

A Genetic Resource for Rice Improvement Introgression Library of Agronomic Traits for All AA Genome Species in Genus *Oryza*

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Original article

Keywords: Rice, AA genome, IL (Introgression line), Grain size, Allelic variation

Posted Date: February 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-174988/v1>

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Abstract

Background: Rice improvement depends on the availability of genetic variation, and AA genome *Oryza* species are the natural reservoir of favorable genes which are useful for rice breeding. Developing the introgression library using multiple AA genome species was rarely reported.

Results: In this study, to systematically evaluate and utilize potentially valuable QTLs/genes or allelic variations, based on the evaluation and selection of agronomic traits, 6372 introgression lines (ILs) were raised by crossing 330 accessions of 7 AA genome species as the donor parents, with three elite cultivars of *O. sativa*, Dianjingyou 1, Yundao 1 and RD23 as the recurrent parents, respectively. Further, twenty-six, twenty-six and nineteen loci were detected in the multiple donors using 1,401 ILs in the Dianjingyou 1 background for grain length, grain width, and the ratio of grain length to grain width, respectively. Interestingly, ten loci had opposite effect on grain length in the different donors, so did grain width. Moreover, one locus for grain width, *qGW3.1*, was validated using the segregation population derived from the donor of *O. glumaepatula*.

Conclusions: This introgression library provided the powerful resource for future rice improvement and genetic dissection of allelic variations. Selections of favorable alleles that are present in wild relatives proceed the driving force of the rice domestication.

Background

Rice is one of the most important staple crops for almost half of world's population. The Food and Agriculture Organization of the United Nations predicts that rice yield will have to be increased 50 to 70% by 2050 to meet human's demands, increasing rice yield is still central for maintaining global food security (Ray et al. 2013). Whereas rice yield potential has been stagnant since the introduction of semi-dwarf gene into cultivated rice and the utilization of heterosis (Virmani et al. 1982; Monna et al. 2002; Sasaki et al. 2002). The narrow genetic basis of parental materials led to yield bottleneck in rice breeding (Tanksley and McCouch, 1997).

Genus *Oryza*, contains twenty-four wild species and two cultivated rice species representing 11 genomes: AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, HHKK and KKLL (Khush, 1997). Among these, six wild rice species (*O. nivara*, *O. rufipogon*, *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*) and two cultivated species (*O. sativa* and *O. glaberrima*) were classified into the AA genome. Asian cultivated rice (*O. sativa* L.) was domesticated from wild species *O. rufipogon* thousands of years ago (Khush, 1997; Huang et al. 2012). Previous reports indicated that 40% of the alleles of *O. rufipogon* was lost during the domestication from common wild rice to cultivated rice (Sun et al. 2002), and only 10%-20% of the genetic diversity in *O. rufipogon* and *O. nivara* was retained in two subspecies of cultivated rice (Zhu et al. 2007). Since sharing the same AA genome, *O. glaberrima* and the six wild rice species are the most accessible gene pool for rice improvement (Ren et al. 2003). Thus, the exploitation and utilization of the useful alleles of AA genome relatives may overcome yield plateaus of *O. sativa* (Xiao et al. 1998). However, it is difficult to utilize the natural genetic diversity because of reproductive isolation, linkage drag and background noise. Moreover, many important agronomic traits including yield are controlled by quantitative trait loci (QTL) with smaller effect, which can be influenced by the environment. It is difficult to understand the QTL-controlled agronomic traits because of their complex inheritance and background noise.

Introgression lines are genetic resource in which the whole genome of a donor genotype is represented by different segments in the genetic background of elite varieties. Genetic background noise of ILs can be eliminated significantly, which can be evaluated for any traits improvement over the recurrent parents for rice breeding, also for QTL mapping and gene discovering as a single Mendelian factor, in addition, potential favorable genes hidden in the background of related species could be expressed in the genetic background of cultivated rice (Ballini et al. 2007; Eizenga et al. 2008; Bian et al. 2010; Rama et al. 2015; Jin et al. 2016; Yang et al. 2016; Bhatia et al. 2017; Yamagata et al. 2019; Bhatia et al. 2018). Thus, ILs eliminating hybrid sterility, linkage drags and background noise, are one of the most important genetic resource for QTL mapping, gene identification and discovery and rapid utilization for commercial breeding. Though many introgression lines were obtained from the intersubspecific crosses between *indica* and *japonica* varieties, and from the interspecific crosses between Asian cultivated rice and wild relatives of *Oryza sativa* (Bhatia et al. 2017; Divya et al. 2018), systemically developing the ILs with multiple donors from AA genome species for transferring the yield-related traits into the elite cultivar varieties remained limited. Moreover, interspecific hybridization is an important driving force for rice domestication, and new genetic variations were introduced via introgression (Petr et al. 2018;

Michael et al.2019). Introgression hybridization from early *japonica* to proto *indica* and proto *aus* resulted in *indica* and *aus* subspecies (Choi et al. 2017)

In this study, in order to explore and utilize cultivated rice relatives for rice improvement, we systematically introduced foreign segments from eight different AA genome species (*O. longistaminata*, *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara*, *O. rufipogon*, *O. glaberrima* and upland rice of *O. sativa*) into three elite, highly productive *O. sativa* varieties (Dianjingyou 1, Yundao 1, RD23) and to provide introgression lines (ILs) as the basis for QTL mapping, gene and allelic variations discovery, and rice breeding. One thousand four hundred and one of 6372 ILs in the Dianjingyou 1 background were used to analysis genotype and discovery novel alleles for grain size. Two QTLs for grain width were identified in the BC₄F₂ and BC₄F₃ populations from the cross between *O. glumaepatula* and Dianjingyou 1. Thus, this introgression library provided the powerful resource for future rice improvement and genetic dissection of agronomic traits.

Methods

Plant Materials

The plant materials included 1 accession of *O. longistaminata*, 13 accessions of *O. barthii*, 6 accessions of *O. glumaepatula*, 8 accessions of *O. meridionalis*, 19 accessions of *O. rufipogon*, 20 accessions of *O. nivara*, 103 accessions of *O. glaberrima* and 160 upland rice varieties (Supplemental Table S1). Three elite varieties, Dianjingyou 1, Yundao 1 and RD23 were used as the recurrent parents.

Three hundred and twenty-nine donor accessions except for 1 accession of *O. longistaminata* as male parents were crossed with the two recurrent parents Dianjingyou 1 and Yundao 1 as female parents followed by successive backcrosses and selfing to generate ILs. The F₁ plants were used as female parents to backcrossing to their respective recurrent parents to produce BC₁F₁ generation. More than 200 BC₁F₁ seeds were generated for each of the combinations. The heading date of plants that were too early or too late were discarded, and then about 30 plants from each cross combination were selected to backcross to the recurrent parents, and about 200 BC₂F₁ seeds were obtained. From each of the BC₂F₁ progeny, individuals that showed significant agronomic difference from the recurrent parents were selected for further backcrossing or selfing.

After 2-3 times backcrossing and 2-7 times selfing, the progeny with stable target traits were developed as ILs.

The ILs derived from the cross between 1 accession of *O. longistaminata* as the donor parents and RD23 as the recurrent parent were generated as above procedure.

Agronomic traits evaluation

Each introgression line was planted in randomized complete block design under two environments. Observation on agronomic traits was recorded from five randomly chosen plants for three replications. Border plants were excluded for phenotype evaluation.

To measure the grain size, seed was selected from primary panicle and stored at room temperature for at least 3 months before testing. Twenty grains were used to measure grain width (GW) from each plant. Grains per individual were taken photographs using stereomicroscope, and then grain width was measured by software Image J. The average width of 20 grains was used as phenotypic value. 1,000-grains weight was measured by weighting fertile, fully mature grains from five panicles;

Prostrate growth habit was observed for the tiller angle in three main stages, including booting stage, heading stage and grain filling stage.

For spreading panicle, primary branch and second branch angle were observed. Erect panicle or drooping panicle was evaluated according to the angle between the lines connecting panicle pedestal with panicle tip and the elongation line of stem;

For a simply inherited traits, awn, pericarp color and kernel color was observed directly in the field.

Tiller numbers were recorded from five random plants; spikelets per panicle (SPP) were measured as the total number of spikelets of the whole plant divided by its total number of panicles;

Aerobic adaptation was evaluated by biomass, yield, harvest index, heading date and plant height difference between upland and irrigated environments; plant height was measured from the ground level to the tip of the tallest panicle.

