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Identifying neutral allele *Sb* at pollen-sterility loci in cultivated rice with *Oryza rufipogon* origin

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Pollen sterility is commonly found in the intra-specific hybrids of *indica* and *japonica* rice, which is one of the main constrains for the utilization of heterosis between *indica* and *japonica*. Six loci controlling the pollen sterility of F_1 between *indica* and *japonica* have been identified from previous studies. Neutral alleles at each locus are potential to overcome the F_1 pollen sterility associated with the locus. Therefore, exploitation and utilization of neutral alleles are of significant importance. The present research was based on fine mapping of the F_1 pollen-sterility gene *Sb* and the abundant genetic diversity of *Oryza rufipogon* Griff. indigenous to Gaozhou, Guangdong Province (referred to as Gaozhou wild rice). Crosses were made using Taichung65 (with the genotype of $S_b^jS_b^j$ and referred to as E_1) and its near-isogenic line of F_1 pollen sterility gene *Sb* (with the genotype of $S_b^iS_b^i$, E_2) as female parents, and 12 different accessions of Gaozhou wild rice as male parents. F_1 pollen fertility was examined to identify the materials having the neutral alleles at the F_1 pollen-sterility locus. Segregation of 4 molecular markers tightly linked with the *Sb* locus was analyzed in the F_2 populations derived from the F_1s carrying the neutral gene. The pollen fertility related to the 3 genotypes of the molecular markers was also checked by statistical test to determine whether it was consistent with the hypothesis. The results showed that the pollen fertility of two F_1s from one accession of Gaozhou wild rice (GZW099) with E_1 and E_2 was (89.22±1.07)% and (85.65±1.05)%, respectively. Both of them were fertile and showed no significant difference by *t*-test. Segregation of the 3 genotypes of the 4 molecular markers followed the expected Mendelian ratio (1:2:1) in the F_2 populations. There was no significant difference for the averaged pollen fertility of the plants related to the 3 genotypes, suggesting that no interaction exists between the alleles at the *Sb* locus in GZW099 and Taichung65 or E_2. Evidentially, GZW099 carried the neutral gene (named $S_b^nS_b^n$) at the *Sb* locus, which provides valuable theoretical basis and resources for further studying and overcoming the sterility of *indica-japonica* hybrids.

Oryza rufipogon Griff, F_1 pollen sterility, *Sb* locus, neutral gene, Gaozhou wild rice

Vigorous heterosis exists between *indica* and *japonica* hybrids of Asian cultivated rice (*Oryza sativa* L.), but the hybrid sterility between the subspecies has hindered the commercial utilization of the heterosis[1,2]. *Indica-japonica* hybrid sterility is mainly caused by female (embryo sac) or male (pollen) gamete abortion, and studies have indicated that both of them are equally affecting the spikelet fertility of the F_1 hybrids between *indica* and *japonica*[3]. So far, a few loci controlling female and male sterility have been identified[4]. Chinese scientists have made significant progress in studying *indica-japonica* hybrid sterility genes in recent years[5]. Chen et al.[6] successfully cloned the S_5 gene controlling the sterility and the wide compatibility between *indica* and *japonica* hybrids, through map-based cloning. The

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gene encodes Histidine-day radon protease relating to embryo sac fertility. The *indica* allele (S_5^i) and *japonica* allele (S_5^j) differed only by two nucleotides. Compared to typical *indica* and *japonica* rice, the sequence of the wide compatible gene S_5^n lost 136 bp in the functional area of TAAT box. The deletion of a particular sequence made the gene nonfunctional, as a result, the F_1 hybrids showed a normal fertility. Long et al.[4] successfully cloned the *Sa* gene about pollen sterility of the *indica-japonica* hybrid and reported that two adjacent genes (*SaM* and *SaF*) jointly controlled pollen sterility and compatibility in *indica-japonica* hybrids, suggesting that SaM^+/SaM^- interaction leads to the abortion of male gamete (SaM^-) when at least one allele SaF^+ was available. Compatibility gene Sa^n (genotypes for SaM^+/SaM^+// SaF^-/SaF^-) was also found. The results of the research have a strong influence on overcoming the *indica-japonica* hybrid sterility along with the origin and evolution of rice.

