Characterization of a Recombinant Inbred Line Population of Rice Using SSR Markers

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ABSTRACT

A population of 269 F$_{10-11}$ recombinant inbred lines (RILs), from a cross between ‘Kaybonnet’ low phytic acid 1-1 (KBNT/lpa) and ‘Zhe733’, was molecularly characterized using simple sequence repeat (SSR) markers. One hundred and seven markers were mapped on 12 rice chromosomes, representing a total of 1016.3 cM of genetic distance. The average frequencies of overall genome heterozygous and non-parental alleles per RIL were 1.3% and 0.4%, respectively. Thirteen heterozygous RILs at ≥5 marker loci and nine RILs with ≥5 non-parental alleles were identified, representing 5.1% and 3.5% of the 255 RILs population. Two hundred and thirty-eight RILs were clustered as 10 sub-groups based on Nei’s (1972) genetic distance. Sixty-nine RILs were selected for field evaluation of rice water-weevil resistance based on the similarities of their genetic background. This linkage map would facilitate developing DNA markers to tag genes resistant to rice pests, agronomically important traits, and also for marker-assisted selection.

INTRODUCTION

Kaybonnet low phytic acid 1-1 (KBNT/lpa) is an irradiation-induced mutant of a tropical japonica cultivar Kaybonnet with the phytic acid portion of seed phosphorus reduced from 71% to 39%. KBNT/lpa possesses a single recessive gene, lpa1-1, for low phytic acid and resistance genes to several U.S.-predominant races of rice blast (Magnaporthe grisea) such as IB-1, IB-49, IC-17, and IG-1 (Rutger et al., 2004). Zhe733 from China is a high-yielding, early-maturing indica rice cultivar and also resistant to M. grisea (Yan and Cai, 1991) and to straighthead, a physiological disorder of rice in the
U.S. (Yan et al., 2005). Using these parental cultivars, Rutger and Tai (2005) developed the F$_{10-11}$ generation of recombinant inbred lines (RIL) population of KBNT/lpa×Zhe733, which is used in this study.

RIL populations have been extensively used for constructing molecular marker-based genetic linkage maps using SSR markers in many crops. Similarly, rice RILs also have been used in mapping qualitative and quantitative traits. Therefore, molecular characterization of KBNT/lpa×Zhe733 RILs population is useful in mapping of rice blast resistance genes, the genes associated with low phytic acid composition, and other agronomically important traits or difficult traits such as reaction to rice water weevil (RWW), *Lissorhoptrus oryzophilus* Kuschel. The objectives of this study were to evaluate heterozygosity in this RIL population using SSR markers, to construct an SSR-based genetic linkage map, and to cluster and select the RILs according to their SSR genotypes.

**PROCEDURES**

The KBNT/lpa×Zhe733 population (Rutger and Tai, 2005) of 269 F$_{10-11}$ RILs was used in this study. The KBNT/lpa×Zhe733 RILs (KZRILs) were planted in plastic pots. DNA extraction was performed based on the method by Tai and Tanksley (1990). DNA samples were qualitatively determined, quantified and normalized to 5 ng/μL prior to DNA amplification. One hundred and sixty SSR markers were tested on the parents and 109 polymorphic markers were used to test KZRILs population. PCR amplification was performed following the standard procedure. The samples were run on an ABI Prism 3700 DNA analyzer according to the manufacturer’s instructions. SSR fragment sizing was performed using the software GeneScan® and Genotyper®. Alleles were binned manually.

Data analysis for all marker loci was based on successful marker amplification and DNA product analysis on the DNA analyzer. All loci used in this study were polymorphic with a frequency of less than 0.94. Genetic linkage analysis of SSR markers was performed using the software JoinMap®. Loci were assigned to linkage groups by the program default settings with likelihood-odds-ratio (LOD) scores equal to or higher than 3.0. The “fixed order” command was used to identify the most probable marker order within a linkage group. Genetic distance and cluster analysis were conducted using the software PowerMarker (http://statgen.ncsu.edu/powermarker). Nei’s (1972) genetic distance was used to calculate pair-wise genetic distance among all the KZRILs. Unweighted pair-group method using arithmetic average (UPGMA) method was used for cluster analysis. Cluster tree was constructed using the program Mega (http://www.megasoftware.net). The KZRILs in cluster sub-groups representing genetic diversity of the KZRIL population were selected using the function of “Line Selection” of PowerMarker and the selected KZRILs were re-clustered.
RESULTS AND DISCUSSION