To evaluate blast resistance, introgression lines were inoculated with *Magnaporthe oryzae* for 3 weeks after sowing by spraying with conidial suspension. After seven days, lesion types on rice leaves were observed and scored according to a standard reference scale based on dominant lesion type (Xu, et al. 2015).

DNA extraction and PCR protocol

The experimental procedure for DNA extraction was performed as previously described (Edwards et al. 1991); 168 SSR markers were selected from the Gramene database (<http://www.gramene.org>) or previously published polymorphic SSR markers within the *Oryza* AA genome species (McCouch et al. 2002; Orjuela et al. 2010). PCR was performed as follows: a total volume of 10 μ l containing 10 ng template DNA, 1 \times buffer, 0.2 μ M of each primer, 50 μ M of dNTPs and 0.5 unit of Taq polymerase (Tiangen Company, Beijing, China). The reaction mixture was incubated at 94°C for an initial 4 min, followed by 30 cycles of 94°C 30 s, 55°C 30 s and 72°C 30 s, and a final extension step of 5 min at 72°C. PCR products were separated on 8% non-denaturing polyacrylamide gel and detected using the silver staining method.

Determination of the length of substituted segment in ILs

The substituted segment was counted based on the SSR markers distributed on twelve chromosomes (Paterson et al. 1988; Young and Tanksley, 1989; McCouch et al. 2002). Intervals between two markers homozygous for the donor genotype (DD) were regarded as 100% introgression segment and a chromosome segment flanked by one marker of the donor genotype and one marker of the recurrent type (DR) was considered as 50% introgression segment, whereas intervals between two markers homozygous for the recurrent genotype (RR) represented the background genotype. Thus, the physical distance of both DD and half of DR was used to estimate the length of introgression segments. The expected introgression length of genome is divided by the total genome size to yield the expected proportion of introgression.

Statistical analysis

Statistical analyses were performed on the SAS software package. Linkage between loci and traits were estimated by Binomial distribution based on the genotype and phenotype between ILs and the recurrent parent. The potential locus was inferred when there is a significant difference between each IL and recurrent parent using the Dunnett's t- test at $P < 0.0001$ (Eshed and Zamir, 1995; Xu, et al. 2014).

Confirmation of loci for grain width

Segregating population was built for validating the loci for grain width above identification. A BC₃F₇ IL containing introgression segment on chromosome 3 and 6 was derived from the cross between an *O. glumaepatula*, IRGC.100184, from IRRI, as a donor parent, and a temperate *japonica* variety, Dianjingyou 1, from Yunnan province, P. R. China, as a recurrent parent, which was used for confirming population. The IL was backcrossed again to produce BC₄F₁, which was self-crossed to produce BC₄F₂ and BC₄F₃ populations. Thus, three hundred BC₄F₂ individuals and 283 BC₄F₃ families were used to validate QTLs associated with grain width.

For QTL validation, linkage maps were constructed using MAPMAKER 3.0 with a minimum logarithm of odds (LOD) score of 3.0 (Lincoln et al. 1992). Kosambi function was used to calculate genetic distance. The interval mapping was performed for QTL analysis by using WinQTL Cartographer v2.0 (Basten et al. 1998). Thresholds were estimated by permutation tests with 1,000 replicates (Churchill and Doerge, 1994). In BC₄F₂ population, the threshold values for grain width at genome-wide significance of 0.05 were 2.57.

Results

Agronomic traits diversity in introgression library from AA genome donors

To systematically explore potentially valuable genes hidden in the AA genome wild relatives and cultivated species, 1 accession of *O. longistaminata*, 13 accessions of *O. barthii*, 6 accessions of *O. glumaepatula*, 8 accessions of *O. meridionalis*, 19 accessions of *O. rufipogon*, 20 accessions of *O. nivara*, 103 accessions of *O. glaberrima* and 160 upland rice varieties in *O. sativa* as the donors were used to raise the introgression line library. Of these accessions, all accessions except for *O. longistaminata* were used for generating ILs in the Dianjingyou 1 background, 233 except for the accessions of *O. glaberrima* and *O. longistaminata* were used for developing ILs in the background of Yundao 1, and 1 accessions of *O. longistaminata* were used for raising ILs in the RD23 background. A total of 6372 introgression lines with multiple donors showed significant difference in the agronomic traits, including spreading panicle, erect panicle, dense panicle, lax panicle, awn, prostrate growth, plant height, pericarp color, kernel color, glabrous hull, grain size, 1,000-grain weight, drought resistance and aerobic adaption, compared with their respective recurrent parents (Figure 1B-D). Among these, 74, 61, 179, 824, 135, 251 and 1561 ILs showing distinguished traits in the Dianjingyou 1 background were selected from the donors of *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. rufipogon*, *O. nivara*, *O. glaberrima* and upland rice, respectively (Figure 1A, 1C, Table S3-S4). Similarly, 244, 85, 547, 714, 858 and 825 ILs exhibiting different phenotype in the Yundao 1 background were developed from the donors of *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. rufipogon*, *O. nivara*, and upland rice, respectively (Figure 1A, Table S5). And 265 ILs were derived from the cross between 1 accession of *O. longistaminata* as the donor and an *indica* variety RD23 as the recurrent parent (Figure 1D, Table S5). Thus, the introgression library derived from the multiple donors in the different backgrounds showed the abundant genetic variations.

For the same donor parent, phenotype variations for agronomic traits were significantly different between Dianjingyou 1 background and Yundao 1 background. The numbers of ILs showing erect panicle, dense panicle, lax panicle, awn, plant height, pericarp color, 1,000-grain weight, drought-resistance and aerobic adaption in Yundao 1 background were more than those in Dianjingyou 1 background, whereas the number of ILs exhibiting spreading panicle, prostrate growth, kernel color, glabrous hull, grain length, grain width in Yundao 1 background were less than these of Dianjingyou 1 background (Figure 1B). It was suggested that target traits expression depended on the background of recurrent parent. Developing introgression library in the different backgrounds will be benefit to express hidden genes in the donor parents and discover more genetic variations for further study.

Characteristics of chromosome substituted segments in the introgression library

A total of 168 SSR markers distributed on 12 chromosomes were selected to genotype the introgression library in Dianjingyou 1 background. The length of the interval between two markers ranged from 0.2-5.5 Mb, with an average of 2.22 Mb on the rice physical map (Figure 2; Table 1). The polymorphism rate displayed from 82.74%-98.43% between 7 AA genome species and Dianjingyou 1 (Table 1).

One thousand four hundred and one ILs in the Dianjingyou 1 background were used to detect the characteristics of chromosome segments from seven AA genome species, including 29 of *O. barthii*, 30 *O. glumaepatula*, 76 of *O. meridionalis*, 380 of *O. nivara*, 74 of *O. rufipogon*, 81 of *O. glaberrima*, and 731 of upland rice (Table S2). Average Coverage rate ranged from 73.11% to 99.10%, indicating that this introgression library contained abundant genetic information from 7 AA genome species.

In addition, different distribution in introgression segments on 12 chromosomes from 7 AA genome donor species was observed. The number of introgression segments on chromosome 3 was more than those on other chromosomes (Figure S1-S7), which may be related to the chromosome structure and location. We also found that introgression segments with the donor of upland rice on chromosome 1 were detected in almost ILs, it may be because this donor segment was tightly linked with some agronomic traits under selection pressure (Figure S7).

Detection of allelic variations for grain size in the introgression library

Seed size plays an important role in rice yield (Xing and Zhang, 2010). Seed size not only determines seed appearance, but also affects milling, cooking and eating quality of rice (Fan et al., 2006). Significant variations were observed for grain length (GL), grain width (GW) and the ratio of grain length to grain width (RLW) in the introgression library with multiple donors in the background of Dianjingyou 1. Some ILs for grain length (GL), grain width (GW) and the ratio of grain length to grain width (RLW) were found to be significantly superior to the recurrent parent Dianjingyou 1. For grain length, 133 and 125 ILs were found to be significantly longer than the Dianjingyou 1 in two seasons, respectively. For grain width, 412 and 508 ILs were observed to be significantly wider than the recurrent parent in two environments. For the ratio of grain length to grain width, 277 and 178 ILs were found to be higher than

the Dianjingyou 1 in both seasons (Figure. 3). These results suggested that abundant genetic variations for grain size existed in the wild and cultivated accessions of rice.