Concerning the pollen sterility in F_1 hybrids of *indica* and *japonica*, Zhang and Lu[7,8], and Zhang et al.[9] found at least 6 loci (*Sa*-*Sf*) controlling F_1 hybrid pollen sterility. At these loci, typical *indica* and *japonica* rice carry S^iS^i and S^jS^j alleles, respectively, with multiple alleles existing between typical S^i and S^j. Obvious differences in genetic effects were found, i.e., degrees of gene differentiation if multiple alleles of S^i and S^j have different origins. The alleles from some intermediate types and widely compatible varieties show good compatibility with the *indica* and *japonica* varieties. Zhang[10], Zhang et al.[11] and Zhuang et al.[12] found that these genes were not related to embryo sac sterility but mainly to pollen sterility, and the cytological mechanism of pollen sterility was different at different loci. At present, the *Sa* gene has been cloned[4], the *Sb*, *Sc* and *Sd* loci fine mapped[13–15], and the *Se* locus preliminarily targeted[16]. Among them, the *Sb* locus has been finally de-limited to a region of 27 kb between A8 and A14 markers, and seven ORFs have been identified in the region based on the annotations of the RiceGAAS system.

Gene interaction at each pollen-sterility locus could lead to partly abortive pollen. Therefore, the alleles that do not interact with S^j and S^i alleles (neutral genes for pollen fertility, S^n) have significant importance to overcome the pollen sterility caused by each locus[7–9]. Ding et al.[17,18] verified the presence of neutral gene (S_b^n and S_c^n) for pollen sterility in some *japonica indica*-compatible lines by test crosses. Gaozhou wild rice is a large population in China and has rich genetic diversity[19,20]. Previous studies from our laboratory showed the presence of some neutral genes in Gaozhou wild rice responsible for pollen fertility [21–23]. However, few neutral genes related to pollen fertility from the ancestral *O. rufipogon* have been identified.

Sb locus has an important effect on the pollen sterility in F_1 between *indica* and *japonica*. Oka[1] bred a few near isogenic lines (NIL) of F_1 pollen-sterility genes by successive backcrossing of 11－13 generations, using Taichung65 as recipient parent and five *indica* varieties as donor parents. Among them, the NIL of *Sb* locus E_2 had the most similar genetic background with the recipient parent Taichung65[12], except some genotypic differences at *Sb* locus, i.e. genotypes S^jS^j for Taichung65 and S^iS^i for E_2. Both Taichung65 and E_2 carry the genotype S^jS^j at the other five F_1 pollen sterility genes loci[24]. The model of gene interaction for the *Sb* locus fitted the one-locus sporo-gametophytic interaction model[7–9]. To explore the neutral gene for pollen fertility at the *Sb* locus in Gaozhou wild rice, Taichung65 and its NIL E_2 were used as the genetic testers in this study. Twelve accessions of Gaozhou wild rice were selected to cross with the genetic testers. Analyses were made on pollen fertility of F_1 and F_2 within the pairs of testcross and the segregation of four molecular markers linked tightly with *Sb* locus to identify the neutral allele (S_b^n) at the *Sb* locus. The objective of the study was to provide a theoretical basis for identifying neutral genes responsible for pollen sterility in *O. rufipogon* and offer new germplasms for overcoming sterility in *indica-japonica* hybrids.

1 Materials and methods

1.1 Materials

A *japonica* variety Taichung65 and its NIL of *Sb* locus E_2 were used. 141 accessions of Gaozhou wild rice were collected by our laboratory with the sampling strategy of population in the territory of Gaozhou, Guangdong Province, unified label as GZW001－GZW141, and maintained in the *Oryza* genus germplasm resources conservation base at South China Agricultural University[22]. Twelve accessions namely GZW005, GZW006, GZW011, GZW019, GZW034, GZW075, GZW087,

GZW099, GZW101, GZW124, GZW133 and GZW137 were selected for the study. These accessions are belonging to five populations (Heyakeng, Libecun, Xiangshandong, Shuikudi and Shanditan). E_1 and E_2 were used as the maternal parent to cross with twelve accessions of Gaozhou wild rice to generate F_1s from which F_2 populations were obtained by selfing.

