QTL analysis for hybrid sterility and plant height in interspecific populations derived from a wild rice relative, *Oryza longistaminata*

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Hybrid sterility is a serious barrier in the utilization of wild rice for breeding, but little is known regarding hybrid sterility between the cultivated rice, *Oryza sativa*, and its wild relative, *O. longistaminata*. In order to understand further the nature of interspecific hybrid sterility, pollen and spikelet fertility were investigated in two BC1F2 populations derived from a semisterile individual of BC1F1 between *Oryza sativa* L. and *O. longistaminata*. One main-effect QTL for pollen and spikelet fertility *apsf6* was detected on the short arm of chromosome 6 close to RM587, around it favoring *O. longistaminata* allele was found. Comparing the position and effect with other studies indicated that this QTL coincides with the gamete eliminator, *S1*. It suggests that there exists an orthologous hybrid sterility locus that controls the reproduction barrier between *O. sativa* and its AA genome relatives. QTL mapping for plant height was also conducted in one of the BC1F2 populations. One QTL, *qph1*, was detected on the long arm of chromosomes 1 close to RM6333 and coincides with the semi-dwarf gene, *sd-1*. These new QTL information will increase the efficiency of cultivar development via interspecific hybridization involving *O. longistaminata*, and offset the stage for fine mapping of these QTL.

Key Words: *Oryza longistaminata*, hybrid sterility, plant height, quantitative trait locus (QTL), *S1*, *sd-1*.

Introduction

Considerable attention has been given to wild relatives of crop species in germplasm collections, as they are known to contain a large proportion of the existing genetic variation for these genera. In rice, a large number of accessions have been collected; however the majority of genetic variation in the genus *Oryza* still lies untapped in wild relatives (Tanksley et al. 1997). *Oryza longistaminata* Chev. (*2n*=24, AA) is broadly distributed throughout Africa, and is a tall, rhizomatous, allogamous, perennial and highly diverse relative of *O. sativa*. Some genes such as *Rhz2*, *Rhz3*, and *Xa21* have been identified and have been used for developing perennial rice (Hu et al. 2003, Sacks et al. 2003) and blight disease resistance (Song et al. 1995), but so far *O. longistaminata* has been little utilized for rice improvement. It is considerably diverged from *O. sativa* as compared to the other AA species (Ren et al. 2003, Duan et al. 2007), and is reproductively isolated from the others by hybrid sterility and embryo abortion (Causse et al. 1994, Sacks et al. 2003). To obtain F1, inter-specific progeny, embryo rescue is usually required, and hybrid sterility is a common phenomenon in backcross generations.

With the advent of genetic maps and molecular markers, mapping of quantitative trait loci (QTL) becomes routine. Moreover, referring to the strategies such as advanced backcross QTL analysis (AB-QTL), chromosome segment substitution lines (CSSLs) and near isogenic lines (NILs), favorable QTL from the wild and unadapted germplasm have been transferred into elite breeding lines (Tanksley et al. 1996, Taguchi et al. 2006, Xi et al. 2006). Li et al. (2008) developed forty-four hybrid sterile NILs between *O. sativa* and *O. glaberrima*, and identified four genes for stable hybrid sterility and an epistatic QTL. However, little is known regarding loci affecting the hybrid sterility between *O. sativa* and *O. longistaminata*. Until now, only two pollen killers *S13* and *S34(t)* in *O. longistaminata* on chromosome 1, 2 respectively (Li et al. 2005, Sano 1994) have been identified. As is the case with most other quantitative traits, the phenotype of hybrid sterility is controlled by multiple genes and is conditioned by environment. Thus, we undertook this study to map additional QTL for hybrid sterility and to improve the understanding of the genetic basis of...
hybrid sterility between *O. sativa* and *O. longistaminata*.

In this study, two BC₁:F₂ populations derived from a cross between a rice cultivar RD23 of *O. sativa* and an accession of *O. longistaminata*, were used to detect QTL for hybrid sterility. QTL for plant height were also investigated. These QTL information will increase the efficiency of cultivar development via interspecific hybridization involving *O. longistaminata* and will be used for further research of fine mapping.

