (Attere *et al.* 1983), blast (Silue *et al.* 1991), bacterial leaf blight (Khush 1989), African rice gall midge (Olga 2002), *Heterodera sacchari* (Reversat *et al.* 1995) and leafhopper (Khush 1989), and it is also tolerant for abiotic factors, such as drought, salinity and acidity (Ghesquiere *et al.* 1997, Lorieux *et al.* 2000, Sano *et al.* 1984). Meanwhile, high-yield genes or alleles of *O. glaberrima* could be used to break the yield ceiling of *O. sativa* cultivars. Thus, it was considered as an excellent gene pool to improve Asian cultivated rice (Jones *et al.* 1997, Xu *et al.* 2005).

However, hybrid sterility between *O. sativa* and *O. glaberrima* hinders the transfer of useful genes between the two species. To overcome hybrid sterility, genetic factors affecting sterility must be identified and characterized to better understand the nature of the sterility barrier.

So far, several genetic models have been proposed for hybrid sterility in plants, including the one-locus allelic interaction model (Ikehashi *et al.* 1986, Oka 1974, Sano 1990, Sano *et al.* 1979), duplicate gametophytic lethal model (Oka 1974) and Bateson-Dobzhansky-Muller (BDM) model (Bateson 1909, Dobzhansky 1936, Muller 1942). The one-locus allelic interaction model can explain the genetic behavior of most hybrid sterile loci in *indica-japonica* or *O. glaberrima-japonica* hybrids. Recently, with the development of rice genomics and molecular markers, a large number of loci affecting hybrid sterility between *O. sativa* and *O. glaberrima* were identified, such as gamete eliminator *S1* and pollen killer *S3*, *S18*, *S19*, *S20*, *S21, S29*(t) (Doi *et al.* 1998, 1999, Hu *et al.* 2006, Sano 1983, 1986, Taguchi *et al.* 1999); however, only a few genes have been cloned and characterized in rice. *S5* is a major gene controlling female fertility with wide compatibility in *O. sativa* L. ssp. *indica-japonica* hybrid. Chen *et al.* (2008) proposed a tri-allelic system to explain the molecular interactions among *indica* (*S5-i*), *japonica* (*S5-j*) and wide compatibility (*S5-n*) alleles, but Yang *et al.* (2012) found a killer-protector system at the *S5* locus encoded by three tightly linked genes regulating fertility in *indica-japonica* hybrids. *Sa*, a locus for *indica-japonica* hybrid male sterility, comprises two adjacent genes, *SaM* and *SaF*, a two-gene/three-component interaction model was proposed for *Sa* (Long *et al.* 2008).

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Both *S27* and *S28*, the reciprocal loss of duplicated genes, encode a mitochondrial ribosomal protein *L27* (*mtRPL27*), which controls hybrid pollen sterility in F_1 hybrids between *O. sativa* and its wild relative *O. glumaepatula* (Yamagata *et al.* 2010). Additionally, a model in which incompatibilities in epistatic interactions between *S1* and additional factors are the cause of the female sterility barrier between *O. sativa* and *O. glaberrima* was developed to explain female sterility and the transmission ratio distortion mediated by *S1* (Garavito *et al.* 2010).

These efforts allow further understanding of the nature of hybrid sterility. Meanwhile, it would be interesting and useful to find an interspecific neutral allele or wide compatibility allele, such as *S5-n*, which does not cause gamete abortion in hybrids (Ikehashi *et al.* 1986, Tao *et al.* 2003). Unfortunately, there is still no publication report regarding this issue (Deng *et al.* 2010). An alternative method of solving this problem is to use a bridge parent with a sterile gene allele (Deng *et al.* 2010); however, when a single sterile gene or a pyramid of several sterile genes was transferred to Asian cultivar species as a bridge parent to cross with African species, F_1 was still highly sterile (Heuer *et al.* 2003, Sigrid *et al.* 2003). These results suggested that our understanding of the nature of interspecific hybrid sterility is far from complete. Currently, most hybrid sterility studies using one *O. glaberrima* experimental population can only obtain a few individuals due to high sterility in F_1 and BC_1F_1 of interspecific hybrids; thus, it is likely that the current diverse studies are not sufficient to cover the range of interspecific hybrid sterile loci. Therefore, it is necessary to adopt a multiple donor approach to determine the number of loci and allelic variations responsible for hybrid fertility (Zhou *et al.* 2010) within accessions of *O. glaberrima* (Semon *et al.* 2005).

