Introduction

The Asian cultivated rice species (*Oryza sativa* L.) is the major food crop around the world. Narrow genetic base in the rice breeding materials confines the genetic gain during improvement of rice (Tanksley and McCouch 1997). The wild relatives of Asian cultivated rice with the same AA genome and similar sequence arrangement as *O. sativa* were deemed to be of valuable source for the genetic improvement of rice (Chang 1976, Ohmido and Fukui 1995). *O. meridionalis* is an annual diploid relative of cultivated rice, which was reported in the northern parts of Australia and Irian Jaya. This wild relative with the same AA genome as the cultivated rice contains favorable genes to the resistance to white-backed planthopper, rice tungro disease, stem elongation ability and resistance to seasonal dry (Thanh et al. 2006, Vaughan 1989, Xu et al. 2005). Therefore, *O. meridionalis* has been widely considered as one of the most accessible genetic resources for the genetic improvement of rice.

However, reproductive barriers frequently exist between *O. sativa* and its wild relatives including *O. meridionalis* (Li et al. 2007). Hybrid sterility is a major form of postzygotic reproductive barriers. The F₁ hybrids barely have enough fertile pollen grains and thus the valuable genes in *O. meridionalis* will naturally disappear along with hybrid sterility. So hybrid sterility is one of the main constrains to transfer favorable genes from *O. meridionalis* to *O. sativa*.

Several genetic models were proposed to explain hybrid sterility. The typical models are the one-locus allelic interaction model (Ikehashi and Araki 1986, Sano 1990), duplicate gametophytic lethal model (Oka 1974) and Bateson-Dobzhansky-Muller (BDM) model (Bateson 1909, Dobzhansky 1936, Muller 1942). With the development of rice genomics and molecular markers, more than 50 loci affecting hybrid sterility in rice have been revealed and a few of them have been cloned (Ouyang et al. 2010, 2013). The S₅, a locus related to embryo-sac fertility in *O. sativa* L. ssp. *indica-japonica* hybrid, is composed of three tightly linked genes regulating fertility (Chen et al. 2008, Yang et al. 2012). Similarly, Sa, a locus for *indica-japonica* hybrid male sterility, consists of two adjacent genes of SaM and

Mapping five novel interspecific hybrid sterility loci between *Oryza sativa* and *Oryza meridionalis*

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*Oryza meridionalis* is a potential source for improving Asian cultivated rice *O. sativa* via direct hybridization and backcrossing. However, hybrid sterility between *O. sativa* and *O. meridionalis* is the main barrier of reproduction hindering the transfer of favorable genes from *O. meridionalis* to *O. sativa*. To investigate the nature of hybrid sterility between *O. sativa* and *O. meridionalis*, three accessions of *O. meridionalis* were used as male parents to cross Dianjingyou 1, an *O. sativa* subsp. *japonica* cultivar following the backcross with the recurrent parent of Dianjingyou 1. Twenty pollen sterility NILs (BC₆F₁) were obtained and genotyped by using simple sequence repeat (SSR) markers distributed across the 12 rice chromosomes. The heterozygous markers were employed to genotype the corresponding segregation populations for mapping sterility genes. As a result, five novel loci for pollen sterility between *O. sativa* and *O. meridionalis* were identified and designated as S₅₁(t), S₅₂(t), S₅₃(t), S₅₄(t) and S₅₅(t), respectively. The genetic behavior of five novel loci followed one-locus allelic interaction model. The disharmonious interaction between Asian cultivated rice allele and wild relative allele led to the partial or full abortion of male gametes for one parent allele in the heterozygotes. These results will be useful for elucidating the mechanism of interspecific hybrid sterility and further utilizing favorable genes from *O. meridionalis* for enhancement of rice breeding.

Key Words: *Oryza sativa*, *Oryza meridionalis*, hybrid sterility, sterility locus.
SaF. The two-gene/three-component interaction model is presented in the effect of Sa on hybrid sterility (Long et al. 2008). The hsa1 locus containing two tightly linked genes causes both F1 and F2 hybrid sterility in Asian cultivated rice (Kubo et al. 2015). OgTPR1 gene plays a positive role on the SI-mediated interspecific hybrid sterility and loss-of-mutation of this gene can rescue male and female fertility in hybrids between O. sativa and O. glaberrima (Xie et al. 2017). The genetic behavior of SI, S5, Sa and hsa1 fitted well with one-locus allelic interaction model. A combination of loss-of-function alleles at two independent loci, S27/S28, was reported as the responsible cause for the F1 pollen abortion was made in the Late Crop Season of 2004 (July–November). F1 hybrids were planted in the Winter Crop Season of 2004 (December 2004–April 2005). The backcross cycles were carried out during the period of 2005 to 2008 in order to obtain BC6F1. BC6F2 and BC6F3 mapping population were planted in the Winter Crop Season of 2008 and Late Crop Season of 2009, respectively.