1.2 Pollen fertility analysis

Pollen fertility was assayed according to Zhang and Lu[7], with some minor modifications. Six spikelets per plant were collected from the upper part of the panicle and fixed in FAA solution (Ethanol, Formaldehyde and Acetic acid with a ratio of 89∶6∶5, respectively) and then shifted into 70% ethanol after 24 h. Six anthers from the spikelets were crushed on a microscope slide for pollen fertility. Pollen was stained with 2% I_2-KI solution, and then was observed by the Motic BA200 microscope under 10 × 10 multiple and taken pictures. Fertile and abortive pollens were counted by Motic Images Advance 3.2 software. Pollen grains were classified as fertile and sterile pollen based on their shape, size and staining color. Three spikelets per plant were selected and three fields per spikelet were observed, and the rate of pollen fertility per plant was calculated.

1.3 Molecular markers selection

On the basis of fine mapping of the *Sb* locus, four pairs of molecular markers were used, which were polymorphic and closely linked with the *Sb* locus. Genetic distance between microsatellite markers (PSM59 and PSM9) and *Sb* locus was 0.9 cM, while that of PSM215 was less than 0.9 cM[13,25]. Indel marker A07-55 was 0.15 cM[26] away from *Sb* locus.

1.4 Molecular markers analysis

The DNA extraction and PCR were according to Zheng et al.[27] and Panaud et al.[28], respectively, with some minor modifications. The reaction reagent of 20 μL included 0.15 μmol/L SSR primer, 200 μmol/L dNTP, 1×PCR buffer, 50—100 ng template DNA and 1 U *Taq* polymerase; PCR amplification was performed with the PTC-100 PCR machine with the following profile: 5 min at 94℃ to denature; followed by 33 cycles of 1 min at 94℃, 1 min at 55℃, and 1 min at 72℃; and a final extension period of 72℃ for 5 min to complete the reaction. PCR products were separated with 6% polyacrylamide gels and detected according to Li et al.[24].

1.5 Identification method for the neutral gene S_b^n for pollen fertility

The neutral gene for pollen fertility was identified according to the method described by Ding[17] with some modifications. The candidate tested line was supposed to carry the $S_b^xS_b^x$ genotype at *Sb* locus. Firstly, the candidate tested line was crossed with both E_1 and E_2 to make a pair of test combinations. Because E_1 and E_2 had the same genetic background and differed only at *Sb* locus, the significant difference of pollen fertility in the same pair of test combination was due to the allelic interaction at *Sb* locus and the allelic interactions at the other loci were the same. Thus the influence of the genetic background was reduced. The interaction of the testcross at *Sb* locus would be of the following three conditions.

(i) If $S_b^xS_b^x$ was $S_b^iS_b^i$, the genotype of the F_1 from the testcross between the candidate tested line and E_1 was $S_b^jS_b^i$. Owing to the allelic interaction, the F_1's pollen and the gametes carrying S^j were partly abortive. Correspondingly, the genotypes of the molecular markers linked with the *Sb* locus in F_2 population would show skew distribution with the reduced numbers of the alleles from the E_1 (S^j gamete). The genotype of the F_1 from the testcross between the candidate tested line and E_2 was $S_b^iS_b^i$. Due to the lack of allelic interactions, the pollen of the F_1 was fertile, and the genotypic segregation ratio of the corresponding molecular markers linked with the *Sb* locus in F_2 population would show the normal Mendelian ratio (1∶2∶1).

(ii) If $S_b^xS_b^x$ was $S_b^jS_b^j$, the genotype of the F_1 from the testcross between the candidate tested line and E_1 was $S_b^jS_b^j$. Because there was no allelic interaction, the pollen of F_1 was fertile, and the genotypic segregation ratio of the corresponding molecular markers linked with *Sb* locus in F_2 population would show the normal Mendelian ratio (1∶2∶1). The genotype of the F_1 from the testcross between the candidate tested line and E_2 was $S_b^jS_b^i$. Due to the allelic interaction, the pollen and the gametes carrying S^j allele were partly abortive, and the genotypic segregation ratio of the molecular markers linked with the *Sb* locus in F_2 population would show skew distribution.