In this study, 59 accessions of *O. glaberrima* were used as donors to detect interspecific hybrid sterile loci. The results partially confirm previously described sterility loci and allow the identification of some new loci. This research will be benefit for further understanding the range of interspecific hybrid sterility, overcoming the sterility barrier between *O. sativa* and *O. glaberrima* and transferring genes controlling desirable traits such as high yield and drought tolerance from *O. glaberrima* to Asian cultivated rice varieties.

Materials and Methods

Materials

Fifty-nine accessions of *O. glaberrima* from the International Rice Research Institute (IRRI) as the maternal and donor parents, one *O. sativa* ssp. *japonica* variety, Dianjingyou 1 (DJY 1), from Yunnan province, P. R. China, as the paternal and recurrent parent, were used to obtain F_1 (Late Crop Season in 2001, July to November), BC_1F_1 (Late Crop Season, 2002), BC_2F_1 (Winter Crop Season, November–March 2002) and BC_3F_1 (Early Crop Season, March–June 2003) progenies in Sanya, Hainan province, P. R. China. At BC_1F_1, about 20 plants of each cross were grown and used for backcrossing. Between BC_2F_1 and BC_3F_1, 10 plants of each family were grown, and about 3 plants of each family were randomly selected for backcrossing without selection. From BC_4F_1 (2003 Late Crop Season) after investigation of pollen grain fertility, sterile individuals (pollen grain fertility below 90%) were selected to backcross with the recurrent parent DJY 1 to obtain BC_5F_1 and BC_6F_1 (2003 Winter Crop Season, 2004 Late Crop Season). In BC_6F_1, 135 semi-sterile plants selected from 142 families of 59 cross combinations of *O. glaberrima*/Dianingyou 1/7/Dianjingyou 1 were used for genotyping (7 families without any sterile individual, sterile individuals from each donor ranged from 1 to 9), and raising BC_7F_1 mapping populations. BC_7F_1 populations were planted in Sanya in the 2004 Winter Crop Season.

Based on marker-assisted selection, NILs for the homozygous sterile gene were obtained from self-fertilized progenies of sterile plants from corresponding BC_7F_1 mapping populations. NILs were backcrossed with the recurrent parent DJY 1 to obtain BC_8F_1 for phenotype confirmation. Data for NILs and BC_8F_1 were collected in Late Crop Season, 2009.

Pollen grain and spikelet fertility check

Pollen grain fertility was measured using anthers collected from spikelets 1 to 2 days before anthesis and stored in 70% ethanol (Doi *et al.* 1998). Pollen grain fertility was estimated as the percentage of pollen grains that could be stained with 1% I-KI solution. Sterile types were further classified as typical, spherical and stained abortion types (Li 1980). Five independent microscopic fields were scored for counting the percentage of fertile pollen grains in each plant. Spikelet fertility was only investigated for parents and mapping populations.

Genotype and segmental linkage group construction

Leaves from each plant were sampled to extract DNA. The 135 BC_6F_1 semi-sterile plants were genotyped with 225 SSR markers based on the results of polymorphism detection for 344 SSR primer pairs tested between two parents, *O. sativa* DJY 1 and *O. glaberrima* IRGC102203. The selected markers were evenly distributed on 12 chromosomes. Since interspecific sterility was heterozygous sterility and selection was according to sterility, if the heterozygous rate of a marker in advanced backcross progeny is significantly higher than that of the theoretical predication from binomial distribution, this marker is likely to be linked to a QTL for sterility (Bernardo 2004, Li *et al.* 2008, 2011), provided there is no differentiation among these 59 cultivars in regards to hybrid sterility, and sterility is controlled by a dominant gene(s). In order to avoid the high probability of false QTL from multiple tests, a high significance level of 0.0005 was used.