Investigation of pollen and spikelet fertility

Pollen fertility for all materials was investigated following the instructions of Zhu (1979). Pollen fertility was measured using the anthers collected from the spikelet at one to two days before the anthesis and stored in 70% ethanol (Doi et al. 1998b). Three to four anthers per floret from each plant were mixed and stained with 1% I2-KI solution and more than 300 pollen grains were observed by light microscope. Sterile types were further classified as typically abortive pollen (irregular shape and unstained), spherically abortive pollen (round and unstained) and stained abortive pollen (round and partial brown, or smaller and stained) (Fig. 1) (Li 1980). Three independent microscopic fields were scored for estimation of the percentage of the four types of pollen grains in each plant.

Spikelet fertility of the parents and mapping populations was investigated and scored as seed setting rate of three to five panicles on each plant.

Pollen sterility loci mapping

Leaves from each plant were sampled to extract genomic DNA following the method of Edwards et al. (1991). The

Table 1. The pollen fertility in F1 hybrids between Oryza meridionalis accessions and Dianjingyou 1, an O. sativa subsp. japonica cultivar

| Cross                  | Typical abortion (%) | Spherical abortion (%) | Stained abortion (%) | Sterility (%) |
|------------------------|----------------------|------------------------|---------------------|--------------|
| DJY1/Acc.104498        | 24.06                | 70.28                  | 5.66                | 100.00       |
| DJY1/Acc.101145        | 22.87                | 62.33                  | 14.80               | 100.00       |
| DJY1/Acc.104085        | 34.00                | 59.00                  | 7.00                | 100.00       |

Materials and Methods

Plant materials

Three accessions of O. meridionalis introduced from the International Rice Research Institute (IRRI) as the paternal and donor parents, one cultivar of the Asian cultivated rice (O. sativa L. ssp. japonica “Dianjingyou 1” designated DJY1) as the maternal and recurrent parent, were used to produce F1 plants (Table 1). About ten F1 plants of each cross were grown and used for backcrossing as the maternal parents to obtain BC1F1 progenies. After investigation of pollen fertility, one to ten sterile (pollen fertility <90%) individuals for each donor accession were selected to make backcross with the recurrent parent DJY1 from BC1F1 until BC3F1 (Supplemental Tables 1–3). Totally 20 semi-sterile plants from BC3F1 were obtained including four from Acc.101145, seven from Acc.104085 and nine from Acc.104498. The 20 semi-sterile NILs were used for genotyping and raising BC6F2 or BC6F3 mapping populations through self-pollination of BC6F1 or BC6F2 semi-sterile plants.

All plant materials were planted at Winter Breeding Station, Yunnan Academy of Agricultural Sciences (YAAS), Sanya, Hainan Province, P. R. China. Interspecific hybridization was made in the Late Crop Season of 2004 (July–November). F1 hybrids were planted in the Winter Crop Season of 2004 (December 2004–April 2005). The backcross cycles were carried out during the period of 2005 to

![Fig. 1. Three types of sterile pollen. (a) typically abortive pollen, (b) spherically abortive pollen, (c) stained abortive pollen. The picture is from one of the BC1F1 individual, 2005H3W97-3-2, derived from the donor of ACC.104498.](image-url)
20 BC₆F₁ semi-sterile NILs were genotyped with 237 simple sequence repeat (SSR) markers which are evenly distributed across the 12 rice chromosomes. The PCR protocol was as described by Chen et al. (1997). PCR products were separated on an 8% non-denaturing polyacrylamide gel and detected using the silver staining method of Sanguinetti et al. (1994).

Because the interspecific sterility was heterozygous sterility and selection was based on pollen sterility, the heterozygous markers from NILs were employed to genotype either BC₆F₂ or BC₆F₃ mapping populations for mapping the pollen sterility loci.

Candidate markers linked with pollen sterility were detected based on the ANOVA analysis. Small-scale genetic linkage groups were constructed using MAPMAKER version 3.0 (Lander et al. 1987), with a logarithm of odds (LOD) score of >3.0 and the recombination frequency were converted to cM using the Kosambi function. Small-scale genetic linkage groups with target loci were drawn by Map Chart 2.2 (Voorrips 2002).

