Introduction

Rice is one of the most important staple food crops and feeds more than half of the world population. In recent years, rice yield became plateau due to the narrow genetic basis of parental materials (Tanksley and McCouch 1997). Two cultivated rice species *Oryza sativa* and *O. glaberrima* domesticated from wild species, only retained 10–20% of genetic diversities in wild species (Zhu et al. 2007). Besides the two cultivated species, six wild species belonging to AA genome are the most accessible genetic resources for the improvement of cultivated rice. But hybrid sterility hinders the transfer of useful traits from wild species to cultivated species. Mapping hybrid sterility locus and understanding the genetic nature of hybrid sterility can help to break reproductive barrier and introgress the useful alleles from AA genome wild species for rice breeding. Up to now, several sterility loci have been reported, and *SI* gene, an eliminator for both male and female gametes in the presence of modifier genes (Sano 1990), was found to be responsible for hybrid sterility in the cross combination between *O. glaberrima* and *O. sativa*.

Materials and Methods

Yundao 1 and Dianjingyou 1 are *japonica* irrigated variety from Yunnan province, P. R. China. *O. nivara* (IRGC102167), *O. rufipogon* (IRGC106138), *O. barthii* (IRGC105987) were introduced from the International Rice Research Institute (IRRI). *O. nivara* (IRGC102167) and *O. rufipogon* (IRGC106138) as donor and male parents, were crossed with Yundao 1. Two self-fertilized populations were developed, 2012H2E836 (Acc. 102176/Yundao 1/4/Yundao 1, BC4F2) and 2012H2E840 (acc. 106138/Yundao 1/4/Yundao 1, BC4F2). The population size are 219 and 163.
plants, respectively. *O. barthii* (IRGC105987) as donor and male parent, was crossed with Dianjingyou 1, and 116 individuals of a back-crossed population (2010H3E855, Acc. 105987/Dianjingyou 1/10/Dianjingyou 1, BC10F1) were developed. All plant materials were grown in the paddy field at the Winter Breeding Station, Yunnan Academy of Agricultural Sciences (YAAS) located in Sanya, Hainan Province, P. R. China. Field management followed essentially the agricultural practice in this district.

**Phenotyping**

Pollen fertility was determined using anthers collected 1 to 2 days before anthesis and stored in 70% ethanol (Doi et al. 1998). Six anthers of a spikelet was stained with 1% I2-KI solution, three views were observed by light microscope and pollen grains were classified as typical abortion, spherical abortion, stained abortion and fertile grain. Pollen fertility was the percentage of fertile grains in all pollen grains. The spikelet fertility was determined for each plant by counting fertile and sterile spikelets on the upper half of three panicles after mature.

**DNA extraction and PCR amplification**

DNA was extracted from fresh leaves of each plant following the method of Edwards et al. (1991). The SSR markers were selected based on published molecular map of rice (McCouch et al. 2002). Amplification reactions were performed in 10 ul containing 10 ng DNA template, 1 × buffer, 0.5 μmol/L of each primer, 50 μmol/L of dNTPs and 0.5 U of Taq polymerase. PCR programmed as 94°C for 5 min, followed by 32 cycles of 94°C 30 s, 55°C 30 s, 72°C 40 s, final extension of 7 min at 72°C. The products of PCR amplification were electrophoresed on 8% polyacrylamide gel in 1 × TBE.

**QTL analysis**

For the QTL analysis, linkage maps of the three different populations were constructed using MAPMAKER 3.0 with a minimum LOD score of 3.0 (Lander et al. 1987). The QTL responsible for pollen fertility and spikelet fertility was identified by the interval mapping analysis using the QTL CARTOGRAPHER software package (Basten et al. 1998). The LOD threshold significance level was determined from 1,000 permutation tests, as implemented by the QTL Cartographer (Churchill and Doerge 1994).