In BC_7F_1 mapping populations, targeted markers and their linked markers for corresponding BC_6F_1 plants were employed to construct segmental linkage groups. Linkage

analysis was performed using MAPMAKER version 3.0 (Lander *et al.* 1987), with a logarithm of odds (LOD) score of >3.0 for the segregating markers. The recombination frequency was converted to cM using the Kosambi function. Segmental linkage maps of target genes were drawn by Map Chart 2.2 (Voorrips 2002).

Results

Distribution of pollen grain fertility from BC_4F_1 to BC_6F_1 in the progenies of O. glaberrima/Dianjingyou 1

Even though several plants from each cross combination were randomly selected for backcrossing from BC_1F_1 to BC_3F_1 without selection, the results showed enough sterile plants for selection in BC_4F_1. The distribution was continuous in BC_4F_1 and BC_5F_1 and fertile, semi-sterile and sterile plants could still be found, but only fertile and semi-sterile plants could be found in BC_6F_1 (Table 1). These results indicate that interspecific hybrid sterility controlled by polygenes in the preliminary populations was gradually dissected as a simple inheritance mode after three times phenotypic selections and continuous backcrossing; thus, it was easy to detect and confirm QTLs in advanced backcross populations.

Detection of QTLs for hybrid sterility in BC_6F_1

According to binomial distribution, significant probability was observed in six regions on chromosome 1, 2, 3, 4, 6, 12 and two regions on chromosome 7. We deduced that there were eight hybrid sterile QTLs corresponding to these regions and denoted them tentatively as *qSS1*, *qSS2*, *qSS3*, *qSS4*, *qSS6*, *qSS12* and *qSS7a*, *qSS7b* (Table 2). Among these, *qSS1*, *qSS4* and *qSS12* were not reported between *O. sativa* and *O. glaberrima* in previous publications and these three new QTLs were detected from 3, 5, 5 different donors, respectively. Thus, three BC_6F_1 sterile plants 2004H2E137-2 (donor, IRGC101854), 2004H2E245-1 (donor, Acc102528), 2004H2E185-2 (donor, IRGC103466), harboring *qSS1*, *qSS4* and *qSS12*, respectively and fewer heterozygous markers were used to raise the corresponding

Table 1. Distribution of pollen grain fertility from BC_4F_1 to BC_6F_1 in *O. glaberrima*/DJY 1 in 2003 Late Crop Season, 2003 Winter Crop Season and 2004 Late Crop Season, respectively, in Sanya, Hainan, P. R. China

Generation	Population	Pollen grain fertility																					Population size	Means of pollen grain fertility (%)	STDEV of pollen grain fertility
		0	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5	92.5	97.5			
BC_4F_1	Preliminary population	47	4	19	14	17	20	18	14	42	34	30	32	59	41	33	15	6	6	2	1	222	676	60.33	33.39
	Selected population	35	1	7	5	4	8	6	4	15	8	10	9	24	12	15	1	1	0	0	0	3	168	36.99	25.67
BC_5F_1	Preliminary population	5	2	6	10	13	14	24	61	51	70	123	117	91	79	44	23	16	13	14	12	431	1219	67.78	27.06
	Selected population	1	1	1	1	2	3	3	9	5	12	20	25	25	17	10	3	2	1	1	0	0	142	50.79	14.79
BC_6F_1	Preliminary population	0	0	1	1	5	8	6	10	40	58	87	150	73	38	10	6	1	1	3	11	285	794	67.74	34.43
	Selected population	0	0	1	1	3	2	1	3	14	12	25	37	23	8	3	1	0	0	1	0	0	135	48.78	11.43

Table 2. Heterozygous markers and QTLs detected in 135 sterile individuals of BC_6F_1 derived from 59 interspecific hybridization cross combinations between *Oryza sativa* and *O. glaberrima* in 2004 Late Crop Season in Sanya, Hainan, P. R. China

Marker	Chromosome	Position (Mb)[a]	No. of heterozygous plants	Percentage of heterozygous plants (%)	No. of donors	PH[b]	QTL	Deduced gene
RM562	1	14.62	9	6.7	9	1.26E-06	*qSS1*	New
RM595	1	15.11	9	6.7	9	1.26E-06		
RM24	1	18.97	12	8.9	11	9.11E-10		
RM236	2	21.06	7	5.2	6	9.01E-05	*qSS2*	*S29*(t)
RM22	3	15.00	13	9.6	13	6.79E-11	*qSS3*	*S19*
RM518	4	20.22	12	8.9	9	9.11E-10	*qSS4*	New
RM586	6	14.77	69	51.1	42	6.90E-107	*qSS6*	*S1*
RM587	6	22.92	68	50.4	36	9.00E-105		
RM295	7	0.41	20	14.8	8	1.10E-19	*qSS7-a*	*S20*
RM3589	7	25.05	8	5.9	7	1.14E-05	*qSS7-b*	*S21*
RM5568	12	0.71	7	5.2	5	9.01E-05	*qSS12*	New

