Note

Development of backcross recombinant inbred lines between *Oryza sativa* Nipponbare and *O. rufipogon* and QTL detection on drought tolerance

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Backcross populations at advanced generations are useful in evaluating the agronomic traits from unadapted germplasms, such as wild species. In this study, we generated 159 backcross recombinant inbred lines (BRILs) at the BC₂F₈ generation between *Oryza sativa* Nipponbare (a recurrent parent) and *O. rufipogon* W630 (a donor parent). These BRILs were found to have nearly homozygous genome constitutions based on the marker genotypes at 180 SSR loci covering whole chromosomes. Since their selfed progenies have almost identical genotypes in each line, they were further used for QTL analysis of drought tolerance. Out of six QTLs detected, three were found to have wild alleles with favorable effects on drought tolerance. The present BRILs with known marker data can be applied to more QTL analyses of traits specific to wild species.

Key Words: rice, *Oryza rufipogon*, backcross recombinant inbred lines, QTL analysis, drought tolerance.

Introduction

The genus *Oryza* comprises two cultivated species and more than 20 wild species (Vaughan 1994). Based on the studies on morphological traits and hybrid fertility, Asian common rice (*Oryza sativa*) was found to have originated from the Asian wild species, *O. rufipogon* (Morishima *et al.* 1963, Oka and Chang 1961). Since *O. rufipogon* has a higher cross-affinity with *O. sativa* than other wild species, it provides most accessible wild genetic resources for improvement of rice cultivars. In fact, many useful traits have been identified and transferred to elite breeding lines (Brar and Khush 1997). These traits are usually controlled by single genes and can be easily identified by phenotypic screening. On the other hand, quantitative traits, such as plant size and yield, are associated with many genes, and their manner of inheritance seems to be very complicated. Tanksley and Nelson (1996) proposed an advanced backcross QTL analysis as a molecular breeding method to identify and transfer agronomically useful QTLs from unadapted germplasms. Using BC₂F₁ and BC₂F₂ populations, many wild QTL alleles have been found to have the potential of substantially increasing the quality or productivity of cultivated tomato and rice species (Tanksley *et al.* 1996, Xiao *et al.* 1998). However, the marker data used for QTL analysis were applicable only with the population examined. Since heterozygous chromosomal segments are still maintained in the backcross

plants at BC₂F₁ and BC₂F₂ generations, their selfed progenies show further segregation of genotypes.

Recombinant inbred lines (RILs) are produced by continually selfing the progeny of each F₂ plant. They constitute a permanent population in which segregation is fixed. In rice, several RILs were produced between *O. sativa* and *O. rufipogon*, and QTL analyses of domestication-related traits were successfully performed (Cai and Morishima 2002, Onishi *et al.* 2007). However, it is difficult to evaluate agronomical traits with RILs because large morphological variations are expected to occur among RILs and many undesirable wild traits would mask the effects of beneficial QTL alleles.

In this study, we generated backcross recombinant inbred lines (BRILs) at the BC₂F₈ generation between *O. sativa* Nipponbare (a recurrent parent) and *O. rufipogon* W630 (a donor parent). These BRILs were found to have nearly homozygous genomes based on the fixation levels of marker genotypes at SSR loci. Since their selfed progenies have almost identical genotypes in each line, they can be applied for the trait evaluation that requires many individuals or replications. *O. rufipogon* is a wild species naturally surviving in tropical Asian countries, and it sometimes encounters severe natural stresses such as drought. In a preliminary experiment, we found *O. rufipogon* W630 had high degree of drought tolerance. Therefore, the BRILs having small wild chromosomal segments in the genetic background of *O. sativa* Nipponbare were further used for QTL analysis of drought tolerance to survey the useful wild alleles.

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Materials and Methods

Plant materials

A Japonica rice cultivar, *O. sativa* Nipponbare, and a wild annual accession of *O. rufipogon* W630 from Myanmar having high degrees of drought tolerance were used in this study. The wild seeds were provided by the National Institute of Genetics, Japan. BRILs were produced as follows: A single wild plant of *O. rufipogon* W630 was crossed twice with *O. sativa* Nipponbare, and 18 BC₁F₁ plants were obtained. They were further backcrossed, and 216 BC₂F₁ plants (12 plants each from 18 BC₁F₁ plants) were generated. From them, BRILs at the BC₂F₈ generation were developed by the single-seed-descendant method. The plants were not artificially selected during the selfing process, but almost 50 lines were eliminated because of weak growth and sterility. As a result, 159 BRILs, which theoretically contain 1/8 of the wild genome in the genetic background of *O. sativa* Nipponbare, were generated.

