Development and molecular characterization of a doubled haploid population derived from a hybrid between *japonica* rice and wide compatible *indica* rice

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Doubled haploid (DH) populations, particularly those from subspecies crosses possessing the wide compatible gene S5n, are important germplasm resources for rice genetic studies and breeding, but their feature and potential have not been fully assessed and explored. In the present study, we produced a DH population from the hybrid of *japonica* 668B and wide compatible *indica* T23. Genotyping of the S5 locus with allele-specific markers for ORF3, ORF4 and ORF5 revealed a potential recombination hot spot in the ORF3-ORF4 region. Haplotype analysis revealed that 21/34 subspecies specific Indel markers segregated in distortion in the DH population, with a few lines having *indica* alleles either extremely low (1.7%) or high (98.3%), with little effect of the S5 allele. While DH lines with the S5n allele had higher frequency of *indica* alleles, no effect of the S5n allele was observed on all agronomic traits but flowering time. Taken together, the present study advanced understanding of the genetics of wide crosses in general, and DH production in particular between the two rice subspecies, and the new DH population generated will become a useful resource for rice genetic study and breeding in the future.

**Key Words:** doubled haploid, wide compatibility, S5 locus, segregation distortion, Indel markers, haplotyping.

## Introduction

Asian cultivated rice (*Oryza sativa* L.) is a very important food crop in the world. Traditionally it was classified into *indica* and *japonica* subspecies (Oka 1988, Vaughan et al. 2008). Recent genomic studies have shown that cultivated rice can be subdivided into 5 groups, i.e. *indica*, tropical *japonica*, temperate *japonica*, Aus and aromatic, with *indica* and temperate *japonica* being the most diverged groups (Garris et al. 2005, Vaughan et al. 2008). The *indica* and *japonica* subspecies hence hold great genetic variations and have been used for generating various genetic populations for genetic studies, such as gene and quantitative trait loci (QTLs) mapping.

Hybrids of typical *indica* and *japonica* rice are usually highly sterile. Early studies indicated that the reproductive barrier between the two subspecies was conditioned by the

S5 locus located on chromosome 6 (Ikehashi and Araki 1986), and a neutral allele (S5n) can overcome this barrier because hybrids of either *indica* (with an *indica* allele S5j) or *japonica* (with S5j) rice crossed to rice carrying S5n is fertile, hence S5n is also referred to as a wide compatibility gene (Ikehashi and Araki 1986). Yang et al. (2012) has uncovered the molecular mechanism of wide compatibility in rice. They showed that the S5 locus contains three tightly linked genes (i.e. open reading frames ORF3, ORF4, ORF5) that encode proteins of different function. The three genes jointly determine the compatibility of two rice lines in a system nicknamed as “killer-protector system”, with the ORF5 as “killer”, ORF4 as “partner” of ORF5, and ORF3 as “protector” (Yang et al. 2012). In addition to the S5 locus, there are a number of other loci that are associated with segregation distortion (SD) in various populations derived from *indica* and *japonica* crosses (Nakagahara 1972, Reffinur et al. 2014 and references within).

As permanent populations, doubled haploid (DH) populations are unique genetic resources that have various applications in plant genetics and genomics studies. In rice, DH populations have been developed from a number of *indica*...
and *japonica* hybrids through anther culture and have been extensively used for QTL mapping and other studies (see review by Forster et al. 2007). However, no DH population is known to have a parental line that carries the wide compatible gene S5\(^n\). The lack of S5\(^n\) would limit the usage of DH lines, e.g. in studies of heterosis involving subspecies because the S5\(^n\) is critical for the fertility of *indica/japonica* hybrids (Ikehash and Araki 1986). Also, the S5 locus is a locus that showed significant segregation distortion (SD) in progenies of subspecies crosses due to selective abortion of female gametes (Yang et al. 2012), but it is yet unknown whether it could also affect male gametes development in anther culture, hence affecting the genetic structure of the DH populations. Therefore for use in both practical breeding and genetic studies, it is highly desirable to develop new DH populations that carry S5\(^n\).