**Results**

**Phenotype analysis of three mapping populations**

In the two BC4F2 populations, the pollen fertility and spikelet fertility showed continuous and bimodal distribution (Fig. 1). In the 2012H2E836 population derived from *O. nivara*, peak values of pollen fertility were observed at 50% and 100%, respectively, and peak values of spikelet fertility were observed at 50% and 80%, respectively (Fig. 1A). In the 2012H2E840 population derived from *O. rufipogon* (BC4F2), peak values of pollen fertility were observed at 50% and 100%, respectively, and peak values of spikelet fertility were observed at 50% and 80%, respectively (Fig. 1B). In the 2010H3E855 population derived from *O. barthii* (BC10F1), pollen fertility and spikelet fertility showed bimodal and trimodal distribution, respectively. Peak values of pollen fertility were observed at 60% and 100%, respectively, but peak values of spikelet fertility were observed at 20%, 50% and 100%, respectively (Fig. 1C). Correlation analysis showed that the pollen fertility was significantly correlated with the spikelet fertility in
Mapping QTLs for hybrid sterility in three AA genome wild species

Breeding Science
Vol. 66 No. 3

Table 1. Correlation between pollen fertility and spikelet fertility in the three populations derived from crosses of rice and three AA genome wild species of *Oryza*

| Populations         | Cross combinations      | Donor parent     | Generation | Population size | Population size | Coefficient of correlation | Level of significance |
|---------------------|-------------------------|------------------|------------|-----------------|-----------------|---------------------------|----------------------|
| 2012H2E836          | IRGC102167/Yundao 1/1/1  | O. nivara        | BC$_4$F$_2$ | 219             | 0.79**          | 0.01                      |
| 2012H2E840          | IRGC106138/Yundao 1/1/1  | O. rufipogon     | BC$_4$F$_2$ | 163             | 0.59**          | 0.01                      |
| 2010H3E855          | IRGC105987/Dianjingyou 1/10/Dianjingyou 1 | O. barthii    | BC$_4$F$_1$ | 116             | 0.68**          | 0.01                      |

Table 2. QTLs detected for pollen fertility and spikelet fertility in the three populations

| Populations         | QTL         | Flanking marker | Trait        | LOD | Variance explained (%) | Additive effect |
|---------------------|-------------|-----------------|--------------|-----|------------------------|----------------|
| 2012H2E836          | qHS6-a      | RM190-RM510     | Pollen fertility | 80.52 | 88.24                  | –0.61          |
|                     |             |                 | Spikelet fertility | 56.19 | 80.60                  | –0.23          |
| 2012H2E840          | qHS6-b      | RM190-RM3414    | Pollen fertility | 30.47 | 61.52                  | –2.84          |
|                     |             |                 | Spikelet fertility | 15.35 | 35.20                  | –1.68          |
| 2010H3E855          | qHS6-c      | RM190-RM587     | Pollen fertility | 13.95 | 44.46                  | 26.15          |
|                     |             |                 | Spikelet fertility | 7.72  | 29.01                  | 42.09          |

Mapping of QTLs for hybrid sterility

A set of 452 SSR markers distributed in rice genome were used to detect polymorphism between parents. In the 2012H2E836 population, 13 SSR markers were polymorphic and distributed on chromosome 6, 8, 11, respectively. Genotyping was carried out on 219 individuals besides pollen fertility and spikelet fertility investigation, one major QTL for pollen fertility and spikelet fertility was detected on chromosome 6, designated as qHS6-a. qHS6-a was restricted to the region between the SSR markers RM190 and RM510, explaining 88.24% of the phenotypic variance in pollen fertility and 80.60% of the phenotypic variance in spikelet fertility (Table 2). In the 2012H2E840 population, 44 SSR markers were polymorphic and distributed on chromosome 1, 2, 3, 5, 6, 7, 9, 10, 11, 12, respectively. Genotypes of 163 individuals were investigated besides pollen fertility and spikelet fertility. QTL analysis indicated that a QTL for pollen fertility and spikelet fertility close to RM190 on chromosome 6, designated as qHS6-b, was mapped into the region between the SSR markers RM190 and RM3414, explaining 61.52% of the phenotypic variance in pollen fertility and 35.20% of the phenotypic variance in spikelet fertility. In the 2010H3E855 population, four SSR markers on chromosome 6 were polymorphic, genotypes and phenotypes of 116 individuals were investigated. QTL analysis indicated that a QTL for pollen fertility and spikelet fertility close to RM190 on chromosome 6, designated as qHS6-c, was located in the region between the SSR markers RM190 and RM587, explaining 44.46% of the phenotypic variance in pollen fertility and 29.01% of the phenotypic variance in spikelet fertility. qHS6-a, qHS6-b and qHS6-c were all mapped close to RM190 on chromosome 6 (Fig. 2), where the known *S1* was mapped (Sano 1990, Zhu et al. 2005).