(iii) If $S_b^xS_b^x$ was $S_b^nS_b^n$, the genotypes of the F_1s from the pair of testcrosses between the candidate tested line and E_1 or E_2 were $S_b^jS_b^n$ and $S_b^iS_b^n$. Because the allele S_b^n is compatible with both S^i and S^j, no gene interaction would exist at *Sb* locus. Thus the pollen of F_1s was fertile

and showed non-significant difference between the two crosses. The genotypic segregation ratio of the corresponding molecular markers linked with the *Sb* locus in F_2 populations would show a normal Mendelian ratio (1:2:1).

The above method was used to identify the neutral gene for pollen fertility.

2 Results

2.1 Preliminary screening of materials carrying neutral genes for pollen fertility

Based on the results of Yang et al.[29,30] and Lian et al.[22,23], some accessions of Gaozhou wild rice which had relatively high pollen fertility, embryo sac fertility and seed setting rate or their F_1s between Gaozhou wild rice and E_1 having high fertility were selected from 141 accessions of Gaozhou wild rice. A total of 12 accessions, namely GZW005, GZW006, GZW011, GZW034, GZW035, GZW075, GZW087, GZW099, GZW101, GZW124, GZW133 and GZW137 were selected as male parents. E_1 and E_2 were used as female parents. Twelve pairs of testcrosses were obtained between them. The pollen fertility of F_1s is shown in Table 1.

According to the identification method of neutral genes for pollen fertility, the average pollen fertility of F_1s obtained from the testcrosses of GZW006, GZW011, GZW034, GZW101, GZW137 with E_1 was significantly higher than the corresponding F_1s between these wild rice and E_2, showing that the genotype at the *Sb* locus for the five accessions of wild rice was $S_b^jS_b^j$. The average pollen fertility of F_1s from the testcrosses between GZW005, GZW075, GZW133 and E_1 was significantly lower than the corresponding F_1s between these wild rice and E_2, indicating that the genotype at the *Sb* locus of the three accessions was $S_b^iS_b^i$. Among them, the F_1 pollen fertility between E_1×GZW075 and E_2×GZW075 was significantly different but high (>83%), which showed that further investigations are needed to confirm that GZW075 might have the neutral gene for pollen fertility at the other loci. The average pollen fertility of F_1s crossed between GZW087, GZW124 and E_1 or E_2 was high, but the pollen fertility of individual plant in the same testcross was quite different, which showed that the genotype at the *Sb* locus of the two accessions was heterozygous. The F_1 pollen fertility between E_1×GZW019 and E_2×GZW019 was low (about 40%), but had no significant difference, which showed that the genetic background of GZW019 was *indica* type and the reason for low pollen fertility might be due to the highly genetic interaction at some other loci except the *Sb* locus. It might carry the neutral gene for pollen fertility at *Sb* locus, but needed further investigation.

Pollen fertility of the pair of F_1s (E_1×GZW099 and E_2×GZW099) was above 85%, and the pollen fertility of their parents (E_1, E_2 and GZW099) was more than 90% (Tables 1 and 2), showing the normal fertility. There was no significant difference for the pollen fertility of the pair of F_1s by *t*-test (t = 2.199, P = 0.07). Moreover, the pollen fertility was much higher than that of the control group (E_1×E_2, 58.53%). The seed setting rate of the pair of F_1s was above 85%, which showed that other genes for pollen and embryo sac sterility had little effect on the pollen fertility and the seed setting rate of the pair of F_1s. The F_1 pollen fertility for the plants in the same cross had a low standard error, and the genotypes for individual F_1 plants in the same cross were the same, checked by 4

Table 1 Pollen fertility in the F_1s between different accessions of Gaozhou wild rice, E_1 and E_2