[a] Chromosome map location of marker in Mb (Ref. International Rice Genome Sequencing Project (IRGSP)).

[b] The probability of heterozygote genotypes scored by the binomial test.

BC_7F_1 populations 2004H3E142 (*qSS1*), 2004H3E244 (*qSS4*) and 2004H3E188 (*qSS12*) for the confirmation and mapping of QTLs.

Segregation of pollen grain and spikelet fertility in BC_7F_1 populations

Pollen grain fertility showed similar bimodal distribution divided into semi-sterile (about 50% fertility) and fertile (about 100% fertility) groups in the three populations, spikelet fertility presenting with normal distribution tended to be fertile in population 2004H3E244 (*qSS4*) and 2004H3E188 (*qSS12*), but spikelet fertility of 2004H3E142 (*qSS1*) showed bimodal distribution (Fig. 1).

Mapping of sterile QTLs in BC_7F_1 populations

Three target SSR markers, RM562 on chromosome 1 linked to *qSS1*, RM518 on chromosome 4 linked to *qSS4*, RM5568 on chromosome 12 linked to *qSS12* and their linked polymorphic markers were used to survey the genotypes of individuals in population 2004H3E142 (*qSS1*), 2004H3E244 (*qSS4*) and 2004H3E188 (*qSS12*) for fine mapping of the target gene, respectively.

In population 2004H3E142 (*qSS1*), the introgression segment was about 3.8 cM between RM562 and RM446 and nine polymorphic SSR markers in the introgression region were used for mapping the target gene. The sterile gene was restricted to a 1.0 cM region flanked by RM449 and RM113 on the long arm of chromosome 1. In population 2004H3E244 (*qSS4*), the introgression segment was about 3.0 cM between RM16251 and RM518 and eight polymorphic SSR markers were used to locate the target gene. The sterile gene was mapped to a 2.7 cM region flanked by RM16251 and RM16260 on the short arm of chromosome 4. In population 2004H3E188 (*qSS12*), the introgression segment was about 8.3 cM between RM1208 and RM5927 and eight polymorphic SSR markers were used for mapping the target gene. The sterile gene was located in a 4.3 cM region flanked by RM5568 and RM7582 on the short arm of chromosome 12. Since sterile genes were not reported in these regions between *O. sativa* and *O. glaberrima* in previous studies, new genes, *S37*(t), *S38*(t), *S39*(t) on chromosome 1, 4 and 12, respectively, were named tentatively (Fig. 2).