Nature of three hybrid sterility QTLs

A common segregation distortion was found in the mapping region for all three populations (Table 3). Both 2012H2E836 and 2012H2E840 are BC$_4$F$_2$ populations in Yundao 1 background. Segregation ratio of Yundao 1 homozygote, heterozygote, and wild rice homozygote did not follow 1:2:1 ratio, which indicates that for qHS6-a and qHS6-b, the interaction between Asian cultivated rice allele and wild rice allele leads to the partial abortion of male and female gametes carrying the allele of cultivar in the heterozygotes. However, a number of homozygotes of Yundao 1 existed in the two BC$_4$F$_2$ populations indicated that qHS6-a/qHS6-b wild alleles did not eliminate Yundao 1 alleles completely (Table 3). For qHS6-c, the number of homozygotes of cultivated rice Dianjingyou 1 at RM190 was significantly lower than that of heterozygotes (Table 2), which indicated that most cultivar alleles were eliminated...
to correspond to the $SI$ locus (Fig. 2). They had good co-linear and might be orthologous loci, which means a common and conserved hybrid sterility locus, exited in AA genome species, is highly possible orthologous before divergence of these species from their common ancestor.

Similarly, good co-linear relationships were observed between $S22$ from $O. glumaepatula$ and $S29(t)$ from $O. glaberrima$ on chromosome 2 (Hu et al. 2006), between $S21$ from $O. glaberrima$ and $O. rufipogon$, and $S23$ from $O. glumaepatula$ on chromosome 7, between $S39(t)$ from $O. glaberrima$ and $S36$ from $O. nivara$ on the short end of arm of chromosome 12 (Doi et al. 1999, Miyazaki et al. 2007, Sobrizal et al. 2000, Xu et al. 2014), indicating that those loci for interspecific hybrid sterility were conserved in AA genome species, and might play an important role in species maintenance and reproductive isolation.

### Hybrid sterility genes for improving interspecific hybrid sterility

In interspecific hybridization, hybrid inviability and sterility are major obstacles for the utilization of closely related species in breeding programs, impairing the exploitation of the rich genetic diversity found within the genus *Oryza*. Breaking the reproductive barriers is necessary to transfer the favorable genes from rice relatives to the cultivated rice. There are two ways to overcome interspecific hybrid sterility. One is to use the interspecific neutral allele or wide compatibility allele, which does not cause gamete abortion in the hybrids. But unfortunately, the neutral alleles, except for $S6$, have no any effect on overcoming interspecific hybrid sterility (Heuer et al. 2003, Koide et al. 2008). Another way is to introgress the hybrid sterile allele from wild rice into the recurrent parent (cultivated rice) as the introgression lines (ILs), then it might be more accessible to overcome the hybrid sterility by crossing between the ILs as the genetic bridge parents and the wild rice (Tao et al. 2003). Previous report confirmed that $O. sativa$ lines carrying $S1-g$ allele from $O. glaberrima$ can be used as bridge parents to significantly improve the fertility of hybrids between $O. glaberrima$ and $O. sativa$ (Deng et al. 2010). In this study, three major QTLs were identified. Introggression of $qHS6-a$, $qHS6-b$, $qHS6-c$ into the cultivated rice as the bridge parents will help to overcome interspecific hybrid sterility between $O. sativa$ and $O. nivara$, $O. rufipogon$, $O. barthii$, respectively, and to introgress the favorable genes from wild rice to cultivated rice.

### Acknowledgements

This research was supported by National Natural Science Foundation of China (Grant Nos. U1502265, U1036605, 31000704, 31201196), Yunnan Provincial National Science Foundation, China (Grant Nos. 2013FA056, 2011FB118, 2015HB079).
Mapping QTLs for hybrid sterility in three AA genome wild species

Literature Cited

Basten, C.J., B.S. Weir and Z.B. Zeng (1998) QTL CARTOGRAPHER: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh.

Chen, Z.W., Y.F. Hu, P. Xu, J. Li, X.N. Deng, J.W. Zhou, F. Li, S.N. Chen and D.Y. Tao (2009) QTL analysis for hybrid sterility and plant height in interspecific populations derived from a wild rice relative, Oryza longistaminata. Breed. Sci. 59: 441–445.

Churchill, G.A. and R.W. Doerge (1994) Empirical threshold values for quantitative trait mapping. Genetics 138: 963–977.

Deng, X.N., J.W. Zhou, P. Xu, J. Li, F.Y. Hu and D.Y. Tao (2010) The role of S1-g allele from Oryza glaberrima in improving interspecific hybrid sterility between O. sativa and O. glaberrima. Breed. Sci. 60: 342–346.