In order to explore favorable allelic variation for grain size, the potential loci were detected based on the genotype and phenotype data. Forty-one loci linking with grain length, forty-four loci linking with grain width, thirty-two loci linking with the ratio of grain length to grain width, were identified in both seasons. It indicated that abundant gene pool for grain size exists in the AA genome species (Figure. 4-6). Among these, 26 loci for grain length were detected from multiple donors, 12, 11, 2 and 1 loci were detected from the donors of two species, three species, four species and six species, respectively. It suggested that the same locus contributing to grain length is potential allelic variation from different donors. Moreover, 4 loci from the different donors were only responsible for long grain, 12 loci derived from the multiple donors only contributed to short grain, and 10 loci from the different species controlled both long grain and short grain (Figure. 4); 27 loci for grain width were examined from the multiple donors, including 13 loci from the only two species, 9 loci from three species, 3 loci from four species, and 1 locus from five species (Figure. 5). Moreover, 12 loci from the different donors were only responsible for wide grain, 4 loci from multiple species only contributed to thin grain, and 10 loci from the different species controlled both wide grain and thin grain (Figure. 5). 19 loci for the ratio of grain length to grain width from multiple donors were explored on 12 chromosomes, including 12, 6, and 1 loci detected simultaneously in two, three and four donor species, respectively (Figure. 6). These results indicated that detection of favorable genes using multiple donors could help us find the novel allelic variations. Some potential loci controlled the opposite phenotype, long grain versus short grain, wide grain versus thin grain, validating these loci's functions in forward and reverse directions. Potential allelic genes were detected in the different donors, suggesting that some loci for grain size were conserved in Genus *oryza*. Taken together, these results will provide the information that the loci for grain size from the different donors were the same or different haplotypes; it also indicated that IL library with the donor of 7 AA genome species was an excellent resource and tool to discovery favorable allelic variations and new QTLs/genes for rice improvement.

Confirmation of loci for grain width

In order to confirm the loci for grain width above identification, a BC₃F₇ IL was derived from the cross between an *O. glumaepatula* (IRGC100184) as a donor parent, and a temperate *japonica* variety, Dianjingyou 1, which was highly significant difference in the trait of grain width between IL and Dianjingyou 1 (Figure 7A). In the BC₄F₂ population, phenotypic values of grain width showed a continuous distribution. The GW was skewed toward the large-value parent, IL (Figure 7B). These results indicated that grain width was controlled by quantitative trait locus. This population was used to confirm the locus for grain width.

In order to validate the QTL for grain width, 443 SSR markers distributed on 12 chromosomes were used for polymorphic analysis between Dianjingyou 1 and IL again. Then, a total of 22 polymorphic markers between the two parents, including 15 markers on chromosome 3 and 7 markers on chromosome 6, were selected to survey the genotype of BC₄F₂ population. Based on the phenotype and genotype data, two QTLs for GW were detected in BC₄F₂ and were validated in BC₄F₃ populations. *qGW3.1* was identified in the region between RM186 and RM416, and it explained 3% and 10% of the phenotypic variation with an additive effect of -0.06mm and -0.02mm in two generations, respectively. Another QTL for GW, *qGW6.1*, was detected in the region between RM253 and RM19623 on chromosome 6, explaining 10% and 15% of the total phenotypic variation with an additive effect of -0.06 and -0.05mm, respectively (Table 3). Thus, the location of *qGW3.1* was consistent with the locus for thin grain from *O. glumaepatula*, indicating that exploration of favorable allelic variation using multiple ILs was reliable. Moreover, a novel *qGW6.1*, was found only from segregating population, suggesting that exploring new genes or QTLs using segregating population could acquire more information.

Discussion

Exploration of natural allelic variations from AA genome species

Genetic diversity and allele were lost during the domestication from the wild species of rice to the cultivated rice (Sun et al. 2002), whereas narrow genetic basis led to the yield bottleneck of Asian cultivated rice, thus, exploration and utilization of favorable allelic genes in AA genome species is an accessible approach to improve rice breeding. Recently years, mining and utilization of useful allele genes have made great progress in rice breeding. For example, the allelic variation in the *Wx* gene and *SSS1* were proved to

contribute greatly to the differences in ECQs in the two subspecies (Li et al. 2018). Allelic variation at the *E1/Ghd7* locus allowed expansion of the rice cultivation area through adjusting heading date (Saito et al. 2019). The allelotypes of *BPH9* confer varying levels of resistance to different biotypes of BPH and enable rice to combat planthopper variation (Zhao et al. 2016). The allelic variation at the rice blast resistance (*R*) *Pid3* locus were analyzed based on the 3K RGP sequencing data, and different strategies were developed to apply the functional *Pid3* alleles to *indica* and *japonica* rice breeding (Lv et al. 2017). Thus, exploration of natural allelic variation and artificial shuffling within useful genes may allow breeding to be tailored to control emerging traits.

Though wild accessions from *O. nivara*, *O. rufipogon*, *O. meridionalis*, *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. barthii* and Africa cultivated rice *O. glaberrima* were used as donor parents in the backcross breeding program (Chen et al. 2006; Tian et al. 2006; McCouch et al. 2007; Rangel et al. 2008; Hao et al. 2009; Gutierrez et al. 2010; Ramos et al. 2016; Bhatia et al. 2017), most of introgression lines were derived from a single accession of AA genome species in a single background leading to the loss of some valuable allelic information. In this study, introgression library with multiple donors from different relatives of Asian cultivated rice is a powerful resource platform to discover novel and functional allele QTLs/genes. One locus for grain length and one locus for grain width were explored from the six and five different donor species, respectively. Two loci for grain length, three loci for grain width and one locus for the ratio of grain length to grain width were detected from the donors of four species respectively (Figure 4-5), mining the natural functional variations in the useful genes derived from the multiple donors and combing the different alleles through diversification could be useful for accurate rice breeding program.

By now, more than 20 sets of introgression lines were raised, derived from the cross between the *indica* varieties and *japonica* varieties (Kubo et al. 2002; Ebitani et al. 2005; Chen et al. 2007; Ando et al. 2008; Hao et al. 2009; Bian et al. 2010; Xu et al. 2010a; Lin et al. 2011; Zhang et al. 2011; Kanjoo et al. 2012; Pinson et al. 2012; Uga et al. 2015; Ookawa et al. 2016; Ujiie et al. 2016), 2 sets of ILs from *indica* x *indica* (Chen et al. 2014; Liu et al. 2016), 2 sets of ILs from *japonica* x *japonica* (Hori et al. 2010), 6 sets of introgression lines were developed from the cross between *O. sativa* and *O. glaberrima* (Doi et al. 1997; Ghesquière et al. 1997; Li et al. 2004; Gutierrez et al. 2010; Shim et al. 2010), 5 sets of introgression lines were raised from *O. sativa* and *O. rufipogon* (Chen et al. 2006; Hao et al. 2006; Tian et al. 2006; Cheema et al. 2008; Furuta et al. 2014), 2 sets of introgression lines from cross the between the *O. sativa* and *O. glumaepatula* (Sobrizal et al. 1999; Rangel et al. 2008). One set of introgression line from the cross between *O. sativa* and Zhangpu wild rice (Yang et al. 2016), 1 sets of introgression lines from the cross between *O. sativa* and *O. minuta* (Guo et al. 2013), 1 sets of introgression lines from the cross between *O. sativa* and weedy rice (Subudhi et al. 2015). Above CSSL library were derived from the cross between single donor or few donors and single recurrent parent. And some CSSL library from multiple donors and single recurrent were also built for rice improvement and gene identification in rice (He et al. 2005a; He et al. 2005b; He et al. 2005c; Xi et al. 2006; Yasui et al. 2010; Arbelaez et al. 2015). These CSSL library were developed based on the genotype selection so that the introgression segments could cover the donor genome completely and the donor information were not lost, but some of introgression lines could not show the significant phenotype, compared to the recurrent parent. Thus, it could not be used for the further study and breeding program. Previous reports indicated the IL library was generated by crossing 70 accessions of six AA genome species with two elite cultivars of *O. sativa* (Bhatia et al. 2017). In this study, 334 accessions of AA genome species as the donor parents were transferred into three different cultivars of *O. sativa*. These IL libraries were raised from a large number of the donors in the multiple recurrent parents based on the phenotype selection, which exhibited the desirable agronomic traits and provided abundant information of favorable genes or QTLs and allelic variations. This approach could save a couple of times and cost and obtained more interesting information. We could select the desirable traits from the different donors using this approach, yield component traits and disease resistance were paid more attention during selection (Bhatia et al. 2017), whereas we emphasized on the selection of plant and panicle architecture, grain size and aerobic adaptation. IL libraries derived from the multiple donors have some advantages: 1) An abundant genetic variations were introgressed into the cultivated rice genome; 2) Target genes/QTLs for the same phenotype could be validated by the different donors, and it will provide the information that these target genes/QTLs could be the same haplotype; 3) The genes or QTLs responsible for the opposite phenotypes, for example, long grain size and short grain size, could also be confirmed using the different populations from multiple donors, and it could be the different haplotype; 4) Introduction of new genetic variation, selection of favorable alleles in the wild relatives could speed up to fix the useful genes. Therefore, Constructing IL library is not only the breeding method, but also the domestication power. Thus, these IL libraries will help us improving rice breeding and interesting genes discovery and utilization, as well as the development of rice domestication.