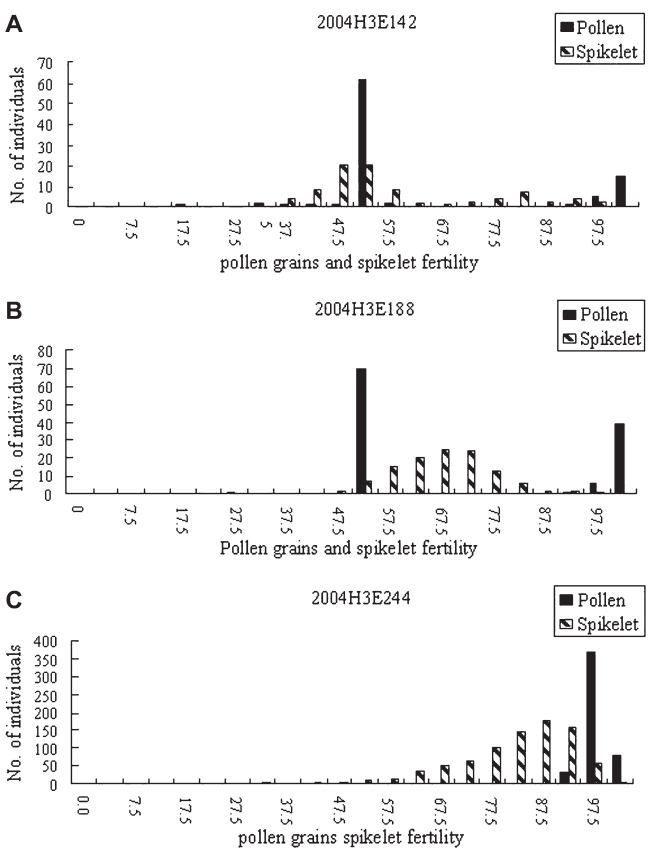

Fig. 1. Distribution of pollen grain and spikelet fertility in three BC_7F_1 mapping populations, 2004H3E142 (IRGC101854/Dianjingyou 1), 2004H3E244 (IRGC102528/Dianjingyou 1) and 2004H3E188 (IRGC103466/Dianjingyou 1), respectively. The data were collected from 2004 Winter Crop Season in Sanya, Hainan, P. R. China.

Genetic pattern and action of new sterile loci

Correlation analysis between pollen grain fertility and spikelet fertility of BC_7F_1 population 2004H3E142 showed that a significant correlation was found for *S37*(t) ($r = 0.780$, $p < 0.0001$), but for *S38*(t) and *S39*(t), the correlation between pollen grain fertility and spikelet fertility was not significant ($r = 0.0097$ and $r = 0.0092$, respectively), indicating that hybrid sterile gene *S37*(t) may synchronously control male and female gamete sterility, or *S37*(t) is a tightly linked pollen and spikelet sterile locus. However, *S38*(t) and *S39*(t) only affect pollen sterility.

Based on the "one-locus allelic interaction model", the semi-sterile phenotype was caused by the allelic interaction between alleles derived from *O. glaberrima* and *O. sativa*. In the backcross population, for a single pollen killer locus, the distribution of semi-sterile/fertile plants should meet a 1 : 1 segregation ratio and for a gamete eliminator locus, all individuals should be semi-sterile. In fact, in population 2004H3E244 (*qSS4*) and 2004H3E188 (*qSS-12*), semi-sterile individuals were significantly fewer than fertile plants, and population 2004H3E142 (*qSS1*) gave some fertile plants (Table 3), indicating that *O. sativa* alleles at these loci are not completely lethal, or sterility was affected by the interaction between detected loci and other unknown loci and further investigations are needed to understand the mechanism.

Pollen and spikelet fertility of NILs and test cross F_1

The closest SSR markers RM449 and RM113 for *S37*(t), RM16251 and RM16260 for *S38*(t), RM5568 and RM7582 for *S39*(t) were used to select homozygous individuals derived from corresponding selfing progenies of BC_7F_1 heterozygous individuals. NIL-*S37*(t), NIL-*S38*(t), NIL-*S39*(t) were raised, which carried 1.0 cM, 2.7 cM, 4.3 cM homozygous introgression segments from *O. glaberrima*,