Doi, K., A. Yoshimura and N. Iwata (1998) RFLP mapping and QTL analysis of heading date and pollen sterility using backcross populations between Oryza sativa L. and Oryza glaberrima Steud. Breed. Sci. 48: 395–399.

Doi, K., K. Taguchi and A. Yoshimura (1999) RFLP mapping of S20 and S21 for F1 pollen semi-sterility found in backcross progeny of Oryza sativa and O. glaberrima. Rice Genet. Newsl. 16: 65–68.

Edwards, K., C. Johnstone and C. Thompson (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19: 1349.

Garavito, A., R. Guyot, J. Lozano, F. Gavory, S. Samain, O. Panaud, J. Tohme, A. Ghesquiere and M. Lorieux (2010) A genetic model for female sterility barrier between Asian and African cultivated rice species. Genetics 185: 1425–1440.

Heuer, S. and K.M. Miezan (2003) Assessing hybrid sterility in Oryza glaberrima × O. sativa hybrid progenies by PCR marker analysis and crossing with wide compatibility varieties. Theor. Appl. Genet. 107: 902–909.

Hu, F.Y., P. Xu, X.N. Deng, J.W. Zhou, J. Li and D.Y. Tao (2006) Molecular mapping of a pollen killer gene S29(t) in Oryza glaberrima and co-linear analysis with S22 in O. glumaepatula. Euphytica 151: 273–278.

Koide, Y., M. Ikenaga, N. Sawamura, D. Nishimoto, K. Matsubara, K. Onishi, A. Kanazawa and Y. Sano (2008) The evolution of sex-independent transmission ratio distortion involving multiple allelic interactions at a single locus in rice. Genetics 180: 409–420.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181.

McCouch, S.R., L. Teytelman, Y.B. Xu, K.B. Lobos, K. Clare, M. Walton, B.Y. Fu, R. Maghirang, Z.K. Li, Y.Z. Xing et al. (2002) Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Res. 9: 199–207.

Miyazaki, Y., K. Doi, H. Yasui and A. Yoshimura (2007) Identification of a new allele of F1 pollen sterility gene, S21, detected from the hybrid between Oryza sativa and O. rufipogon. Rice Genet. Newsl. 23: 36–38.

Sano, Y., Y.E. Chu and H.I. Oka (1979) Genetic studies of speciation in cultivated rice, I. Genic analysis for the F1 sterility between O. sativa L. and O. glaberrima steud. Jap. J. Genet. 54: 121–132.

Sano, Y. (1990) The genic nature of gamete eliminator in rice. Genetics 125: 183–191.

Sano, Y., R. Sano, M. Eiguchi and H.-Y. Hirano (1994) Gamete eliminator adjacent to the wx locus as revealed by pollen analysis in rice. J. Hered. 85: 310–312.

Sobrizal, Y. Matsuzaki, P.L. Sanchez, K. Ikeda and A. Yoshimura (2000) Identification of a gene for male gamete abortion in backcross progeny of Oryza sativa and Oryza glumaepatula. Rice Genet. Newsl. 17: 59–61.

Tankesley, S.D. and S.R. McCouch (1997) Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063–1066.

Tao, D.Y., P. Xu, J. Li, Y.Q. Yang, J.W. Zhou, F.Y. Hu and M.P. Jones (2003) Studies on hybrid sterility inheritance and mapping of sterile genes among near-isogenic lines derived from interspecific hybrid between cultivated rice species Oryza sativa L. and O. glaberrima Steud. Chinese J. Rice Sci. 17: 11–15.

Xu, P., J. Zhou, J. Li, F. Hu, X. Deng, S. Feng, G. Ren, Z. Zhang, W. Deng and D. Tao (2014) Mapping three new interspecific hybrid sterile loci between Oryza sativa and O. glaberrima. Breed. Sci. 63: 476–482.

Zhu, Q.H., X.M. Zheng, J.C. Luo, B.S. Gaut and S. Ge (2007) Multilocus analysis of nucleotide variation of Oryza sativa and its wild relatives: Severe bottleneck during domestication of rice. Mol. Biol. Evol. 24: 875–888.

Zhu, S., L. Jiang, C. Wang, H. Zhai, D. Li and J. Wan (2005) The origin of weedy rice Ludao in China deduced by genome wide analysis of its hybrid sterility genes. Breed. Sci. 55: 409–414.