Testcross	Pollen fertility (%) (M±SE)	Testcross	Pollen fertility (%)(M±SE)
E_1×GZW005	43.14±3.20	E_2×GZW005	59.24±11.02
E_1×GZW006	82.28±10.86	E_2×GZW006	63.60±31.25
E_1×GZW011	43.99±10.17	E_2×GZW011	30.67±4.39
E_1×GZW019	44.03±3.19	E_2×GZW019	40.41±1.84
E_1×GZW034	59.01±7.49	E_2×GZW034	51.63±4.44
E_1×GZW075	83.75±0.05	E_2×GZW075	92.78±2.70
E_1×GZW087	73.51±8.92	E_2×GZW087	79.66±6.39
E_1×GZW099	89.22±1.07	E_2×GZW099	85.65±1.05
E_1×GZW101	84.66±3.80	E_2×GZW101	75.36±10.63
E_1×GZW124	80.37±12.44	E_2×GZW124	80.10±15.14
E_1×GZW133	61.73±23.70	E_2×GZW133	90.14±2.80
E_1×GZW137	95.51	E_2×GZW137	73.82±16.51

Table 2 Pollen fertility and seed setting rate for E_1, E_2, GZW099 and their F_1s

Parents or crosses	Pollen fertility (%) (M±SE)	Seed setting rate (%) (M±SE)
E_1	93.33±1.08	95.32±0.53
E_2	94.81±1.61	94.56±2.25
GZW099	94.24±0.84	85.20±0.51
E_1×E_2	58.53±2.86	74.28±2.55
E_1×GZW099	89.22±1.07	86.86±1.25
E_2×GZW099	85.65±1.05	86.34±0.47

molecular markers tightly linked with the *Sb* locus. It showed that GZW099 was homozygous at the *Sb* locus. The manifestations of pollen fertility with different genotypes showed that the interactive effects between S_b^j and S_b^i alleles lead to the pollen fertility of F_1 being partially sterile, which is in consistence with the previous findings (Figure 1)[13,24]. There was no interaction between S_b^n and S_b^j, S_b^i, which showed that the allele S_b^n had the compatibility to both S^i and S^j. The above results showed that GZW099 might possess neutral gene for pollen fertility, and further studies should be continued.

2.2 Molecular marker analysis in the paired F_2 populations

The molecular marker PSM59 and PSM9 were highly polymorphic between E_1 and GZW099, and co-dominant for their F_1. The segregation of the three genotypes in their F_2 population is shown in Figure 2 and Table 3. The segregation ratio of the three genotypes in their F_2 population followed the expected Mendelian ratio (1∶2∶1) (P = 0.073) by Chi-squared test, and the average pollen fertility corresponding to the three genotypes in the F_2 population showed non-significant difference by the analysis of variance (P = 0.983). The performance of pollen fertility for different genotypes in the F_2 population is shown in Figure 1. The result suggested that the allele at the *Sb* locus in GZW099 did not interact with that in E_1.

The molecular marker PSM215 and A07-55 were highly polymorphic between E_2 and GZW099, and hence used for F_2 genotyping. The distribution of the three genotypes in the F_2 population is shown in Figure 3 and Table 3. The segregation ratio of the three genotypes in the F_2 population was in consistence with the expected Mendelian ratio (1∶2∶1, P > 0.05), and the average pollen fertility corresponding to the three genotypes in the F_2 population showed no significant difference by the analysis of variance (P = 0.823 and 0.380, respectively). The above results suggested that the allele at the *Sb* locus in GZW099 also did not interact with that in E_2. However, the segregation ratio of the three genotypes in the control F_2 population (E_1 × E_2) deviated from the expected Mendelian ratio (1∶2∶1), and the average pollen fertility corresponding to the three genotypes in the F_2 population also differed significantly[24]. According to the method of identifying neutral genes for pollen fertility, the final result indicated that GZW099 carried the neutral gene for pollen fertility at the *Sb* locus ($S_b^n S_b^n$).