Results

Segregation of pollen and spikelet fertility

The F₁ hybrids barely had a fertile pollen grain as shown in Table 1, and the pollen fertility was ranged from 0% to 100% (normal) for all the three donor parents in the offspring of BC₁F₁ populations showing continuous and multiple peaks distribution (Fig. 2a–2e). The result indicated that the sterility was controlled by quantitative trait loci. Furthermore, the frequency distribution of pollen fertility in all of the 20 mapping populations was obviously bimodal distribution, which showed that the QTLs in the BC₁F₁ populations were separated as the simple inheritance factors during the process of successive selection and backcross (Fig. 2d–2h). The spikelet fertility was normal in all of the mapping populations and the pollen fertility was not correlated with the spikelet fertility in all mapping populations, which indicated all loci only related to the pollen fertility instead of spikelet fertility.

Molecular mapping of pollen sterility loci

The heterozygous markers for NILs were employed to genotype the corresponding either BC₆F₂ or BC₆F₃ mapping populations. The ANOVA analysis based on the genotypic and phenotypic data in the mapping populations indicated that there were five loci controlling pollen sterility on chromosome 1, 2, and 7, respectively (Table 2).

Most of the semi-sterile individuals were heterozygous based upon the screening of the target markers and most of the homozygote plants had normal pollen fertility according to the phenotyping. In addition, the average pollen fertility of heterozygotes at target markers was significantly lower than that of homozygotes for O. sativa and O. meridionalis (Table 2). These results indicate that the target SSR markers were linked with the pollen sterility loci.

The linked SSR markers RM5, RM488 and RM3475 on chromosome 1 were significantly correlated with pollen sterility in four mapping populations out of the 20 mapping populations. Linkage analysis revealed that the locus controlling the pollen semi-sterility was located between two SSR markers RM5 and RM488 on the long arm of chromosome 1, with map distances of 2.8 and 1.9 cM, respectively, in the population 2009H2E392 derived from Acc.104498-NIL232-5 (donor, Acc.104498) (Figs. 2d, 3, 4).

The marker RM174 on the short arm of chromosome 2 was obviously related with pollen sterility in only one mapping population. The pollen sterility locus was located in a 5.1 cM region flanked by RM8 and RM6247 on the short arm of chromosome 2 in the population 2008H3E561 derived from Acc. 104498-NIL225-1 (donor, Acc.104498) (Figs. 2e, 3, 4).

RM12368 on chromosome 2 corresponded to pollen sterility in five mapping populations out of the 20 mapping populations. Then, the other three polymorphic markers RM6367, RM110 and OSR17 were further employed to construct the segmental linkage map of the target region. Finally, the pollen sterility locus was located between SSR markers RM110 and OSR17 on the chromosome 2 with the distance of 0.6 and 1.2 cM respectively, in the population 2009H2E382 derived from Acc.101145-NIL209-1 (donor, Acc.101145) (Figs. 2f, 3, 4).

The marker RM1093 was significantly associated with pollen sterility in two mapping populations. One pollen sterility locus was located in a 6.4 cM region flanked by RM1093-RM7454 on the short arm of chromosome 7 in the population 2008H3E555 derived from Acc.104085-NIL220-5 (donor, Acc.104085) (Figs. 2g, 3, 4).

The pollen sterility locus linked with RM234 on the long arm of chromosome 7 was detected in six mapping populations. The pollen sterility locus was finally located between the marker RM234 and RM5623 with the distance of 0.6 and 2.0 cM, respectively, in the population 2009H2E385 derived from Acc.104085-NIL219-1 (donor, Acc.104085) (Figs. 2h, 3, 4).

We could not obtain polymorphic markers correlated with pollen sterility for the rest of two mapping populations from the donor of Acc.101145. For further studies, more markers should be used to detect the polymorphism between the donor parent and DJY1 variety for these two NILs.