In the present study, we developed a DH population by anther culture from the cross of a *japonica* line 668B and a wide compatible *indica* line T23, both are excellent super hybrid rice breeding lines in our breeding program. We further characterized the DH population using subspecies specific molecular markers and allele-specific markers of the S5 locus, and explored some genetic features of inter-subspecies anther culture in rice.

**Materials and Methods**

**Anther culture and DH population development**

668B is a *japonica* rice and is the maintainer line of the Boro-II Type (BT) cytoplasmic male sterile (CMS) line 668A; T23 is a wide compatible *indica* line, which can restore the fertility of both BT- and wild abortive (WA) CMS lines. Both 668A and T23 have been used for commercial hybrid rice breeding production (Gu XH, personal communication). For production of DH population, 668B was artificially emasculated and pollinated with T23, and their F\(_1\) were used for anther culture.

Young panicles covered with leaf sheath were collected at the developmental stage of uninucleate pollens and subjected to cold pre-treatment (5°C, 5–7 d). After surface-sterilization with 70% ethanol (30 s), the outer sheathes were removed and florets with anthers were treated with 20% sodium hypochlorite (15 min), followed by rinsing five times with sterile distilled water. Sterilized florets were dissected and anthers with uninucleate pollens were picked out and cultured on SK3 medium (Chen et al. 1978) supplemented with 2,4-D (1.5 mg/L), casein hydrolysate (0.3 g/L) and sucrose (60 g/L), and phytagel (4 g/L) at pH 5.8 for callus induction in dark at 25°C.

After about 25 days, calli (2–3 mm) gradually emerged on anther surface and were transferred to N6 medium (Chu and Bi 1975) supplemented with 6-BA (3 mg/L), casein hydrolysate (0.3 g/L), NAA (1 mg/L) and sucrose (30 g/L) at pH 5.8. The subcultured calli were cultured in a photosynthetic environment (14 h day with light intensity of 66 \(\mu\)E m\(^{-2}\) s\(^{-1}\) and 8 h in dark) at 28°C for differentiation and regeneration. Regenerated plantlets were then transferred to rooting medium (1/2 N6 medium supplemented with sucrose (20 g/L), colchicine (4 mg/L) and agar (7 g/L) at pH 5.8 under light. Plantlets with good root system were transplanted to paddy field after acclimatization.

Survived plantlets were grown in the Winter Breeding Nursery Farm of Zhejiang University in Lingshui, Hainan in the winter season of 2012, they were grown into mature plants. A good portion (around 70%) of the plants produced seeds in the spring of 2013. Seeds of those plants were harvested on plant basis, which formed the starting lines of the DH population to be further characterized. The whole set of DH lines were again grown in the summer season of 2013 in the Experimental Farm of Zhejiang Zhijiang Seed Co. Ltd. (Yuhang District, Hangzhou, Zhejiang), and in 2014 in the Experimental Farm of Jiaxing Academy of Agricultural Science (Jiaxing, Zhejiang). For the field experiment in Zhejiang Zhijiang Seed Co. Ltd, seeds of DH lines were sown in May 15, 2013 on seedling beds, together with their parental lines 668B and T23; seedlings of 25 d old were transplanted in a paddy field, one seedling per hill. Each line were grown in two rows, each row had 6 plants spacing at 20 cm × 20 cm. Due to the large number of DH lines, the experiment was performed without replication. The heading dates were recorded in the field, the plant height and number of panicles per plant were determined at maturity for three inner plants of each line; the number of florets per panicle and seed-set rate were determined after harvest for the same three plants by counting the five biggest panicles of each plant (if the plants had less than 5 panicles, all panicles were determined).