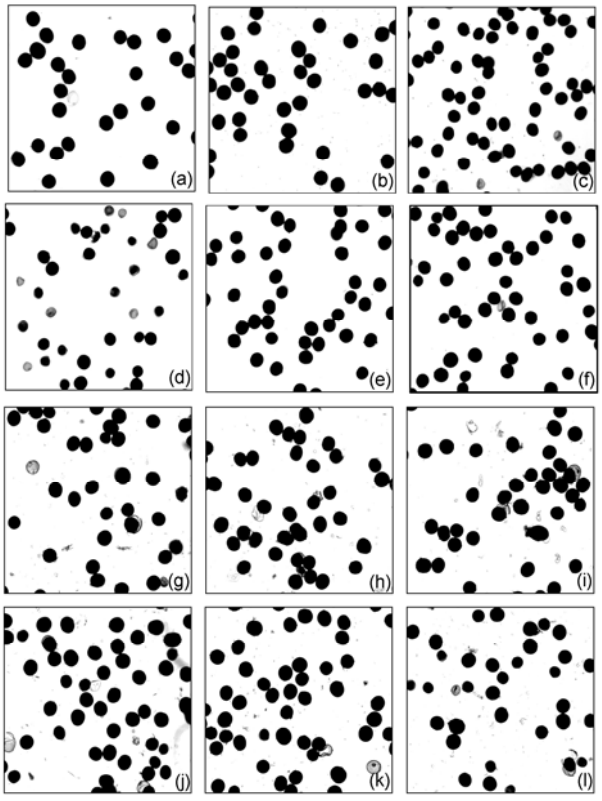

Figure 1 Pollen fertility of different genotypes in E_1, E_2, GZW099 and their F_1s and F_2s. (a) E_1 (genotype $S_b^j S_b^j$); (b) E_2 (genotype $S_b^i S_b^i$); (c) GZW099 (genotype $S_b^n S_b^n$); (d) $F_1$of E_1×E_2 (genotype $S_b^j S_b^i$); (e) F_1 of E_1×GZW099 (genotype $S_b^j S_b^n$); (f) F_1 of E_2×GZW099 (genotype $S_b^i S_b^n$); (g) F_2 of E_1×GZW099 (genotype $S_b^j S_b^j$); (h) F_2 of E_1×GZW099 (genotype $S_b^j S_b^n$); (i) F_2 of E_1×GZW099 (genotype $S_b^n S_b^n$); (j) F_2 of E_2×GZW099 (genotype $S_b^i S_b^i$); (k) F_2 of E_2×GZW099 (genotype $S_b^i S_b^n$); (l) F_2 of E_2×GZW099 (genotype $S_b^n S_b^n$).

Table 3 Genotypic distribution of the molecular markers and their corresponding pollen fertility in the F_2 populations between GZW099, E_1 and E_2

Testcross	Molecular makers	Genotypes	Plant numbers	Pollen fertility (%)(M±SE)	Chi-squared test for genotypes		Analysis of variance for pollen fertility	
					χ^2 value	*P* value	*F* value	*P* value
E_1×GZW099	PSM59	1 (S_b^i S_b^i)	44	84.65±1.90	5.228	0.073	0.017	0.983
		2 ($S_b^i S_b^n$)	87	84.79±1.53				
		3 ($S_b^n S_b^n$)	62	84.29±2.59				
	PSM9	1 (S_b^i S_b^i)	43	84.66±1.95	5.238	0.073	0.017	0.983
		2 ($S_b^i S_b^n$)	88	84.78±1.51				
		3 ($S_b^n S_b^n$)	62	84.29±2.59				
E_2×GZW099	PSM215	1 ($S_b^n S_b^n$)	47	78.60±2.75	1.275	0.529	0.195	0.823
		2 ($S_b^i S_b^n$)	101	76.79±1.96				
		3 (S_b^i S_b^i)	41	78.62±3.34				
	A07-55	1 ($S_b^n S_b^n$)	41	81.29±2.42	1.819	0.403	0.974	0.380
		2 ($S_b^i S_b^n$)	103	76.15±2.04				
		3 (S_b^i S_b^i)	44	77.61±3.23				

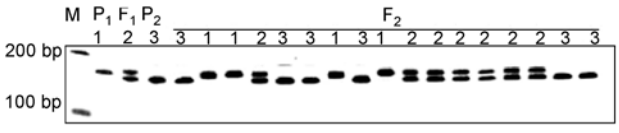

Figure 2 Genotypes of PSM59 in the F_2 population of E_1×GZW099. P_1, Female parent (E_1); P_2, male parent (GZW099); F_1, hybrid (E_1×GZW 099); M, marker, 1−3 indicate maternal genotype (E_1), heterozygous genotype and paternal genotype (GZW099) in F_2 population, respectively.