Since the sterility loci were not reported in these regions between O. sativa and O. meridionalis from previous studies, the new loci name, S51(t), S52(t), S53(t), S54(t) and S55(t) located on chromosome 1, 2, 2, 7, and 7 respectively, were named tentatively (Fig. 4). Two loci identified on chromosome 1 and 2 derived from the donor of Acc.104498 were named as S51(t) and S52(t), respectively. The locus on chromosome 2 derived from the donor of Acc.101145 was named as S53(t). Two loci located on chromosome 7 derived from Acc.104085 were named as S54(t) and S55(t), respectively.
Mapping five novel interspecific hybrid sterility loci

Genetic pattern and action of new sterility loci

In the five mapping populations, we analyzed the segregation of the semi-sterile plants and normal plants. The distribution of semi-sterile/fertile plants met a 1:1 segregation ratio in the mapping populations (Fig. 2d–2h). These results indicated that the genetic pattern of the pollen sterility loci...
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mozygotes of \( S_{51}(t) \)-meridionalis (fertile) as well as few individuals of \( S_{51}(t) \)-sativa were existed. These results indicated that the interaction between \( S_{51}(t) \)-sativa and \( S_{51}(t) \)-meridionalis led to partial abortion of male gametes carrying the allele of \( S_{51}(t) \)-sativa in the heterozygotes. In \( S_{54}(t) \) mapping population 2008H3E555, the same genetic behavior of gametes abortion was observed and only the genotypes of heterozygotes, homozygotes for \( S_{54}(t) \)-meridionalis and few individuals of \( S_{54}(t) \)-sativa were detected. Based on the data of the markers tightly linked to the pollen sterility loci, the genetic behavior of gametes abortion was estimated. In \( S_{51}(t) \) mapping population 2009H2E392, the heterozygotes (semi-sterile) and the homozygotes of \( S_{51}(t) \)-meridionalis (fertile) as well as few individuals of \( S_{51}(t) \)-sativa were existed. These results indicated that the interaction between \( S_{51}(t) \)-sativa and \( S_{51}(t) \)-meridionalis led to partial abortion of male gametes carrying the allele of \( S_{51}(t) \)-sativa in the heterozygotes.

Table 2. The segregation in mapping populations and genetic behavior of gamete abortion of the pollen sterility loci between \( Oryza \) meridionalis and \( O. \) sativa

| Sterility gene | Generation | Donor parent | Marker\(^a\) Chr | Distance from the marker (cM) | No. of individuals | Marker segregation | Chi-square (1:2:1) | Sterile pollen type | Aborted gamete |
|----------------|------------|--------------|-----------------|-----------------------------|-------------------|-------------------|----------------|----------------|---------------|
| \( S_{51}(t) \) | \( BC_{2}F_{3} \) | Acc.104498 | RM5 1 | 2.8 | 166 | 7 | 84 | 75 | 55.74*** | ♂, Empty abortion\(^d\) |
| \( S_{52}(t) \) | \( BC_{2}F_{2} \) | Acc.104498 | RM6247 2 | 2.6 | 106 | 55 | 51 | – | 57.23*** | ♂, Stained abortion |
| \( S_{53}(t) \) | \( BC_{2}F_{3} \) | Acc.101145 | RM6367 2 | 0.6 | 88 | 36 | 52 | – | 32.36*** | ♂, Stained abortion |
| \( S_{54}(t) \) | \( BC_{2}F_{2} \) | Acc.104085 | RM1093 7 | 1.2 | 165 | 19 | 90 | 56 | 17.96*** | ♂, Stained abortion |
| \( S_{55}(t) \) | \( BC_{2}F_{3} \) | Acc.104085 | RM234 7 | 0.6 | 170 | 91 | 79 | – | 98.27*** | ♂, Stained abortion |

\(^a\) Marker information is from the Gramene website (http://archive.gramene.org/markers), \(^b\) DD, DM and MM indicated DJY1-homozygous, heterozygous and \( O. \) meridionalis-homozygous genotypes, respectively, \(^c\) the pollen fertility (%), \(^d\) Both of typical abortion type and spherical abortion type (Zhang and Lu 1989), and ***; significance at \( P < 0.001 \).

Fig. 3. The chromosome segment substitution lines for mapping the five pollen sterility loci. The black bar means the position of the markers with heterozygous genotype in the NILs and the arrow marked the positions of the sterility loci.

Fig. 4. The segmental linkage maps of pollen sterility loci. The numbers on the left of the vertical bars show the genetic distance (cM) that evaluated in the mapping populations. The numbers in the parenthesis following the marker on the right of the vertical bars show the physical distance (Mb) of the corresponding markers in Nipponbare reference sequence. The genetic distance was obtained in the mapping populations from the interspecific hybrid cross. The cause for the unmatched to exist was discussed in the text.
other hand, different genetic behavior of gametes abortion was observed in other three pollen grain sterility loci. The interaction between S-sativa and S-meridionalis led to full abortion of male gametes carrying the allele of wild relative in the heterozygotes at S52(t), S33(t) and S55(t) loci. As the result, only the genotypes of heterozygotes and homozygotes for S-sativa were existed in the mapping populations (Table 2).