**Materials and Methods**

**Plant materials**

The *O. sativa* ssp. *indica* cv. RD23 from Thailand was used as the female and recurrent parent while an unnamed *O. longistaminata* accession, originally collected from Niger and kindly provided by Hiroshi Hyakutake (Institute of Physical and Chemical Research, Saitama, Japan), was used as the male parent to produce the F₁ individual of the RD23/ *O. longistaminata* cross (Tao et al. 2000, Hu et al. 2003). A series of BC₁:F₁ populations were raised from an initial F₁ single plant via consecutive backcrossing. In the process, plants of pollen grain fertility below 90% were selected as female parent, and RD23 as male and recurrent parent. Two NILs of BC₁:F₁, marked as NIL-L2 and NIL-L3, were selected from the selfed progenies of a BC₁:F₁ population derived from a semisterile individual of BC₁:F₁. Two BC₁:F₂ populations, 2006H₁:E1578 and 2006H₁:E1580, which had 189 and 475 individuals respectively, were constructed via further backcrossing.

**Phenotypic evaluation**

The BC₁:F₂ populations and their parents were grown in the Late Crop Season (July–October) in 2006, at the Winter Breeding Station, Yunnan Academy of Agricultural Sciences (YAAS), located in Sanya, Hainan, China. Pollen was collected from the anther of spikelets at 1 to 2 days before anthesis and stored in 70% ethanol, and pollen sterility was measured as the percentage of pollen grains stained with 1% I₂-KI solution (Doi et al. 1998). Spikelet fertility was calculated as the percentage of filled spikelets per panicle for each of the individuals involved. Plant height was investigated as the distance (cm) from the ground to the tip of the tallest panicle (excluding the awn).

**Genotype determination and QTL analysis**

DNA was extracted from the leaf of each plant based on the CTAB method, and Polymerase chain reaction (PCR) was performed according to Temnykh et al. (2000). 415 simple sequence repeat (SSR) markers covering the rice genome (McCouch et al. 2002) were selected to survey the DNA extracted from the parents (RD23 and NILs). Polymorphic markers were used to genotype the two mapping populations. The linkage map was constructed using MAPMAKER/EXP 3.0 (Lincoln et al. 1992) with the Kosambi mapping function at a minimum logarithm of odds (LOD) grouping threshold of 3.0. QTL responsible for the measured traits were identified by the composite interval analysis using the QTL Cartographer software package (Basten et al. 1998) with a minimum LOD score of 2.5. The QTL effects, as the phenotypic variance explained (PVE) were calculated at the same time.

**Results**

**Segregation of traits**

Pollen and spikelet fertility was normal in RD23 and NILs. Semi-sterility occurred in heterozygous F₁ (data not shown), which indicated the NILs carried hybrid sterility gene(s) introgressed from *O. longistaminata*. In F₂ populations, spikelet fertility showed approximately normal distributions, but pollen fertility was nearly bimodal in all populations (Fig. 1A and 1B). Plant height of NIL-L3 nearly equals that of RD23, and little segregation for plant height was observed in the 2006H₁:E1580 population. Plant height of NIL-L2 was higher than RD23, with segregation ranging from 60 to 140 cm with continuous variation in the 2006H₁:E1578 population (Fig. 1C). They both fit for QTL mapping.

**Introgression region**

415 SSR markers from all 12 chromosomes were used for polymorphism survey. Of these markers, 24 (5.78%) markers on chromosomes 1, 2, 5, 6, 7, 8 between RD23 and NIL-L2, and 15 (3.61%) markers on chromosomes 1, 5, 6, 7, 8 and 10 between RD23 and NIL-L3 (ESM 1) gave polymorphic patterns. In the process of forming NILs, true hybrids were verified via selection of sterile phenotypes. Even after 7 times backcrossing, these introgressions were much higher than expected (0.78%) and many regions were not replaced by the recurrent parent. It’s reasonable to deduce that these regions are linked to the selected characters. On the other hand, some regions were common in both NILs, but more introgressions remained in NIL-L2, and NIL-L2 is taller than NIL-L3. The common introgressed regions from the *O. longistaminata* genome may control hybrid sterility and those specific regions in NIL-L2 may be responsible for the plant height segregation.

**QTLs controlling hybrid sterility and plant height**

One major QTL for pollen and spikelet fertility was detected on chromosome 6 close to RM587 (Fig. 2). The phenotypic variance explained by the QTL ranged from 5.91% to 10.93% for pollen fertility and from 1.95% to 12.35% for spikelet fertility, in 2006H₁:E1578 and 2006H₁:E1580 respectively. When comparing with a previously reported result (Sano 1990), this position coincided with the gamete eliminator, *Sl*. Meanwhile, a strong segregation distortion was also found in this region, and most alleles of RD23 were eliminated (Table 1). This phenomenon of genotypic skewness was explained by one-locus sporophyte-gamete interaction mode (gamete elimination) (Sano 1990), although
the phenotype of two families were not all recovered from semi-sterility, especially the spikelet fertility. Undetected minor QTLs and incompletion of elimination of gametes (Sano 1990) can also explain this phenomenon.