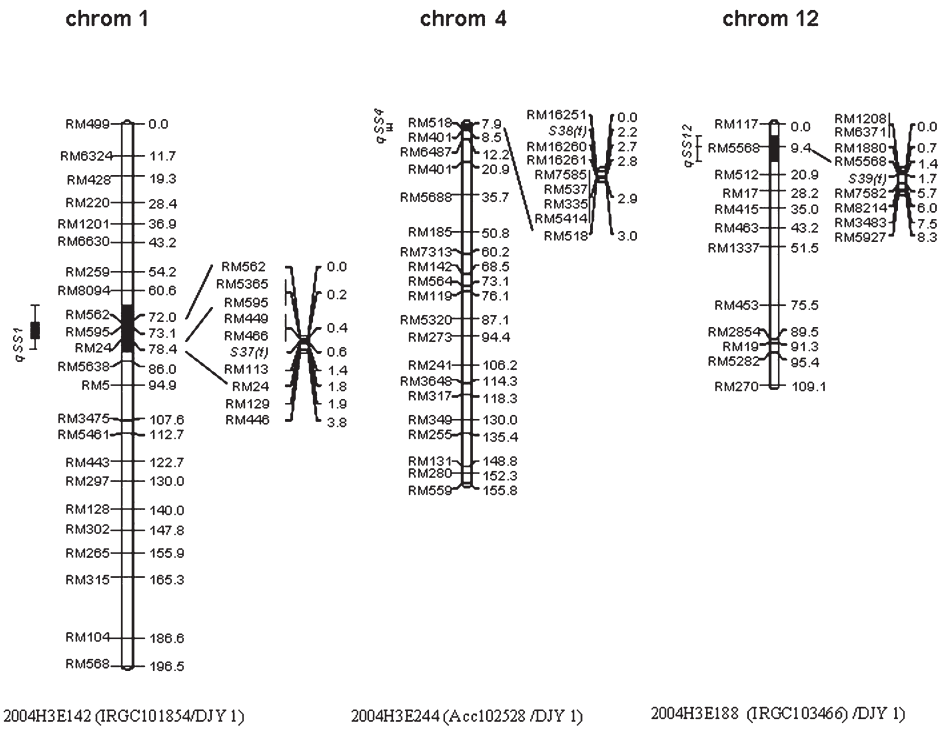

Fig. 2. The positions of QTLs in BC_6F_1 and segmental linkage maps of gene mapped in BC_7F_1 mapping populations for hybrid sterility (black bar represents QTL regions of hybrid sterility deduced with the probability of heterozygote genotypes in BC_6F_1 population. The small-scale linkage map shows the identified sterile loci in BC_7F_1 populations. DJY 1: Dianjingyou 1).

Table 3. Significant test of sterile plants: normal plants ratio by the chi-square test in three mapping populations in 2004 Winter Crop Season in Sanya, Hainan, P. R. China

BC_7F_1 population	Donor	Population size	No. of individuals		X^2 (1 : 1)	P
			Semisterile	Normal		
2004H3E142	IRGC101854	91	70	21	25.32**	X^2 (0.05, 1) = 3.841, X^2 (0.01, 1) = 6.635
2004H3E244	IRGC102528	882	398	484	8.39**	
2004H3E188	IRGC103466	904	420	484	4.53*	

Table 4. Pollen and spikelet fertility of NILs and test cross BC_8F_1 between NILs and DJY 1 in 2009 Late Crop Season in Sanya, Hainan, P. R. China

Materials	Donor	Generations	N	Pollen fertility		Spikelet fertility	
				Mean (%)	STDEV	Mean (%)	STDEV
DJY 1			5	98.73	0.26	98.09	1.13
NIL-*S37*(t)	IRGC101854	BC_7F_3	5	98.82	0.28	95.00	2.16
NIL-*S38*(t)	IRGC102528	BC_7F_3	5	98.82	0.84	94.51	2.24
NIL-*S39*(t)	IRGC103466	BC_7F_3	5	99.18	0.26	92.30	0.89
NIL-*S37*(t)/DJY1		BC_8F_1	5	55.75	2.30	44.12	1.32
NIL-*S38*(t)/DJY1		BC_8F_1	5	46.24	1.42	92.21	0.91
NIL-*S39*(t)/DJY1		BC_8F_1	5	47.91	2.43	93.27	1.47

respectively. Then NILs were crossed with their recurrent parent DJY1 and BC_8F_1 were obtained. All three NILs showed fertile pollen grains and spikelets as the recurrent parent of DJY 1. The test cross F_1, (BC_8F_1), which was obtained by using DJY1 to cross NIL-*S37*(t), NIL-*S38*(t) and NIL-*S39*(t), respectively, showed semi-sterile pollen grains, while the test cross F_1 of NIL-*S38*(t)/DJY1 and NIL-*S39*(t)/DJY1 showed normal spikelet fertility; however, NIL-*S37*(t)/DJY1 exhibited semi-sterile spikelet fertility (Table 4). These results were consistent with the phenotype in BC_7F_1 mapping populations.