The closest SSR markers RM5 and RM488 for S51(t) as well as RM1093 and RM7454 for S54(t) were used to select homozygous individuals derived from the corresponding selfed progenies of heterozygous individuals in mapping populations. Then, homozygous NILs for S51(t) and S54(t) were raised, which carried 4.7cM and 6.4cM homozygous introgression segments on the target regions from O. meridionalis, respectively. Both of the NILs showed fertile pollen grains and normal spikelet fertility as the recurrent parent of DJY1, whereas the test cross F1 obtained using DJY1 to cross NIL-S51(t) and NIL-S54(t) showed semi-sterile pollen grains. We could not develop the homozygous near isogenic lines for S52(t), S33(t) and S55(t) due to the abortion of male gametes carrying the allele of wild relative. As a result, the individuals carried homozygous introgression segments from O. meridionalis with target locus could not be obtained. The heterozygous individuals with the target introgression segments were kept as NILs for further studies.

**Discussion**

In this study, the semi-sterile plants of the BC_{6}F_{1} from the cross combinations of O. sativa and O. meridionalis were used to advance the mapping populations through self-pollination. Five pollen sterility loci on chromosome 1, 2, 2, 7 and 7 respectively were identified as the pollen killers and were tentatively named as S51(t), S52(t), S53(t), S54(t) and S55(t). The male gametes with the allele of O. sativa in the heterozygote were partially killed at S51(t) and S54(t) loci while the male gametes with the allele of O. meridionalis were fully aborted at S52(t), S53(t) and S55(t) loci. Advanced backcross population was frequently employed in QTL/gene identification. However, the genetic behavior of male gametes abortion could not be fully understood since only the heterozygous and homozygous individuals for the recurrent parent were left in all the backcross populations. Even with tightly linked molecular markers, it is difficult to understand which allele was aborted without other experiment design such as population using BC_{6}F_{1} semi-sterile individual as male parent to make backcross with the recurrent parent. For example, when the semi-sterile plants of S52(t), S53(t) and S55(t) loci were used as the maternal and paternal parents to make backcross with the recurrent parent respectively, the progenies would segregate as 1:1 semi-sterile/fertile, and 0:1 semi-sterile/fertile types, respectively, since the male gamete with the allele of O. meridionalis were fully aborted because of the disharmonious interaction. However, the current method, in which only sterile plants were selected for backcrossing, led to sterility loci having high sterility, like S3 and S18 reported by Doi et al. (1998a), and some possible recessive sterility loci based on the BDM model, could not be detected. In this study, either BC_{6}F_{2} or BC_{6}F_{3} population was used as mapping population, the segregation for spikelet fertility was not observed. Therefore, the pollen sterility loci could be mapped, and the abortion pattern and action of male for different alleles could be understood using tightly linked markers.

The S51(t) detected on chromosome 1 shared the same chromosome region with Sa, which was also considered as a pollen killer in O. sativa intersubspecies hybrid, and was cloned (Long et al. 2008). The sterile pollen of both the S51(t) heterozygotes and the Sa heterozygotes showed similarly empty abortion phenotype (typically abortive pollen and spherically abortive pollen) in I2-KI staining. In addition, the japonica alleles, S51(t)-DJY1 and Sa-J, were not transmitted to the progeny via male gametes. Comparison of the location and the mode of locus action, S51(t) and Sa might be the same locus. There was another interspecific hybrid sterility locus, S37(t), which was located on the short arm of chromosome 1 from the cross between O. sativa and O. glaberrima. The heterozygotes of S37(t) presented semi-sterility behavior in both of pollen and spikelet and acted as the gamete eliminator (Xu et al. 2014). Therefore, the chromosome location and genetic action indicated that S37(t) is quite different from Sa and S31(t).