Figure 3 Genotypes of PSM215 in the F_2 population of E_2×GZW099. P_1, Female parent(E_2); P_2, male parent(GZW099); F_1, hybrid (E_1×GZW099); M, marker, 1−3 indicate paternal genotype (GZW099), heterozygous genotype and maternal genotype (E_2) in F_2 population, respectively.

3 Discussion

Hybrid sterility between *indica* and *japonica* subspecies of cultivated rice is a complex biological phenomenon. The level of expression, the extent of hybrid sterility and the quantity of sterile genes varied in different crosses. Rice wide-compatibility gene (WCG) was first proposed by Ikehashi et al.[31], which offered a bright prospect to overcome the sterility in *indica-japonica* hybrids and to utilize the heterosis between the subspecies. The main method for identifying wide-compatibility gene is testcross. A certain quantity of typical *indica* and *japonica* rice as tester lines was used to cross with the candidate tested varieties and the fertility of F_1 spikelets from the testcross was checked to identify the wide-compatible genes[32]. The shortcoming of the method is that it might lose some materials carrying neutral genes for pollen fertility while sterile embryo sac, because spikelet fertility is affected both by embryo sac and pollen fertility and any abnormality in these factors would lead to spikelet sterility. With the rapid development of molecular marker technology and genome sequencing for *japonica* variety Nipponbare and *indica* variety 9311 completed, especially fine mapping and cloning of *indica-japonica* hybrid sterility genes, the neutral gene and wide-compatibility gene can be identified using functional molecular markers and closely linked molecular markers. Yang et al.[33] used the sequence of a cloned S_5^n gene to design the primers at both ends of the deleted DNA fragments and established the functional molecular markers for S_5^n gene. Ten cultivars from Chinese national micro-core rice collection were successfully detected carrying S_5^n. However, sometimes molecular markers are not equivalent to the gene itself, so the phenotype must be identified by testcross. Therefore, the combination of the two methods described above is the most effective way to find out the neutral alleles.

Genetic background has important effects on the identification of the intra-specific hybrid sterility. Using Taichung65 and Guangluai4 as testers, Ding et al.[17] found the neutral genes for F_1 pollen fertility in *indica*-compatible *japonica* lines. However, the significant differences in the genetic background between Taichung 65 and Guangluai4 might affect the accuracy of the test results. From this viewpoint, Taichung65 and its NIL E_2, which have a similar genetic background, were used in the study. NIL E_2 was bred using Taichung65 as a recipient parent and *indica* variety 144 as a donor parent

through successive backcross of 13 generations[1]. Including 187 RFLP markers and 500 RAPD primers, Zhuang et al.[12] analyzed the polymorphism between Taichung65 and E_2 and found only two RAPD markers linked to *Sb* locus that exhibited polymorphism. It suggests that the genetic background between Taichung65 and E_2 is very similar except for the *Sb* locus after multiple backcrosses[24]. For the wild rice under test, GZW099 with a homozygous S_b locus and consistent F_1 pollen fertility was selected to be crossed with Taichung65 and E_2 in the study thus reduced the impact of the genetic background. Therefore, if one candidate tested accession of wild rice was crossed with the two tester lines simultaneously, the significant difference for the pollen sterility in the pair of testcross was the main cause of allelic interaction at *Sb* locus because of the same allelic interaction at the other loci. The effects of S_b^n could be accurately determined through the experimental design. Results would be more accurate if the wild species under test were the near-isogenic lines. Our research team is constructing single segment substitution lines (SSSL) of F_1 pollen sterile gene from *O. rufipogon*, and BC_4 generation has been bred for further studies.