**Genotyping of DH lines for the S5 locus and subspecies specific markers**

Genomic DNAs were extracted from seeds of each DH line according to Liu et al. (2012). For genotyping of the S5 locus, three markers, i.e. S5-3 and S5-4 were used to differentiate ORF3\(^+\) from ORF3\(^–\), and ORF4\(^+\) from ORF4\(^–\), respectively, and S3-5 for distinguishing ORF\(^{\text{Sn}}\) from ORF\(^{\text{Sn}}\) (Supplemental Table 1, Fig. 1A), according to Yang et al. (2012). The 40 Indel markers that were proposed to effectively differentiate *indica* and *japonica* subspecies of rice (Lu et al. 2009, Xiong et al. 2010, 2011) were also adopted. After testing the 40 subspecies specific Indel markers for 668B and T23, thirty four of them were selected for genotyping the DH lines based on their reproducibility. All primers were synthesized in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

PCRs were performed in a 20-\(\mu\)L volume on a PTC-200 thermocycler (Bio-Rad laboratories, Inc.), containing 1 mmol/L buffer, 1 mmol/L each of dNTP, 10 mmol/L of forward and reverse primers, 20 ng of genomic DNA and 0.6 units of Taq polymerase (TaKaRa Inc.). The PCR program was set as following: 4 min at 94°C followed by 35 cycles of 40 s at 94°C, 30 s at 55°C and 40 s at 72°C, and then 7 min at 72°C for final extension. PCR products were
separated through electrophoresis on 3% agarose gels, or on 8% polyacrylamide gels and stained with silver nitrate method (Bassam et al. 1991).

The two varieties, 93-11 and Nipponbare, both of which the genome has been sequenced in the International Rice Genome Sequencing Project (IRGSP 2005), were used as standard indica and japonica rice in the present study, as were in the studies that established the subspecies specific Indel markers (Lu et al. 2009). The Indel markers were scored by allele size and used for assessment of the frequency of indica (Fi) or japonica (Fj) specific alleles for each DH line.

Results

DH population development

A total of 282 independent lines, each containing one or a few plantlets that were regenerated from separated calli, were produced through anther culture of the 668B × T23 F1 hybrid. Among them 202 lines produced seeds (doubled haploids, DHs) while the remaining did not (haploids). The DH2 seeds were grown into plant lines and subjected to genotyping using the subspecies specific and S5 locus specific molecular markers. Among the 202 lines, six lines were heterozygous for several Indel markers and at the S5 locus, e.g. line 46 in Fig. 1B, 1C. Those six lines were excluded from further analysis and the remaining 196 lines were subsequently grown up to DH4 generation and showed consistent performance.

Subsequent haplotype analyses (see below) revealed that a number of DH lines have identical haplotypes to one or a few other lines, i.e. lines 3, 31 and 98; lines 8, 14, 36 and 39; lines 35 and 42; lines 57 and 58; lines 67 and 68; lines 70 and 72; lines 75 and 113; lines 128 and 135; lines 144, 148, 151, 172 and 177; lines 153 and 173; lines 156 and 188; lines 199 and 200 (Supplemental Table 1). The haplotype grouping is supported by some basic phenotypes, particularly, the plant height (Data not shown). They may be siblings derived from the same callus during the regeneration process. Therefore, the redundant lines were excluded and only 178 unique lines formed the new DH population.

Haplotype analysis of the S5 locus

The S5 locus contains three tightly linked genes, i.e., ORF3, ORF4, ORF5, the ORF4 and ORF5 are located only 0.8 kb apart in opposite transcription directions, and the ORF3 is 9.2 kb away from ORF4 (Fig. 1A). Three ORF specific Indel markers (Fig. 1B) were developed and used for genotyping the DH lines. Majority (174/178) of the DH lines had the haplotype identical to that of either 668B (ORF3+/ORF4+/ORF5+), e.g. lines 9 and 10, Fig. 1B) or T23 (ORF3+/ORF4+/ORF5n, e.g. lines 1 and 2, Fig. 1B), but