Of 255 KZRILs detected by 109 markers, 172 KZRILs (67.5%) were homozygous; 42 KZRILs (16.4%) were heterozygous at a highest marker loci of 42; 30 KZRILs (11.8%) had up to 9 non-parental alleles; and 11 KZRILs (4.3%) were heterozygous and had non-parental alleles. A KZRIL detected as having heterozygosiy or non-parental alleles at more than 5 marker loci was defined as a heterozygous KZRIL or a non-parental KZRIL. Thus, 13 heterozygous KZRILs and 9 non-parental KZRILs were found, representing 5.1% and 3.5% of the 255 KZRILs population, respectively. The average frequencies of overall genome heterozygosity and non-parental alleles per KZRIL were 1.3% and 0.4%, respectively. Theoretically, the average frequencies of heterozygous loci in a F10 and F11 RIL population should be 0.2% and 0.1%, respectively. Even though the frequency of heterozygous loci in this study (1.3%) was higher than the theoretical values, it is still obviously lower than the average frequencies of 3.6% by Xiao et al. (1996) and Cho et al. (1998).

A genetic linkage map of 107 marker loci was constructed based on the analysis of 109 SSR markers (Fig. 1). RM1 and RM408 were not linked to other markers in the linkage map. The mapped markers covered 12 rice chromosomes in 1016.3 cM of genetic distance with an average of 9.3 cM between two markers. This is shorter than the genetic distance of 1565.9 cM for the same number of SSR markers from the database of “Cornell2001” in Gramene (http://www.gramene.org). The total genetic distance in this population was 64.9% of Cornell map (2001). Similarly, He et al. (2001) reported that the genetic distance of each chromosome in an RIL population was shorter than that in a double haploid (DH) population. The total genetic distance in the RIL population of ZYQ8/JX17 (indica/japonica) was 70.5% of that in the DH population derived from the same rice cross. The order of the markers on chromosomes 1, 2, and 4-11 agreed with Cornell2001. However, there were some disagreements on marker order with Cornell2001 in this study. The high percentage of skewed markers towards Zhe733 on chromosome 3 and the relatively small number of markers on chromosome 12 might result in these disagreements.

Excluding heterozygous and non-parental KZRILs, cluster analysis was applied to 238 KZRILs using UPGMA method. The dendrogram showed a clear separation of the KZRILs into 10 sub-groups (Fig. 2). Clustering of KZRILs is particularly useful to select representative RILs of the whole population for mapping RWW resistance. Reducing the number of RILs by means of line selection is necessary to study such a trait that is so difficult to evaluate. Screening for resistance to RWW in the greenhouse has not been possible due to the difficulty in culturing RWW in an environment-controlled condition (Zhang et al., 2004). However, field evaluation of RWW resistance is feasible with a small number of test entries. For this purpose, 69 representative KZRILs were selected based on the similarities of their genetic background for field phenotyping RWW resistance (Fig. 3).
SIGNIFICANCE OF FINDINGS

Clustering and selection of KZRILs are essential steps towards phenotyping difficult traits such as RWW resistance. The KBNT/lpa/Zhe733 RIL population has been confirmed as an excellent mapping population. The genetic linkage map generated in this study will be useful for mapping and cloning genes of agronomic interest and for marker-assisted selection in rice improvement.

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LITERATURES CITED


Fig. 1. A genetic linkage map of 107 SSR markers on 12 rice chromosomes based on 269 RILs of KBNT/lpa/Zhe733 population. *The genetic distances of SSR markers in cM were shown on the left side of each chromosome.*
Fig. 2. Clustering of 238 RILs of KBNT/lpa/Zhe733 population using UPGMA method based on Nei’s (1972) genetic distance. The RILs indicated with the symbol of “●” were the representative RILs selected for further field evaluation for rice water weevil resistance.
Fig. 3. Clustering of the selected representative RILs in the KBNT/pai/Zhe733 population using the UPGMA method.