Introgression library for QTL mapping and cloning

Introgression lines harboring one or more donor chromosome segments exhibited distinguish traits, compared to the recurrent parent. Since the background of introgression lines is similar to the recurrent parent and it is easier to correlate a particular chromosomal region to phenotypic variation, they were used for mapping and cloning QTLs or genes for complex traits. Many QTLs or genes have been mapped based on introgression lines, such as *GS2* for grain size and weight (Hu et al. 2015), *GL7* for grain size (Wang et al. 2015), *DEP1* for dense and erect panicle (Huang et al. 2009), *GS3* for grain length and weight (Fan et al. 2006), *qPGWC-7* for grain chalkiness (Zhou et al. 2009), QTLs for heterosis (Tao et al. 2016), QTLs for seed dormancy (Marzougui et al. 2012), *qGRH9* for green rice leafhopper (Fujita et al. 2010), *Pi54rh* for rice blast (Das et al. 2012), *qSPP2.2* controlling spikelet per panicle (Kaur and Singh, 2018).

Previous reports indicated that some genes or QTLs for agronomic traits were identified based on this introgression library, such as *Spr5(t)* for spreading panicle from *O. glaberrima* (Xu et al. 2010b), *qph1* for plant height from *O. longistaminata* (Chen et al. 2009), *EP4* for erect panicle from *O. glumaepatula* (Zhang et al. 2015), *Er1* for erect panicle from *O. glaberrima* (Zhou et al. 2008), *GS3* for grain length from *O. nivara*, *O. glumaepatula*, *O. longistaminata* and *O. glaberrima* (Zhang et al. 2014), *HS1* for hybrid seed shattering between *O. barthii* and *O. sativa* (Zhang et al. 2019). In this study, 41 loci for grain length, 44 loci for grain width on 12 chromosomes, 42 loci for the ratio of grain length to grain width were explored on 12 chromosomes (Figure 4-6), and some loci were identified in the same location with published genes, such as *GW2* (Song et al. 2007), *GL2* (Hu et al. 2015), *PGL2* (Heang and Sassa, 2012b), *PGL1* (Heang and Sassa, 2012a), *GL3.2* (Xu et al. 2015), *GS3* (Fan et al. 2006; Takano-Kai et al. 2009; Mao et al. 2010), *qGL3-1* (Qi et al. 2012), *qGL3.3* (Hu et al. 2018), *GS6* (Sun et al. 2013), *TGW6* (Ishimaru et al. 2013), *GL7* (Wang et al. 2015), *OsSPL13* (Si et al. 2016). In addition, 29 loci might be new QTLs or genes controlled for grain size from the different AA genome donors, and most of published genes for grain size were found based on the introgression library. Moreover, a novel QTL for grain width, *qGW6.1*, was identified from the *O. glumaepatula* based on BC₄F₂ and BC₄F₃ populations. It suggested that this library is an effective tool to systematically discover and map novel genes and allelic variations. Moreover, introgression lines showing superior agronomic traits could accelerate the improvement of rice breeding. The ILs with the desirable traits could be used to improve rice breeding program through marker-assisted selection (Ashikari and Matsuoka, 2006).

Hybrid sterility is the major barrier to develop interspecific introgression library, which is also an ideal model for studying the relationship between the reproductive isolation and speciation

The major barrier is hybrid sterility exhibiting complete or partial pollen sterility and/or spikelet sterility in the crosses between AA genome species and *O. sativa*. We observed that pollen fertility of F₁ varied from 1.92% to 93.19% dependent on the different accessions of *O. nivara* and *O. rufipogon*, whereas all the crosses with the accessions of *O. barthii*, *O. glumaepatula* and *O. meridionalis* showed complete pollen sterility in the F₁ combinations (data not shown). For the accessions of *O. longistaminata*, the crosses using the *japonica* varieties Dianjingyou 1 and Yundao 1 as the recurrent parents were failed, only the crosses using an *indica* variety RD23 as the recurrent parent was obtained by embryo rescue. Thus, hybrid sterility between *O. sativa* and AA genome species is the main difficulty to transfer favorable genes between them. Fortunately, the female gametes from the interspecific hybrids were partially fertile, and some backcross seeds in the different combinations could be obtained by backcrossing F₁ as the female parent with *O. sativa* as the male parent.

Genus *Oryza* probably originated at least 130 million years ago and spread eventually in Asia, Africa, Americas, Australia and Antarctica, which contains twenty-four wild rice species and two cultivated rice species representing 11 genomes (Khush, 1997). The AA genome includes six wild rice species (*O. nivara*, *O. rufipogon*, *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*) and two cultivated species (*O. sativa* and *O. glaberrima*). Though reproductive isolation, especially hybrid sterility, was existed in the hybrids between Asian cultivated rice and AA genome species, direct cross and backcross could be made for raising introgression library. Thus, introgression library from AA genome species is an ideal model to study the relationship between reproductive isolation and AA genome species divergence. Using this resource platform, we identified a series of QTLs or genes for interspecific hybrid sterility, including *S1*, *S29(t)*, *S37(t)*, *S38(t)*, *S39(t)*, *S40*, *S44(t)*, *S51(t)*, *S52(t)*, *S53(t)*, *S54(t)*–*S55(t)*–*S56(t)* and *qHMS7* (Hu et al. 2006; Zhao et al. 2012; Xu, et al. 2014; Chen et al. 2017; Xie et al. 2017; Li, et al. 2018; Zhang, et al. 2018; Xie et al. 2019). Moreover, we found some orthologous genes for hybrid sterility across the species and populations. For examples, *S29(t)* from *O. glaberrima*, *S53(t)* from *O. meridionalis*, and *S22B* from *O. glumaepatula* had good co-linear relationship on chromosome 2 (Hu et al. 2006; Sakata et al. 2014; Li et al. 2018). *S56(t)* locus from *O. glumaepatula* was mapped around *S20* and *qSS-7* region identified in the cross between *O. sativa* and *O. glaberrima* (Doi et al. 1999; Li et al. 2011). In addition, It was

observed that *S23* from *O. glumaepatula*, *S21* from *O. glaberrima* and *O. rufipogon*, *qHMS7* from *O. meridionalis* were located into the similar region on chromosome 7 (Doi et al. 1999; Sobrizal. et al. 2000; Miyazaki et al. 2007; Yu et al. 2018). These results suggested that alleles of *S29* (t) / *S53*(t) / *S22B* were required for the divergence among *O. sativa* and *O. glaberrima*, *O. meridionalis*, *O. glumaepatula*, *S56*(t) / *S20* / *qSS-7* played an important role in the species formation of *O. glumaepatula*, *O. glaberrima* and *O. sativa*, *S23/S21/qHMS7* are necessary for the speciation of *O. glaberrima*, *O. rufipogon*, *O. glumaepatula* and *O. meridionalis*. Thus, introgression library with the multiple AA genome donors is an excellent resource for studying the reproductive isolation and speciation in rice.