Most of the pollen or embryo sacs sterility genes for inter-specific or inter-subspecific hybrids coincide with the one locus sporo-gametophytic interaction model[4]. As far as the cloned S_5 gene is concerned, Chen et al.[6] developed a triallelic system where the interaction of three alleles S_5^i, S_5^j and S_5^n at S_5 gene locus controlled the sterility and wide compatibility of hybrids, as the lone locus sporo-gametophytic interaction model. Studies of Zhang and Lu[7,8], Zhang et al.[9], Zhuang et al.[12] have showed that Taichung65 and E_2 only interacted at the *Sb* locus and that the interaction of S_b^i and S_b^j led to the S_b^j allele abortion and stainable abortive pollen. The gene had no effect on the female gamete and fitted to one locus sporo-gametophytic interaction model. Long et al.[4] cloned a pollen sterility *Sa* gene in *indica-japonica* hybrids, and proposed a "two genes/three-component interaction" model in which there were two tightly linked genes *SaM* and *SaF* jointly controlling the pollen sterility and compatibility. In the existence of SaF^+ allele ($SaM^+/SaM^-//SaF^+/SaF^-$ or $SaM^+/SaM^-//SaF^+/SaF^+$), the interaction of SaM^+/SaM^- led to the male gametes carrying the SaM^- allele abortion. When rice varieties having genotypes $SaM^+/SaM^+//SaF^-/SaF^-$ crossed with *japonica* rice ($SaM^-/SaM^-//SaF^-/SaF^-$) and *indica* rice ($SaM^+/SaM^+//SaF^+/SaF^+$), the combinations will be compatibile because of the absence of SaF^+ or SaM^- in hybrids. The genotype is defined as compatibility gene Sa^n. Molecularly, the *Sa* locus consists of two tightly linked genes, and SaF^+ plays the role of an epistatic gene. However, from the viewpoint whether it has the compatibility or not, they can be treated as one functional unit. That is to say, if the genotypes have no interaction with those of *indica* and *japonica*, it can be treated as one neutral gene Sa^n. Therefore, *Sb* locus was also treated as one functional unit in the study. Thus, the *Sb* locus in the wild rice GZW099 has no significant interaction with those of Taichung65 and E_2 and showed good compatibility.

Common wild rice is the ancestral species of cultivated rice (including *indica* and *japonica*). From the phylogenetic point of view, neutral genes for pollen and embryo sac sterility should theoretically exist in common wild rice. Oka[34] found high F_1 hybrid fertility in the crosses between common wild rice from Asia and various cultivated rice. Lu and Pan[35] reported good compatibility of Dongxiang wild rice from Jiangxi Province to both *indica* and *japonica*. After investigation of many cross combinations between cultivated and wild rice, Liang et al.[36] found that the averaged seed set rate of the F_1s between cultivated rice and common wild rice were about 68%, which was obviously higher than that of the hybrids between typical *indica* and *japonica* (10%—30%). Li et al.[21] showed that the pollen and the spikelet fertility of the hybrids between common wild rice and Taichung65 (typical *japonica* rice) or Guangluai4 (typical *indica* rice) was 70%—88% and 61%—89%, respectively, and the correlation between the pollen fertility and the spikelet fertility of F_1 reached a highly significant level. It was presumed that common wild rice and cultivated rice had almost the same loci for the sterility gene, and some alleles in the common wild rice had the compatibility to both of the *indica* and *japonica* alleles. Lian et al.[22] found that the pollen fertility of F_1 between some Gaozhou wild rice and Taichung65 had abundant genetic diversity with some crosses being higher than 85%. It indicated that neutral genes for pollen fertility might exist in Gaozhou wild rice, which can overcome the pollen sterility of hybrids between *indica* and *japonica*. Results from this study indicated the existence of neutral genes ($S_b^nS_b^n$) in Gaozhou wild rice. Other neutral genes at the other gene loci are under further studies.

Sterility genes of hybrids between *indica* and *japonica* play an important role in the evolution of cultivated rice. Systematic study of the genotypes of common wild rice at the F_1 sterility gene loci in *indica* and *japonica* hybrids has a great importance not only for finding the widely compatible genes or neutral genes which can overcome the hybrid sterility between *indica* and *japonica*, but also for clarifying the questions concerning the origin and evolution of cultivated rice.

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