![Fig. 1. Genotyping of part of the DH population derived from a hybrid of 668B and T23 with three S5 locus markers and two representative Indel markers. A, Physical map of the S5 locus and the primer positions of S5-3, S5-4 and S5-5 for ORF3, ORF4, ORF5; B, Genotype of some individuals of the DH population by S5-3, S5-4 and S5-5 markers on 8% polyacrylamide or 3% agarose gels. C, Genotypes of two Indel markers on 8% polyacrylamide gels. P1, P2 are parents. 1, 2, 9, 10, 24, 46, 90, 98 and 180 are DH lines. M indicates DNA standard markers.](image-url)
four lines showed a new haplotype: \( \text{ORF3}^+/\text{ORF4}^+ /\text{ORF}^5 \), i.e. lines 24, 90, 98 and 180 (Fig. 1B), which is likely due to a recombination between \( \text{ORF3} \) and \( \text{ORF4} \). Significantly greater \((P < 0.01)\) numbers of DH line with the wide compatible haplotype \((n = 109)\) than the \( \text{japonica} \) haplotype \((n = 69)\) were observed (Fig. 2). Detailed haplotype information for each DH line at the \( S5 \) locus is provided in Supplemental Table 1.

**Haplotypes of DH lines revealed by subspecies specific markers**

The haplotype of each DH line based on the 34 Indel markers is provided in Supplemental Table 1 and the genotyping results of some representative markers are shown in Fig. 1C. Further analysis revealed that there were significant segregation distortions (SDs) of the \( \text{indica}/\text{japonica} \) alleles in the DH population. Less than half (13/34) of the markers fit the 1:1 segregation ratio, others were either over represented by the \( \text{indica} \) allele (12/34) or by the \( \text{japonica} \) allele (9/34) (Fig. 2). A few marker loci were dominated by either the \( \text{indica} \) alleles (e.g. \( \text{R9M42}: 175/178; \text{R12M16}: 165/178 \)) or by the \( \text{japonica} \) alleles (e.g. \( \text{R2M24}: 174/178; \text{R7M37}: 168/178; \text{R2M50}: 169/178; \text{R6M44}: 175/178 \)) in the DH population (Fig. 2).

To assess effect of the \( S5 \) locus on segregation distortion, the DH population is divided into sub-population 1 with the \( S5^i \) (Sub-5n) and subpopulation 2 with the \( S5^j \) allele (Sub-5j). Overall, majority of the SDs detected in the whole DH population remained in both sub-populations, i.e. 20/21 in Sub-5j (Supplemental Fig. 1) and 18/21 in Sub-5n (Supplemental Fig. 2). In the Sub-5j population, one SD locus (\( \text{R4M17} \)) became fitting the 1:1 segregation, while 3 loci became significantly over represented by either \( \text{japonica} \) alleles (\( \text{R4M43} \) and \( \text{R12M10} \)) or by \( \text{indica} \) allele (\( \text{R6M14} \)) (Supplemental Fig. 1). In the Sub-5n population, three SD loci (\( \text{R1M30}, \text{R3M10} \) and \( \text{R9M10} \)) became fitting the 1:1 segregation, while 3 loci became significantly over represented by either \( \text{japonica} \) allele (\( \text{R12M10} \)) or by \( \text{indica} \) alleles (\( \text{R6M14} \) and \( \text{R8M46} \)) (Supplemental Fig. 2).

**The indica/japonica attributes and phenotypes of DH lines**

The \( \text{indica} \) (Fi) and \( \text{japonica} \) (Fj) attributes of each DH line are calculated according to Lu et al. (2009) and given in Supplemental Table 2. We observed that the DH population had the majority (132/178) of the lines having an intermediate haplotype with an F(i) value between 0.4 and 0.6, only a few lines belong to typical \( \text{indica} \) or \( \text{japonica} \) type (Fig. 3a). However, among the intermediates, more lines...
have greater *indica* attribute [the lines with F(i) 0.5–0.6] than *japonica* [F(i) 0.4–0.5] (Fig. 3a).