Interspecific hybridization is an important driving force for evolutionary process

The process of crop domestication is driven by artificial selection, cultivation practices, as well as agricultural environments (Petr et al. 2018). Large-scale chromosomal structural changes, polyploidy, copy-number variation and changes in transposable-element content exhibited distinguished difference between wild and cultivated plants, which are the important mechanism for crop evolution (Yang et al. 2012; Wang et al. 2015; Salman-Minkov et al. 2016). In addition, Hybridization is major power in crop domestication, increases crop diversification and arises new crop species (Michael, 2019). Archaeological evidence and genomic analysis supported that *indica*, a subspecies of rice evolved from the hybridization between *japonica* and *O. nivara* (Fuller et al. 2010; Fuller, 2011; Silva et al. 2018). Interspecific hybridization can lead to novel allelic variations, gene combinations and/or novel patterns of gene expression, which in turn provide the variation on which natural selection can act. The decrease in diversity caused by artificial selection and bottlenecks could be counteracted by interspecific hybridization, interspecific introgression may provide for novelty, superior quality gene resources which could increase crop yield, quality or adaptive ability. In this study, multiple donors were introgressed into Asian cultivate variety, Dianjingyou 1. ILs library exhibited abundant and diverse traits in plant architecture, seed characteristics, biotic or abiotic resistance (Table S3-S5). Thus, interspecific hybridization could be an important mechanism for rice species diversity and increase adaptive ability in the different environment, which is valuable resource for meeting the demand of breed challenge in rice.

Conclusions

6372 introgression lines (ILs) from BC₂ to BC₆ were developed by crossing 174 accessions of 7 AA genome species, *Oryza longistiminata*, *O. glumaepatula*, *O. barthii*, *O. meridionalis*, *O. nivara*, *O. rufipogon*, *O. glaberrima* and 160 upland rice of *O. sativa* as the donor parents, with three elite cultivars of *O. sativa*, Dianjingyou 1 [a *japonica* variety], Yundao 1 [a *japonica* variety] and RD23 (an *indica* variety) as the recurrent parents, respectively. forty-one loci for grain length, forty-four loci for and thirty-two loci for the ratio of grain length to grain width were identified, and twenty-six, twenty-six and nineteen loci were detected in the multiple donors for grain length, grain width, and the ratio of grain length to grain width, respectively. Interestingly, ten loci for long grain were found in some donors, and vice versa, those ten loci for short grain were also identified in other different donors, and ten loci controlled both wide grain and thin grain in the different donors. One locus for grain width, *qGW3.1*, was validated using the BC₄F₂ and BC₄F₃ population derived from the donor of *O. glumaepatula*. Thus, this introgression library provided the powerful resource for future rice improvement and genetic dissection of agronomic traits.

Declarations

Authors' Contributions

TD designed the experiments. ZY performed the experiments, analyzed the data and drafted the manuscript. ZJ, XP, DX and DW raised the experimental materials and evaluated the phenotype. LJ, YY and YY performed genotype analysis. All authors have read and approved the content of the manuscript.

Funding: This research was supported by National Natural Science Foundation of China (Grant Nos. 31991221, 31660380, 31201196), Yunnan Provincial Science and Technology Department, China (Grant Nos. 202001AS070003, 2015HB079), Yunnan Provincial Government (YNWR-QNBJ-2018-359).

Availability of Data and Materials: All datasets generated for this study are included in the manuscript and additional files.

Ethic approval and Consent to Participate: Not applicable

Consent for publication: Not applicable

Competing Interests: The authors declare that they have no competing interests.

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Tables

Table 1. The description of markers used for genotyping introgression library

Chr	Number of Markers	Density(Mb)	The marker polymorphism rate (%)						Upland rice
			<i>O. barthii</i>	<i>O. glumaepatula</i>	<i>O. meridionalis</i>	<i>O. nivara</i>	<i>O. rufipogon</i>	<i>O. glaberrim</i>	
1	17	2.55	94.12	94.12	100.00	100.00	100.00	100.00	100
2	18	2.00	77.78	77.78	88.89	83.33	88.89	94.44	77.78
3	16	2.28	100.00	100.00	100.00	100.00	100.00	100.00	100.00
4	15	2.37	80.00	100.00	93.33	100.00	100.00	100.00	93.33
5	15	2.00	93.33	60.00	86.67	93.33	93.33	86.67	80.00
6	13	2.40	92.31	84.62	100.00	100.00	100.00	100.00	100.00
7	14	2.12	92.86	78.57	92.86	92.86	92.86	100.00	85.72
8	14	2.03	100.00	57.14	100.00	100.00	100.00	100.00	78.58
9	10	2.30	100.00	90.00	100.00	100.00	100.00	100.00	83.34
10	12	1.93	91.67	100.00	100.00	100.00	91.67	100.00	100.00
11	12	2.42	66.67	66.67	83.33	100.00	83.33	100.00	100.00
12	12	2.29	83.33	83.33	100.00	100.00	100.00	100.00	100.00
Mean(%)		2.22	89.29	82.74	95.24	97.02	95.83	98.43	91.56

Table 2. The characterization of introgression lines

Donors	Number of introgression lines	Number of introgression Segments	Average number of segments per chromosome	Range of Segment length (Mb)	Average length of segments (Mb)	Average background recovery rate(%)
<i>O.barthii</i>	29	349	29.08	2.66-28.98	6.99	88.92
<i>O.glumaepatula</i>	30	210	17.50	0.66-25.60	5.35	91.94
<i>O.meridionalis</i>	76	434	36.17	0.27-23.77	5.83	93.21
<i>O.nivara</i>	380	3332	277.67	0.30-27.43	5.84	92.74
<i>O.rufipogon</i>	74	625	52.08	0.28-23.46	6.37	89.92
<i>O.glaberrima</i>	81	1138	94.83	0.27-24.17	5.80	85.14
Upland rice	731	9198	766.50	0.19-43.27	6.67	84.03

Table 3. QTLs identified for grain width in BC₄F₂, BC₄F₃ population

QTL	Chr.	Interval	BC ₄ F ₂			BC ₄ F ₃		
			LOD	R ²	ADD	LOD	R ²	ADD
<i>qGW3.1</i>	3	RM186-RM416	6.65	10	-0.06	2.80	3	-0.02
<i>qGW6.1</i>	6	RM253-RM19623	9.26	15	-0.06	2.05	10	-0.05

R²: percentage of total phenotypic variance explained by QTL

ADD: Additive effect, negative value mean the *O. glumaepatula* allele increases the trait