One QTL controlling plant height, qph1, near to RM6333 on chromosome 1 was detected in population of 2006H2E1578 (Fig. 2). The percentage of phenotypic variance explained for plant height is 24.79%. By comparing to the previously reported result (Monna et al. 2002), the locus coincided with the semi-dwarf gene, sd-1.

**Discussion**

**Strategy to study sterility between O. sativa and O. longistaminata**

Among interesting traits of wild rice, hybrid sterility can act as a strong barrier to genetic recombination and limit favorable gene transfer during inter-specific crosses. Sterility arising from variable QTL contributions (Xu et al. 1997), epistatic interactions (Kroymann et al. 2005) and different combinations (Sano 1990) may cause a number of effects on sterility. The complexity of hybrid sterility makes study arduous and hard. The basis for identifying and isolating various genes involved in sterility, and analysis of their characteristics separately, require the development of special purpose populations. In primary populations harboring sterile genes derived from O. longistaminata, as F2 or BC1F1, the fertility was quite low and abnormal. However, in BC5F1, the fertility is approximately semi-sterile, which suggested that some major QTL were separated as single factor. Several other studies in our laboratory demonstrated that compared to using primary mapping populations, the use of QTL-NILs and derived segregating populations is a powerful
strategy for estimating the gene action of QTL and for fine mapping of QTL underlying complex hybrid sterility and agronomic traits in rice (Li et al. 2008).

Orthologous locus in A4 genome species of genus Sativa

One significant QTL for hybrid sterility was detected on chromosome 6 (Fig. 2). Comparison of location and effect with sterile genes reported, it coincided with gamete eliminator S1, which was found in O. glaberrima (Lorieux et al. 2000, Koide et al. 2008). Genetic analysis revealed that the action of this gene leads to a violation of Mendelian inheritance by preferential heredity of a particular chromosome or allele of O. longistaminata. This effect on segregation distortion was in accordance with one-locus sporophyte-gamete interaction mode of gamete elimination (Sano 1990). From the analyses of various combinations of O. sativa/ O. glaberrima, hybrid sterility was considered to be caused by allelic interactions, and S1 was one of major sterile genes in many hybrid combinations (Li et al. 1997, Oka 1974, Sano 1983, 1986, 1990). Considering the same location and effect, QTL mapped in this study and S1 are likely to be alternative alleles from different species at the same locus. Similarly, good co-linear relationships were observed between S22 (O. glumaepatula) and S29(t) (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 (Doi et al. 1999, Sobrizal et al. 2000, Miyazaki et al. 2007), between S1 in O. glaberrima and S10 in O. sativa spp. japonica on chromosome 6 (Heuer et al. 2003) have been reported, which indicates that there exists at least some common (orthologous) hybrid sterility loci controlling the reproductive barriers between O. sativa and its AA genome relatives O. glaberrima, O. glumaepatula, O. longistaminata, and O. rufipogon.

Interestingly, the same results for sd-l locus were reported from O. rufipogon (Xiong et al. 1999), which indicated that it is highly possible to have an orthologous locus sd-l for plant height among O. sativa, O. rufipogon, and O. longistaminata.

Segregation distortion and sterility loci

The genetic basis of segregation distortion may be one or several of the abortion of male and/or female gametes, the selective fertilization of particular gametic genotypes, or the selection of zygote (Xu et al. 1997). Strong segregation distortions were observed in some chromosomes (Table 1). From the F2 population of the hybrid used in this study, segregation distortion of allelic frequencies were biased from the expected 1:1 ratio at 73 markers (40%) in 18 genomic regions, including 40 markers on chromosomes 1–6, 9, and 11 favoring the O. sativa allele and 33 on chromosomes 1, 3, 8, 9, and 12 favoring the O. longistaminata allele, respectively (Hu et al. 2003). Around the locus of S1 detected by this study, a segment favoring RD23 alleles occurred. Nevertheless in the present study of BC1F2, the O. longistaminata allele was strongly favored. This is the same as sex-independent transmission ratio distortion system responsible for reproductive barriers between Asian and African rice species by Koide et al. (2008). These results suggested that the mechanism of S1 was complex and the investigation of distorted segregation in advanced populations further improved our understanding of the genetic nature of hybrid sterility, and it is necessary to use secondary populations to study segregation distortion and sterility inheritance besides preliminary populations. The difference could be explained by linkage or/and epistasis since only a short segment was introgressed from the donor O. longistaminata parent.

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