Although both subpopulations had more lines with F(i) > 0.5 than with F(i) < 0.5, this trend is only evident in the Sub-5n population, where 64.2% (70/109) lines had F(i) > 0.5 (Fig. 3a). However, the *indica* type DH lines [with F(i) > 0.8] had the S5n allele, while *japonica* type lines [with F(i) < 0.2] had the S5j allele.

The frequency distribution of DH lines for different agronomic traits is presented in Fig. 3b–3f. Both as a whole population or in subpopulations with different S5 allele, all traits showed more or less normal distribution with single peaks. For all traits except seed-set, there are DH lines that had trait values greater or less than the two parents, although more lines seem to be intermediate between the two parents. Both parents had high seed-set rates; no DH line had seed-set greater than 668B and only 27.5% (49/178) DH lines had seed-set rate greater than T23 (Fig. 3e).

The effect of the S5 locus on frequency distribution of DH lines for all agronomic traits but days from sowing to flowering (DSF) seems to be very limited, if any. The percentage of DH lines having DSF similar to T23 in the Sub-5n population is profoundly greater than in the Sub-5j population (Fig. 3e).

**Discussion**

DH populations are permanent genetic resources that have wide and unique uses in genetic studies, including gene and quantitative trait loci (QTLs) mapping. A few rice DH populations including from inter-subspecies cross have been developed in the past but none of them is known to contain the S5n wide compatible gene. In the present study, we developed a DH population by anther culture of the hybrid of a typical *japonica* variety (668B) crossed to a typical wide compatible *indica* rice line (T23), and subsequently performed molecular characterization for the DH lines. Through the study, we not only developed a new genetic population of great potential to be used in future rice breeding and studies, but also uncovered a number of genetic features related to rice anther culture, particularly related to subspecies anther culture and the effect of the S5n gene.
Selection of a rice DH population without heterozygous and redundant lines

Characterization of DH populations is traditionally relied on morphological characters, while phenotypic data are valuable they are dependent on environmental factors. Therefore, some molecular markers, such as RFLP, RAPD and SSR have been used to characterize DH populations. In the present study, we used three $S_5$ locus-specific markers and a set of Indel markers that are proved to be able to differentiate both subspecies (Lu et al. 2009). Theoretically, the DH population derived from anther culture should be homozygous. However, heterozygous lines are not rare. Cheng et al. (2001) showed 7.7% of DH lines had heterozygous genotypes of RFLP markers. A higher percentage (28/152) of anther culture derived lines being heterozygous at some loci was reported by Grewal et al. (2011) through SSR analysis. In the present study, we also identified 6 lines out of 202 DH lines being heterozygous at some marker loci. The heterozygosity is likely due to the following sources: regeneration of anther somatic tissues, somalonal variations during the tissue culture process and the cross-pollination of DH plants or physical mix-up during seed multiplication.

We further identified individual lines having the same haplotypes (18 lines, 9.2%), which were very likely due to the inclusion of siblings from the same callus. Hence it is advisable to perform molecular analysis for DH lines for removing redundant lines. Inclusion of siblings would otherwise cause high redundancy of the DH population.