Figures

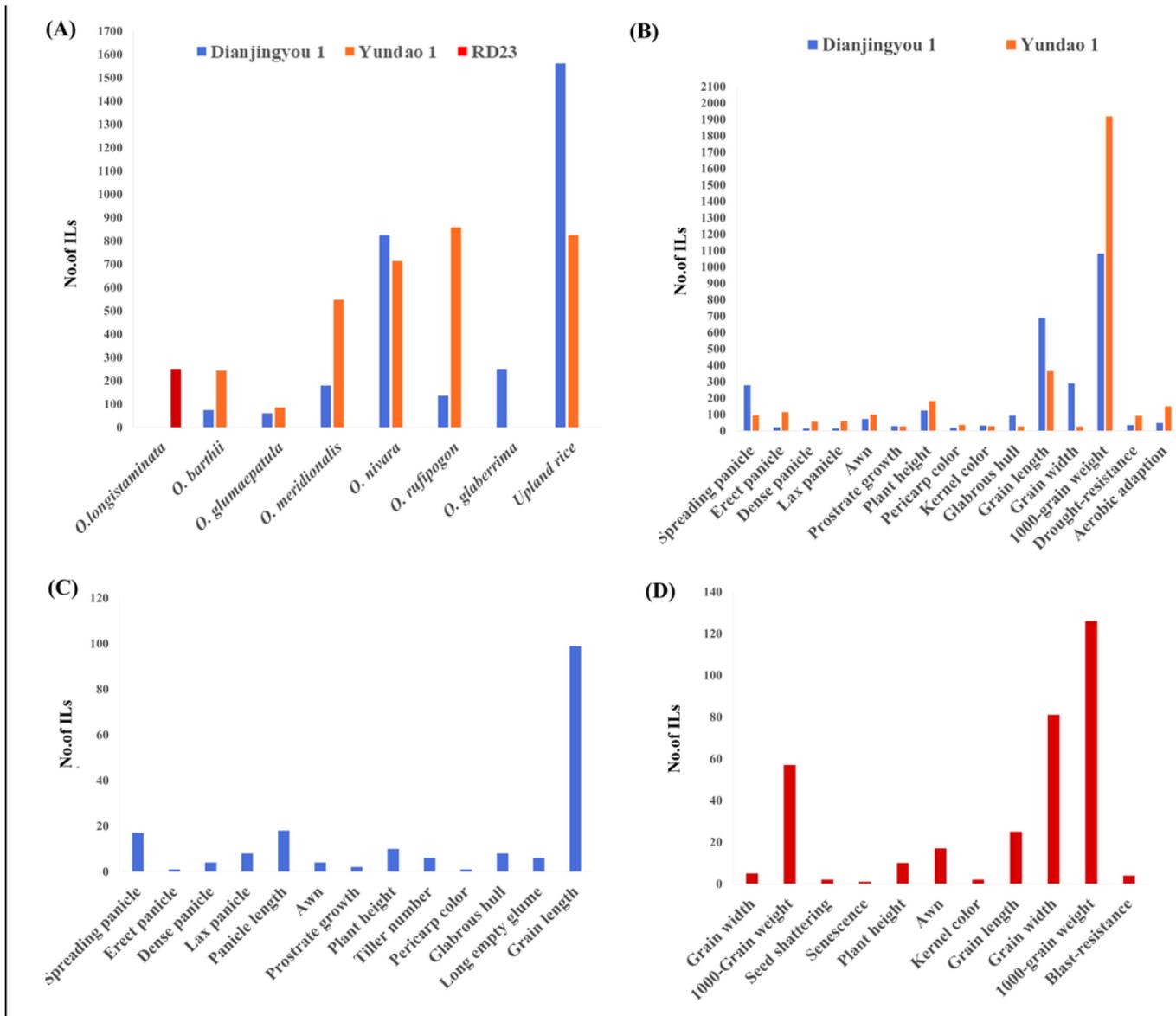


Figure 1

A total of 6372 introgression lines with multiple donors showed significant difference in the agronomic traits, including spreading panicle, erect panicle, dense panicle, lax panicle, awn, prostrate growth, plant height, pericarp color, kernel color, glabrous hull, grain

size, 1,000-grain weight, drought resistance and aerobic adaption, compared with their respective recurrent parents

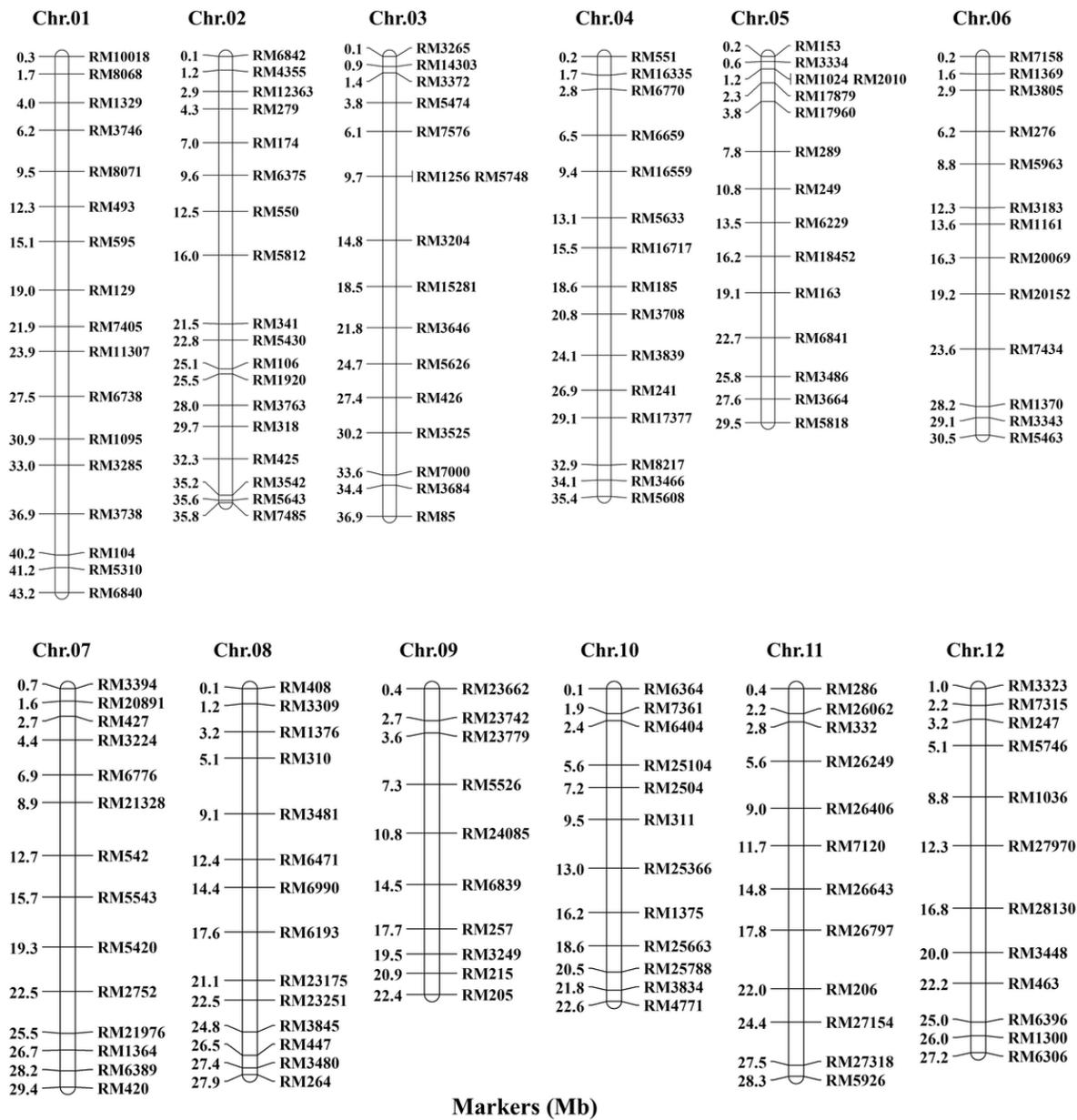


Figure 2

A total of 168 SSR markers distributed on 12 chromosomes were selected to genotype the introgression library in Dianjingyou 1 background. The length of the interval between two markers ranged from 0.2-5.5 Mb, with an average of 2.22 Mb on the rice physical map