Discussion

Diversity of sterility genes and gene action in O. glaberrima

The purpose of this research was to understand the range of interspecific hybrid sterility by using multiple donors of *O. glaberrima*. In this study, we detected 8 sterile QTLs, among which 5 loci, *qSS2*, *qSS3*, *qSS6*, *qSS7a* and *qSS7b*, corresponded to previous sterile loci *S29*(t), *S19*, *S1*, *S20* and *S21* (Doi *et al.* 1999, Hu *et al.* 2006, Taguchi *et al.* 1999, Tao *et al.* 2003) and *qSS1*, *qSS4* and *qSS12*, which were identified as 3 new loci, were named *S37*(t), *S38*(t) and

S39(t), respectively. These results will help to further understand the complexes of interspecific hybrid sterility in *O. glaberrima*. However, the current method, in which only semi-sterile plants were chosen for analysis, led to sterile loci of *S3* and *S18*, which were reported to have high sterility (Doi *et al.* 1998) and some possible recessive sterile loci of *O. glaberrima* based on the BDM model, could not be detected. For the morphology of sterile pollen grains, it was interesting that loci *S19*, *S20*, *S21*, *S29*(t), *S38*(t) and *S39*(t), which were related to male-only function, showed staining abortion with partial starch filling. *S1* and *S37*(t), controlling both male and female gamete fertility, all showed semi-sterility of the spikelet; however, *S1* showed shrunken aspherical pollen grains with typical abortion, whereas *S37*(t) produced nearly 50% small and spherical empty pollen grains and half-stained abortion. *S1* confirmed that epistatic interaction with additional factors was the cause of the female sterility barrier between *O. sativa* and *O. glaberrima* (Garavito *et al.* 2010). *S37*(t) influenced both male and female gamete fertility, but the detailed molecular mechanism needs to be confirmed by further investigation.

Co-linear analysis of detected sterile genes among AA genome species in genus Oryza

Hybrid sterility is the most common sterility of post-zygotic mechanisms between species or subspecies, provides an initial force to maintain the genome stability of species and plays an important role in maintaining species identity (Orr *et al.* 2000, Sano 1986). Several co-linear analyses of hybrid sterility loci between or among species or subspecies have been described using the reported hybrid sterile loci (Chen *et al.* 2009, Hu *et al.* 2006, Lin *et al.* 1993, Sano 1994, Zhao *et al.* 2012, Zhou *et al.* 2010). In this study, *S39*(t) was detected on the end of the short arm of chromosome 12 and comparative mapping indicated that the region of *S39*(t) was close to *S25*(t), which is also a pollen killer in *O. sativa* intersubspecies hybrid and is derived from *indica* IR24 (Kubo *et al.* 2001) and an interspecific sterile gene *S36* was also reported on the end of the short arm of chromosome 12 between *O. sativa* ssp. *japonica* (Taichung 65) and *O. nivara* (IRGC105444). Comparison of map positions of *S36* and *S25* suggested that these two loci might be the same locus (Win *et al.* 2009). These examples of co-linearity among different sterility loci implied that orthologous loci of hybrid sterility might control the reproduction barrier among AA genome species of genus *Oryza*. Further identification and exploration of more hybrid sterile genes from other species of genus *Oryza* will contribute significantly to our understanding of the mechanism of speciation and identify methods of manipulating hybrid sterility between *O. sativa* and its relatives on the AA genome.

Overcoming hybrid sterility for interspecific hybrid between O. sativa and O. glaberrima

One purpose of this study was to overcome hybrid sterility in the process of interspecific hybridization breeding. Overcoming hybrid sterility in early generations will be propitious to introgress more and wider genetic variation. Raising bridge parents of *O. sativa* with hybrid sterility genes from *O. glaberrima* are a convenient tool to surmount or allay hybrid sterility (Tao *et al.* 2003, Xu *et al.* 2005). In our previous research, bridge parents harboring *S1-g* were applied in a breeding program, showing significantly increasing pollen fertility in BC_1F_1 progenies (Deng *et al.* 2010); however, it is still necessary to identify more sterile loci to elucidate their panoramic influence and mutual interaction for controlling sterility. To achieve this, NILs carrying different hybrid sterile locus alleles were considered as a favorable approach. In this research process, a series of NILs (BC_7) carrying different hybrid sterility loci *S1*, *S19*, *S20*, *S21*, *S29*(t), *S37*(t), *S38*(t) and *S39*(t) in a Dianjingyou 1 background were raised, respectively, and these materials will be of benefit to reveal more genetic mechanisms of hybrid sterility.

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