Indica and japonica attributes, and variation of agronomic traits

According to the Indel markers used in the study, the DH population showed normal distribution of indica and japonica attributes. A large proportion (74.2%) of the lines are intermediate types [0.4 < F(i) < 0.6] between the typical indica and japonica genotypes, similar to other rice DH populations (Cheng et al. 2001, Rao et al. 2010). However, among the 178 selected lines, 109 lines showed more indica alleles whereas only 69 lines showed more japonica alleles (Supplemental Table 2). The results were unexpected to a certain extent because a general trend in anther culture ability is that it decreases in the order of japonica/japonica $F_1 >$ japonica $>$ indica/japonica $F_1 >$ indica/indica $F_1 >$ indica (Guiderdoni et al. 1992, Yan et al. 1996), which suggests DH lines may have more japonica alleles than indica ones. Further analysis showed that this might be associated with the $S_5^e$ gene of the indica parental line T23, because this trend is only observed in the Sub-5n population where 70/109 lines had F(i) > 0.5 (Fig. 3a). Similar numbers of DH lines with F(i) less or more than 0.5 (33:36) were observed in the Sub-5j populations.

The variations and frequency distribution of the DH population for agronomic traits are similar to those observed by Grewal et al. (2011) for the DH population derived from the japonica cultivar (IR69428) × indica variety (IR64), indicating that in general the $S_5$ locus does not have effect on agronomic performance. But, we did observe that a high percentage of DH lines with the $S_5^e$ showed early maturity with DSF similar to T23 (Fig. 3e). This might be due to the linkage of $S_5$ locus with a few genes that control flowering time in rice, i.e. $Hd1$ (Os06g0275000), RFT1 (Os06g0157500) and $Hd3a$ (Os06g0157700). However, the actual underlying mechanism has yet to be studied.

Segregation distortion in the DH population

Distorted segregation has been observed in a number of DH populations of indica and japonica subspecies. In our study, we observed that among the 34 Indel markers, there are only 13 (38.2%) followed Mendelian segregation (1:1) while the rest 21 (61.8%) did not. This number is much higher than what has been observed by Xu et al. (1997) and Grewal et al. (2011), where 22–32% and about 10% of markers were shown to be deviated from 1:1 ratio in the DH populations they studied. Nonetheless, as indicated above, the balance of over- and under-representation of indica alleles among these markers resulted in a nonsignificant deviation from the expected 1:1 indica:japonica allele ratio in our population. Of the 21 markers that did not follow Mendelian fashion, six were extremely distorted, i.e. R2M24, R2M50, R6M44, R7M37 had indica allele frequency from 1.7 to 5.6% while R12M16, R9M43 had indica allele frequency of 92.7% and 98.3%, respectively (Fig. 2). This skewness is much higher than what was found by Xu et al. (1997).

When analyzed in subpopulations with and without the $S_5^e$ gene, the overall SD scenario was not different from the DH population as a whole; particularly the loci with extremely SD remained the same (Fig. 2, Supplemental Figs. 1, 2). There were a few loci that either became SD or no more SD in the subpopulations. Among them R6M14 became over represented by the indica allele in the Sub-5n population (68.8%; Supplemental Fig. 1) and by the japonica allele in the Sub-5j population (65.2%; Supplemental Fig. 2), respectively. This is indeed understandable because R6M14 is closely linked to the $S_5$ locus.

Many SD loci have been identified in rice through analysis of different types of genetic population (Kinoshita 1993, Reflinur et al. 2014, Xu et al. 1997). Xu et al. (1997) attributed the SD observed to (i) differential transmission, (ii) post-zygotic selection, (iii) male gametophytic selection, and (iv) environmental effect. Because the DH production process does not involve double fertilization and zygote development but involves in vitro culture, the SDs observed in the present study might have resulted from (i), (iii), and (iv), as well as from culturability difference of male gametes. A number of QTLs for SD and tissue culturability (e.g. Yamagishi et al. 1998, Kwon et al. 2001) have been identified, but many of them were reported in linkage maps that used genetic distance rather than physical positions on chromosomes, which made it difficult for comparative analysis. However, two recent studies reported QTLs for SD and culturability in precise positions thus enabled us for further analysis.
First Reflinur et al. (2014) identified a number SD QTLs. After comparing their results with our present study, we found that a number of male function SD (mSD) QTLs reported by Reflinur et al. (2014) are located around three distorted Indel markers identified in the present study, i.e. the mSD QTL linked with marker S01157B on chromosome 1 (39.8 Mb) is near R1M47, and the mSD QTL linked with marker S02135 on chromosome 2 (31.48 Mb) is next to R2M50, and the mSD QTL linked with markers S05036 and S05045 on chromosome 5 (4.71–6.97 Mb) is around R5M13 (Fig. 2).