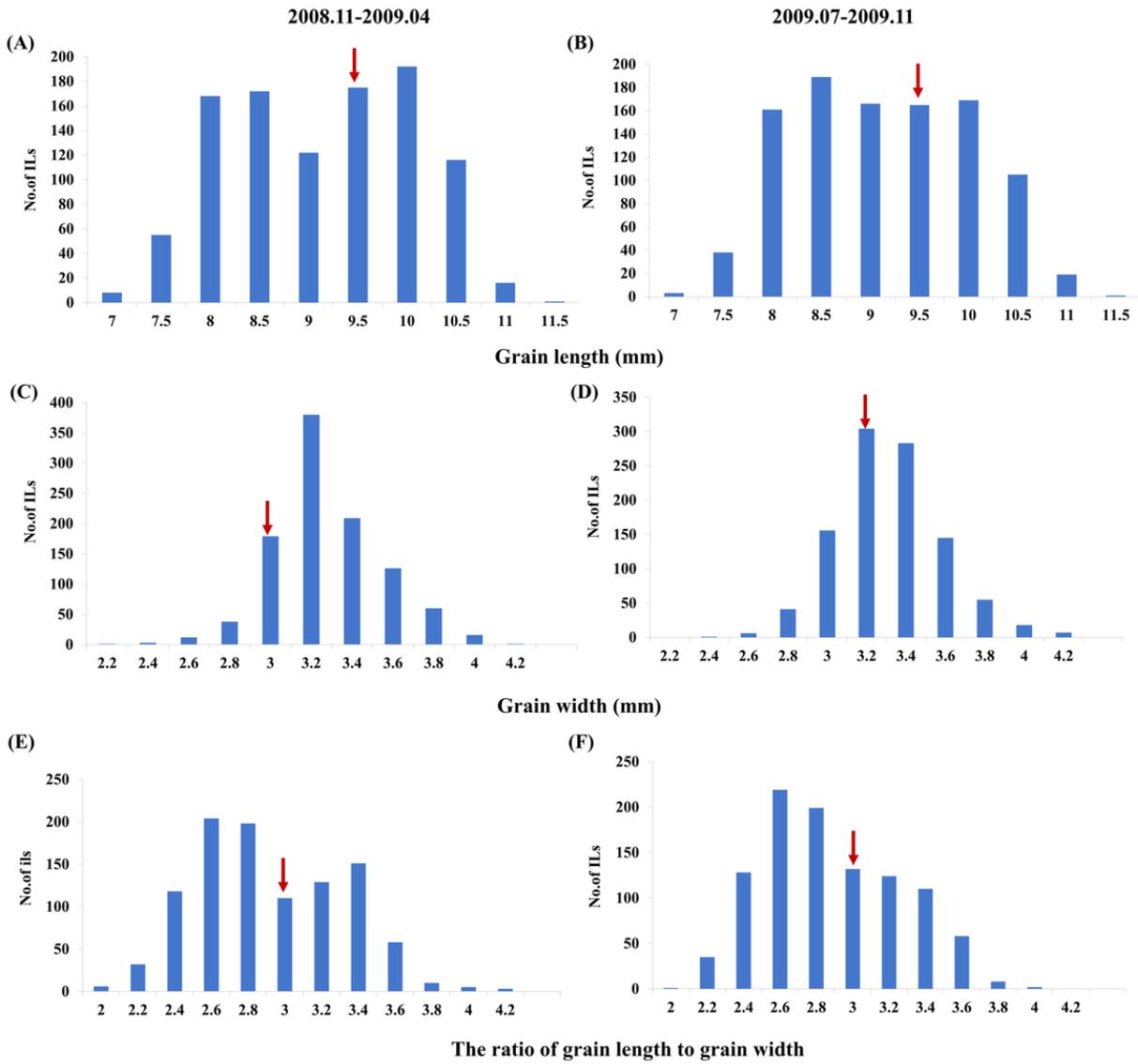


Figure 3

For the ratio of grain length to grain width, 277 and 178 ILs were found to be higher than the Dianjingyou 1 in both seasons (Figure. 3).

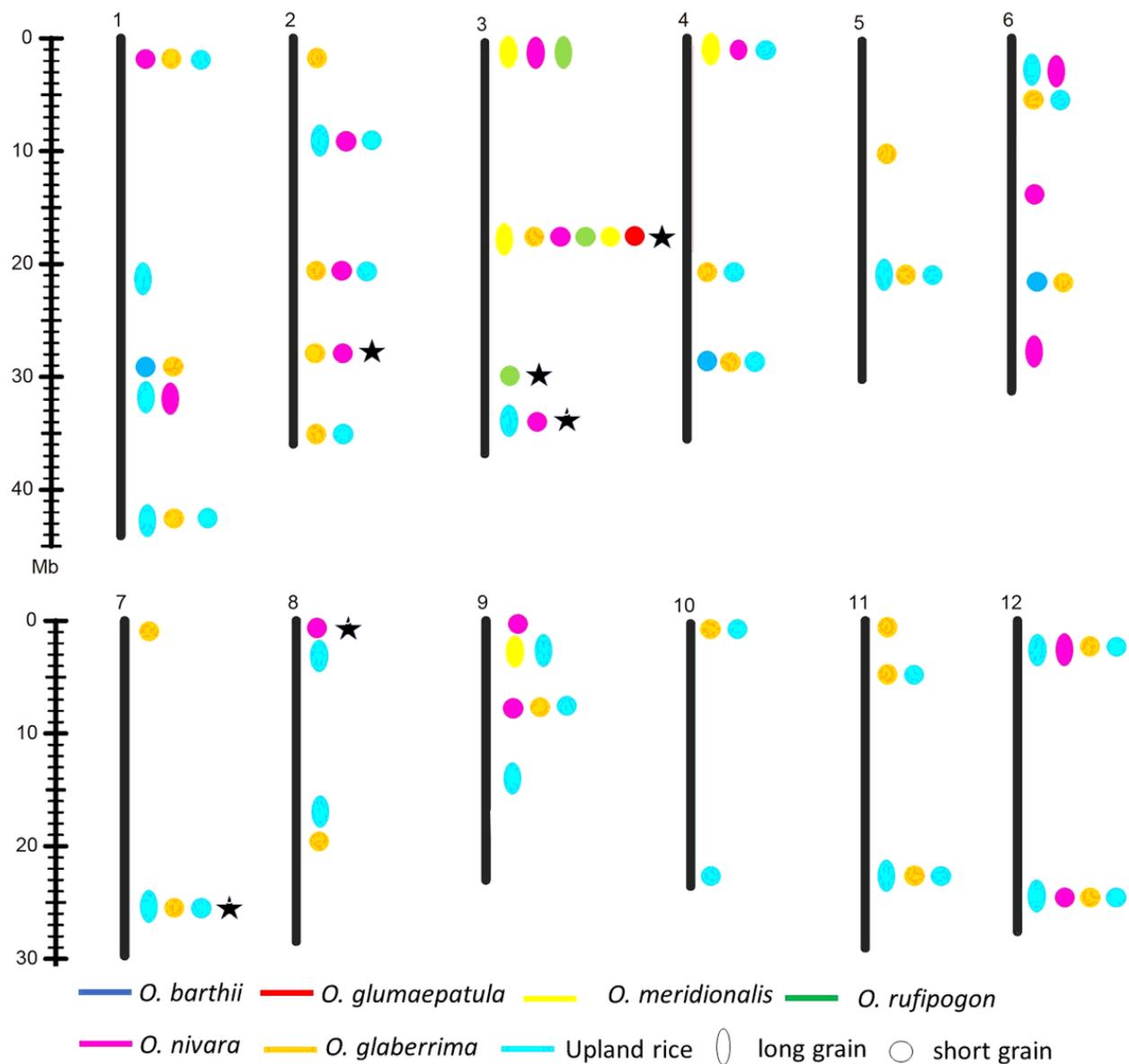


Figure 4

Moreover, 4 loci from the different donors were only responsible for long grain, 12 loci derived from the multiple donors only contributed to short grain, and 10 loci from the different species controlled both long grain and short grain

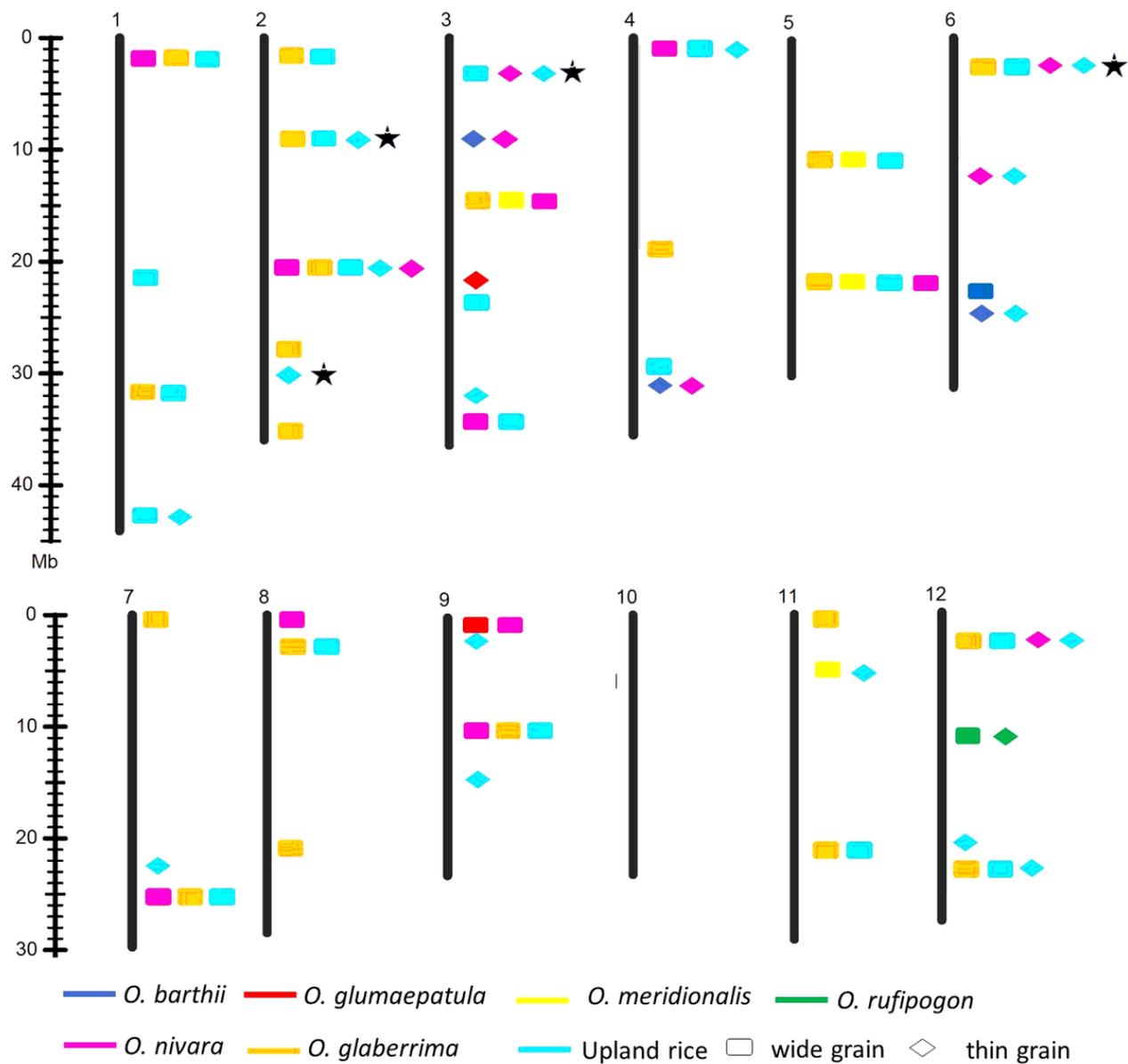


Figure 5

12 loci from the different donors were only responsible for wide grain, 4 loci from multiple species only contributed to thin grain, and 10 loci from the different species controlled both wide grain and thin grain (Figure. 5).