S53(t) was mapped between SSR marker RM110 and OSR17 on the chromosome 2 and comparative mapping indicated that the region of S53(t) was close to S22(t), which was identified in backcross progeny of O. sativa and O. glumaepaula (Sobrizal et al. 2000b), and another interspecific sterility locus S29(t) was also reported on the same chromosome region between O. sativa and O. glaberrima (Hu et al. 2006). S22(t) worked on the hybrid sterility in one-locus allelic interaction model and the gametes of cultivated rice aborted in the heterozygotes. But S53(t) is quite different from S22(t) and S29(t), the male gametes of O. meridionalis aborted in the heterozygotes. Recently, S22(t) was further dissected as S22A and S22B in hybrid between O. sativa and O. glumaepatula, and the genomic region of S22B corresponded to that reported for S29(t) (Hu et al. 2006, Sakata et al. 2014). Thus, whether S33(t) is a new allele of S22(t) or a new locus needs further studies.

Comparison of map positions also revealed that S54(t) on the top of short arm of the chromosome 7 had good co-linear with S20 and S56(t), which were identified from the hybridization between O. sativa and O. glaberrima (Doi et al. 1999) and between O. sativa and O. glumaepatula (Zhang et al. 2018), respectively. Similar to S20 (Doi et al. 1998b, 1999) and S56(t), the japonica alleles of S54(t) were not transmitted to the progeny via male gametes and the plants with normal pollen fertility were homozygous for O. meridionalis allele in the inbreeding population raising from the semi-sterility individual.
The map position of S55(t) was similar to that of S21 identified from O. glaberrima, O. rufipogon and S23(t) identified from O. meridionalis, respectively (Doi et al. 1999, Miyazaki et al. 2007, Sobrizal et al. 2000a, Yu et al. 2018), whereas S55(t) is quite different from S21 that the allele of O. glaberrima reduced pollen fertility in the heterozygous condition (Doi 1998b). For the S23(t) (Sobrizal et al. 2000a), the allele related to pollen fertility was still unclear. But qHMS7 allele of O. meridionalis was aborted. Even so, it is still highly possible for S55(t) to be a new allele of S21 and S23(t), and to be same locus as qHMS7.

The pollen sterility locus, S52(t), was located on the middle of the short arm of chromosome 2 and no sterility locus related to pollen fertility was reported in this region. Thus it was an exclusive locus for hybrid sterility in O. meridionalis.

However, the physical position values of RM8 and RM12368 in chromosome 2 were unmatched with their order of genetic distance (Fig. 4). The physical position of the markers was from the Nipponbare reference sequence, whereas the genetic distance was obtained in the mapping populations from the interspecific hybrid crosses. It is highly possible for this kind of unmatched to exist since the structural variations (SV) exists even in diverse accessions of Asian cultivated rice (Wang et al. 2018) and the SVs may contribute to the varying degrees of hybrid sterility and hybrid breakdown (Shen et al. 2017).

Several good co-linear relationships of hybrid sterility loci were observed either between or among species or subspecies. Comparison of map positions of S36(t) and S25(t) suggested that these two loci might be the same locus on the end of the short arm of chromosome 12 (Kubo et al. 2001, Win et al. 2009) and an interspecific sterility gene S39(t), a pollen killer from O. glaberrima, was also reported on the same chromosome region (Xu et al. 2014). S1 was deemed the most common hybrid sterility locus responsible for both pollen and spikelet sterility in rice and firstly identified in the interspecific cross between O. sativa and O. glaberrima (Sano 1990). Nowadays, S1 locus was also reported in many different hybrid combinations between O. sativa and its AA genome relatives including O. longistaminata, O. nivara, O. rufipogon and O. barthii (Chen et al. 2009, Yang et al. 2016). The evolution study of the nucleotide variation of the interspecific hybrid sterility S1 showed that only a small genetic change occurred in the ancestral Oryza species and the diverged alleles had co-existed in the primitive gene pool of the genus Oryza (Guyot et al. 2011, Xie et al. 2017).

These loci with good co-linear may be orthologous loci before divergence of these species from their common ancestors, which means there exist a few common and conserved hybrid sterility loci in AA genome species. It is a common thought that the reproductive isolation is the by-product of the accumulation of genetic differentiation between diverging populations (Dobzhansky 1970). Genetic differentiation provides opportunities to increase gene diversity during evolution. The accumulation of genetic differentiation can introduce a new function, divide the original function, or loss the original function (Lynch and Force 2000) and thus the new alleles can be produced. These orthologous loci may have sets of differentiation allele. The action between different alleles produced by genetic differentiation is very important to order species pairs by their time of divergence and further understand the complexes of interspecific hybrid sterility between O. sativa and its AA genome relatives.

**Acknowledgments**

This research was funded by grants from the National Natural Science Foundation of China (Grant Nos. U1502265, 31000704, U1036605).

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