Determination of marker genotypes at SSR loci

DNA was extracted from 159 BRILs using the potassium acetate method as described by Dellaporta *et al.* (1983). In total, 180 polymorphic SSR markers between *O. sativa* Nipponbare and *O. rufipogon* W630 were selected to survey the BRIL genotypes in 12 chromosomes. The total map size of these markers was 1362 cM, with an average marker interval of 8.2 cM. The primer sequences of these SSR markers were obtained from the Gramene database (<http://www.gramene.org/>) by Liang *et al.* (2008). PCR was carried out in a 25- μ l reaction, and the amplified products were electrophoresed in 4% polyacrylamide denaturing gel with 0.5 $\times$ TBE buffer. The microsatellite banding patterns were visualized using the silver staining method as described by Panaud *et al.* (1996).

Evaluation of drought tolerance

Drought tolerance evaluation was carried out in 159 BRILs (or BC₂F₉ plants) and their parents. The experiments were performed in the university greenhouse to avoid rainwater. An upland cultivar of IRAT13 was also included as a drought-resistant control. The germinated seeds were sown in the seedling cases, and three-week-old seedlings were transplanted at intervals of 8 cm in a soil plot of 0.8 m $\times$ 5.5 m with three replications. The plot was surrounded by a cement frame, and equal moisture levels in the plot were checked by a model DM-8 tensiometer (Takemura Co., Japan). After supplying the water for 30 days, irrigation was withheld. On the 12th day of drought treatment, some plants started to show rolling leaves. On the 21st day, when the leaf-rolling phenotypes were highly varied among the BRILs, the degree of leaf rolling was visually scored at mid-day on a scale of 0–9 (0: unrolled, 9: tightly rolled) according to the scores of the Standard Evaluation System for rice (INGER 1996). The BRILs were continuously maintained under drought stress for the next 18 days to induce leaf dry-

ing. Irrigation was restarted, and after 10 days the drying of leaves was scored on a scale of 0–9 (0: no symptoms, 9: all leaves apparently dead) (INGER 1996). For both traits, average scores of three replications were calculated for all lines.

QTL analysis

Based on the marker and trait data, QTL analysis for drought tolerance was carried out. Putative QTLs were estimated by composite interval mapping using WinQTL Cartographer ver. 2.5 software (Wang *et al.* 2007). The optimal log of odds (LOD) threshold values obtained from WinQTL Cartographer (with the permutation value set at 1,000) were used to determine the presence of a putative QTL. The percentages of variation explained by the QTL and the additive effect were also estimated using the software.

Results and Discussion

Genome composition of BC₂ backcross inbred lines

BRILs at the BC₂F₈ generation were expected to have the genetic constitution of the recurrent cultivar, *O. sativa* Nipponbare, with the exception of small substitutions of chromosome segments from the wild accession, *O. rufipogon* W630. Theoretically, these BRILs may have 1/8 (12.5%) of the wild genome in the genetic background of Nipponbare. Based on the marker genotypes at 180 microsatellite loci on 12 rice chromosomes covering 1,362 cM, the wild chromosomal segments in each line were estimated. The frequency distribution of the proportion of wild genome introgressed into BRILs is shown in Fig. 1. The percentages of wild genome ranged from 0.0% to 23.6%, with a mean of 11.3%. This value is close to the theoretical ratio of 12.5%. Compared with lines having specific wild chromosomal segments, such as chromosome segment substitution lines and introgression lines, the BRILs have various combinations of wild chromosomal blocks in the genome. Therefore, they are good materials to investigate interactions between wild genes/loci.

The numbers of the lines having wild homozygous alleles at 180 microsatellite loci were examined. The minimum and maximum numbers of the lines having wild homozygous alleles were three and 39, respectively. This shows that no loci were fixed with only Nipponbare alleles, and a set of BRILs used here may carry the complete genomic regions of W630. By the single-seed-descendant method, 159 BRILs were developed from the BC₂F₁ plants that originated from 18 BC₁F₁ plants. Although we can not directly examine the segregation distortion among BRILs because of the bottleneck effect at the BC₁F₁ generation, the following three wild chromosomal regions were found to be covered by a few BRILs (3–5 lines): RM259–RM35 region on chromosome 1, RM417–RM142 on chromosome 4, and RM219–RM321 on chromosome 9. These suggest that some wild genes associated with semi-sterility or hybrid weakness are located in the regions. In this study, the genotypes at 28,620 loci (180 loci $\times$ 159 lines) were surveyed, but 21 could not be

Fig. 1. Frequency distribution of percentage of *O. rufipogon* genome found in the backcross recombinant inbred lines between *O. sativa* Nipponbare and *O. rufipogon* W630.

determined. The remaining 28,599 loci consist of 25,277 Nipponbare homozygous, 161 heterozygous and 3,161 wild W630 homozygous loci. The homozygous fixation value was 99.4%; $(25,277 + 3,161)/28,599$. This result confirmed that the BRILs at the BC₂F₈ generation are almost pure lines and their progenies can be repeatedly used for the trait evaluation that requires many individuals or replications.