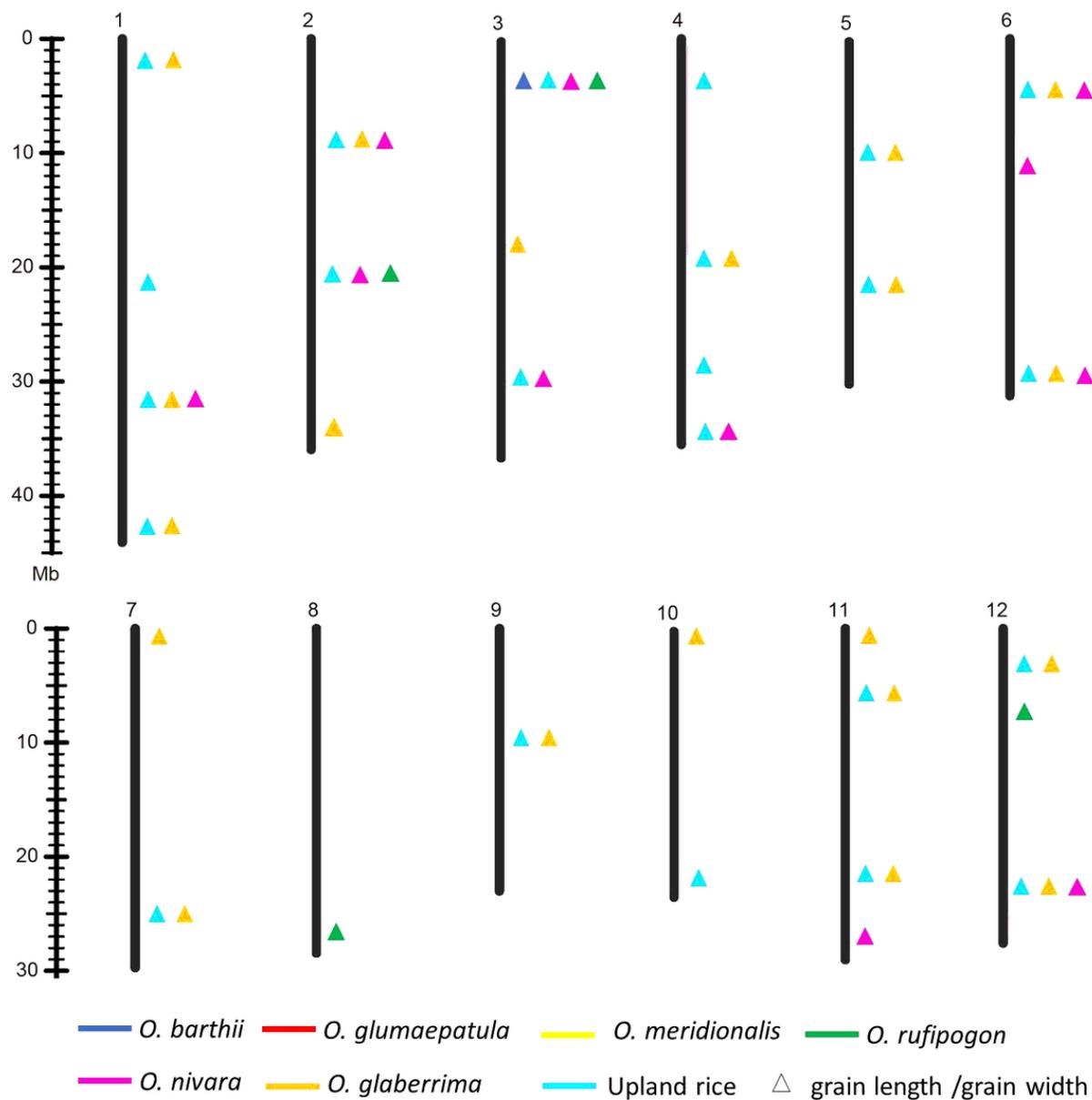


Figure 6

19 loci for the ratio of grain length to grain width from multiple donors were explored on 12 chromosomes, including 12, 6, and 1 loci detected simultaneously in two, three and four donor species,

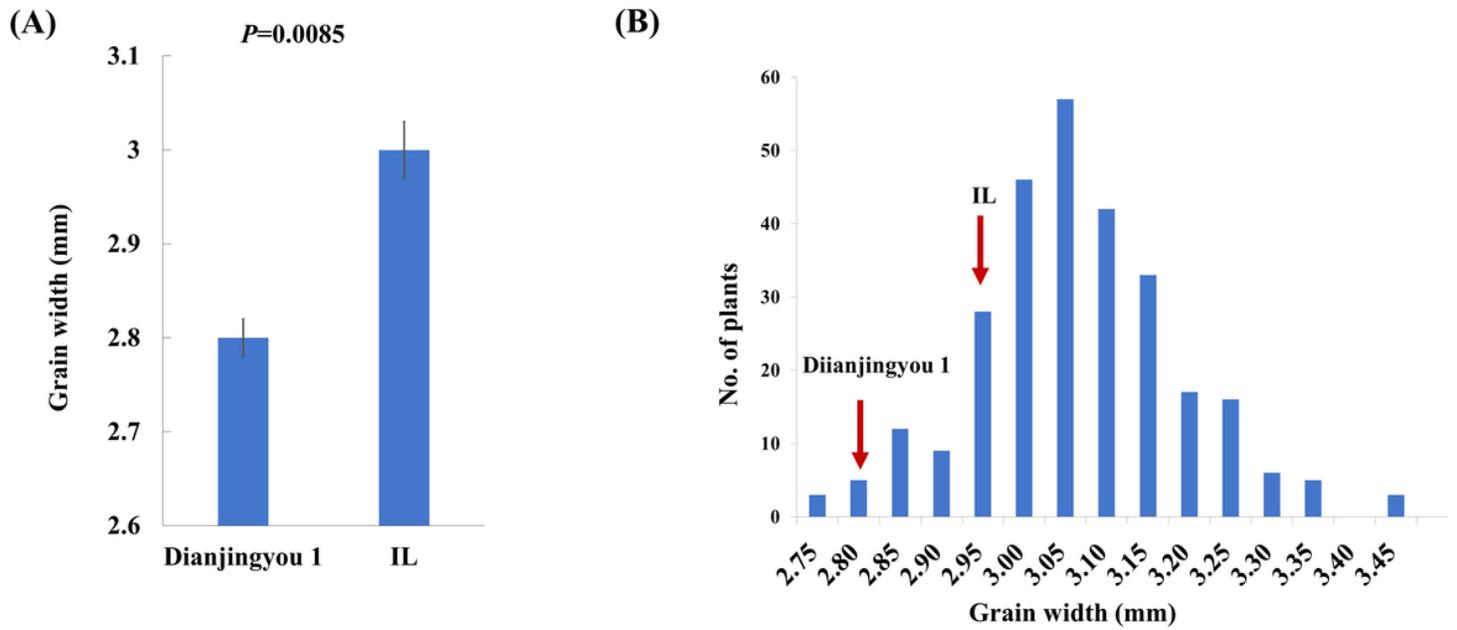


Figure 7

In the BC4F2 population, phenotypic values of grain width showed a continuous distribution.

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