Second, Li et al. (2013) identified a number of QTLs with precise physical positions through sequencing-based genotyping. To our surprise, several QTLs they identified to be associated with tissue culture responses are very close (0.0–0.8 Mb) to the three most distorted markers we identified in the present study (due to over-representation of japonica allele), i.e. R2M50 (qCPA2b, QTL for callus proliferation ability), R6M44 (qICC6, for induced callus color), and R7M37 (qRR7 for regeneration rate, qNRS7 for the average number of regenerated shoots per callus, and qCDA7 for callus greening ability). The coincidence of the tissue culture response QTLs with the marker loci that are extremely over-represented by japonica alleles is very intriguing for more studies to confirm and identify the underlying genes that are possibly beneficial for anther culture.

In the population, the frequency of the indica allele S5n was significantly higher than the japonica allele S5j (Fig. 2). This might have resulted from the following factors alone or combined. First, the S5n allele might be closely linked with a gene that responsive to anther culture in T23 (which is plausible because some indica rice varieties are known to be responsive to anther and tissue culture). Second, although the SD at the S5 locus in cross progenies is mainly affected by gamete selection in the embryo sac (Yang et al. 2012), the possibility of male gametes with the S3n allele being more responsive to anther culture could also not be excluded. Hence further studies are needed to ascertain the underlying factor(s).

Recombination at the S5 locus

In the present study, we identified 4 DH lines having recombinant genotype (+/+/+; lines 24, 90, 98, 180; Fig. 1B). The occurrence of the recombinant genotype (+/+/+) is obviously a result of the recombination between ORF3 and ORF4. Theoretically, an equal number of DH lines with the genotype of “-/–/–” should be identified among the DH lines. In a survey of germplasm accessions, Yang et al. (2012) did identify 4 out of 43 accessions studied being “-/–/–”, which indicates such genotypes are viable and can be produced through normal reproduction. Hence the absence of “-/–/–” lines suggested that the microspores with this genotype might not be competitive or even lethal in in vitro culture.

The identification of 4 recombinant DH lines (and an equal number of missing recombinant lines) among the 178 lines suggests that the S5 region is probably a recombination hot spot, because even the missed recombinant lines are not calculated, the recombinant rate already reached 2.25%, which suggest ORF3 and ORF4 had a genetic distance of 2.25 cM. However, ORF3 is only 9.2 kb away from ORF4, and the total distance between two markers for ORF3 and ORF4 is only 12 kb (Fig. 1A), which means a recombination rate of 188 cM Mb⁻¹.

Through sequencing of F2 plants, Si et al. (2015) revealed that the average recombination rate across all chromosomes is 4.53 cM Mb⁻¹. By using a sliding window of 100 kb, they identified 27 hot spots (regions with recombination rates > 50 cM Mb⁻¹) and 13 cold spots (regions with recombination rates of 0 cM Mb⁻¹) in meiotic recombination in rice. On chromosome 6 they identified one hot spot in the region between 2.1–2.2 Mb with a crossover rate of 52.63 cM Mb⁻¹. The S5 locus is located on chromosome 6 with the physical position of 5.74 Mb, and therefore this region could be a new recombination hot spot in addition to those reported by Si et al. (2015).

In conclusion, the present study not only produced the first DH population that carries the S5n gene, but also revealed a number of interesting features related to this intersubspecies DH population, which awaits further studies to confirm their generality and uncover the underlying mechanism(s). The new DH population and related information are expected to be useful resources for rice genetic study and breeding in the future.

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Inter-subspecies doubled haploid rice