QTL analysis for drought tolerance

Drought tolerance evaluation was carried out using 159 BRILs and their parents in the university greenhouse with three replications. *O. rufipogon* W630 was more tolerant to drought (1.7 and 1.0 for average leaf rolling and leaf drying scores, respectively) than *O. sativa* Nipponbare (9.0 and 6.3 for average leaf rolling and leaf drying scores, respectively). The degree of tolerance of W630 was slightly stronger than that of the drought resistant control cultivar (IRAT13) in this experiment. The frequency distributions for leaf rolling and leaf drying in BRILs are shown in Fig. 2A and 2B, respectively. Using these trait data, QTL analysis for drought tolerance was carried out. In total, four and two QTLs were found for leaf rolling and leaf drying, respectively (Table 1 and Fig. 3). Their LOD score varied from 3.56 to 7.92, and individual QTL explained variance ranged from 6.8% to 23.0%. Desirable wild alleles decreasing drought scores were detected at one locus for leaf rolling in the RM243–RM129 region on chromosome 1 and two for leaf drying in the RM580–RM9 and RM249–RM440 regions on chromosomes 1 and 5, respectively. These useful wild QTL regions on chromosome 1 for both traits were partly overlapping and were close to the QTL for leaf drying reported by Price *et al.* (2002). In the similar region of RM249–RM440 on chromosome 5, Yue *et al.* (2005) detected a leaf rolling QTL with a drought-tolerant allele from IRAT109 (an upland rice cultivar). Although they found QTLs near the regions where we detected, their plant materials and drought treatment procedures were different from those used in this study. More

Fig. 2. Frequency distribution of drought tolerance in the backcross recombinant inbred lines between *O. sativa* Nipponbare and *O. rufipogon* W630. (A) Leaf rolling. (B) Leaf drying. Npb: *O. sativa* Nipponbare. W630: *O. rufipogon* W630.

Fig. 3. Chromosome positions of putative QTLs controlling drought tolerance (LR: leaf rolling, LD: leaf drying) detected in the backcross recombinant inbred lines between *O. sativa* Nipponbare and *O. rufipogon* W630. QTL positions are represented as bars with the LOD peaks shown by arrows.

Table 1. Putative QTLs for drought tolerance (LR: leaf rolling, LD: leaf drying) detected in the backcross recombinant inbred lines between *O. sativa* Nipponbare and *O. rufipogon* W630

Trait	Chr.	QTL region	Nearest marker	LOD	PV ^a (%)	A ^b	Source ^c
LR	1	RM243-RM129	RM23	4.51	8.9	-1.12	W
	1	RM128-RM431	RM265	3.64	6.8	0.80	N
	6	RM30-RM412	RM412	7.92	23.0	1.46	N
	11	RM21-RM144	RM206	5.62	12.9	1.11	N
LD	1	RM580-RM9	RM129	3.93	13.1	-0.59	W
	5	RM249-RM440	RM440	3.56	17.0	-0.62	W

^a Percent phenotypic variance explained by the QTL.^b Additive effect of the allele from *O. rufipogon* W630.^c Favorable allele source for drought tolerance (decreasing effects on LR and LD). W: *O. rufipogon* W630. N: *O. sativa* Nipponbare.

detailed examination is required at different stages to confirm the useful wild alleles for drought tolerance.

Conclusions

Advanced backcross populations are useful in evaluating the agronomic traits from unadapted germplasms, such as wild species. In this study, we generated 159 BRILs at the BC₂F₈ generation between *O. sativa* Nipponbare and *O. rufipogon* W630. Their chromosomal information was determined using 180 SSR markers covering whole chromosomes. BRILs were found to have nearly homozygous genome constitutions, and the same genotypes of the lines are easily produced from their selfed progenies without segregation. They can be applied for the trait evaluation that requires many individuals or replications. Besides the QTL analysis on drought tolerance in this study, we are able to carry out more QTL analyses for traits specific to wild species using the same set of BRILs with known marker genotype data.

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