Mapping of Rice Rf Gene by Bulked Line Analysis

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Abstract

A method, bulked line analysis (BLA), was developed for identification of the RFLP markers associated with a target gene. Instead of segregating progenies, conventional lines sharing the same trait were bulked by the BLA method. This method is an alternative approach to the identification of DNA markers linked with a target gene. A major advantage of this method is time-saving for genetic stock development. The advantage is very significant for organisms having a long generation period. This method has been tested by using fertility restoration of rice cytoplasmic male sterility of wild abortive type as a target trait. A fertility-restoring gene was successfully identified by linkage with RFLP markers. This gene was mapped in the middle of the long arm of chromosome 10 of the rice genome.

Key words: Rf gene; Gene Mapping; Marker Identification; RFLP marker

1. Introduction

The advent of techniques for detecting abundant DNA markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP), have provided powerful tools for genetic map construction and gene location identification. Three linkage maps have been constructed for the rice genome using DNA markers.1–3 All these linkages have been assigned to relevant chromosome arms by cytological techniques.2,4 Therefore, an agronomically important rice gene can be located precisely through the linkage map. Identification of the linkage between interesting genes and DNA markers can be carried out by using various genetic stocks, such as nearly isogenic lines (NILs),5 bulked segregant analysis (BSA),6 and segregating progenies. However, all these genetic stocks are restricted to advancing generations through specific crosses. This disadvantage appears more significant for an organism having a long period for advancing a new generation. If cultivars or advanced lines sharing the same phenotype can be used instead, identification of DNA markers associated with a target gene will be more flexible for genetic stock preparation.

Although a number of cultivars and advanced lines are available as the restorers and maintainers for rice cytoplasmic male sterility of wild abortive type (CMS-WA), identification of more elite restorers and maintainers is needed for making more promising hybrid combinations. If DNA markers tightly linked with a fertility restoring (Rf) gene is identified, a testcross used for restorer evaluation can be replaced by marker-assisted selection, which will speed up the breeding programs. Furthermore, such identification will improve our understanding of the inheritance of fertility restoration. Although it has been reported that restoration is mainly controlled by one or two dominant genes which act sporophytically,7–9 gene location is still not well identified. To date, three Rf gene locations were reported on chromosomes 1, 7, and 10,10,11 respectively. However, there is no molecular evidence which supports their localization on chromosomes 7 and 10.

In this study, we developed a method for the identification of DNA markers linked to desired traits in breeding programs. The method is termed “bulked line analysis” (BLA), and has been used for the identification of DNA markers associated with an Rf gene of rice CMS-WA cytoplasm.

2. Materials and Methods

2.1. Plant materials

Six maintaining and 15 R restoring lines of CMS-WA were used for the marker identification by BLA method
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Table 1. Plant materials

<table>
<thead>
<tr>
<th>Maintainer</th>
<th>Restorer</th>
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<tbody>
<tr>
<td>RD21</td>
<td>SPR 60</td>
</tr>
<tr>
<td>V20</td>
<td>IR 24</td>
</tr>
<tr>
<td>IR64608</td>
<td>RD 23</td>
</tr>
<tr>
<td>KDML105</td>
<td>RD 7</td>
</tr>
<tr>
<td>Basmati4</td>
<td>SPR 90</td>
</tr>
<tr>
<td>IR54752</td>
<td>RD 23</td>
</tr>
</tbody>
</table>

2. Identification of Rice Rf Gene by Bulked Line Analysis

Two segregating populations, one BC1 (RD21A/IR24) and an F2 (RD21A/IR13419-113-1) were used for progeny analysis, and the BC1 population was used for Rf gene mapping. RD21A is a male sterile line possessing CMS-WA cytoplasm. IR24 and IR13419-113-1 are two restoring lines for CMS-WA cytoplasm. The BC1 and the F2 populations consisted of 122 and 197 individuals, respectively.

RFLP clones were obtained from Cornell University (NY, USA), and the Rice Research Genome Program (RGP) in Japan. Chromosomal localization of the clone used was done according to the published map.2,4

2.2. Fertility scoring

Viable pollen percentage of a progeny was examined by staining with 1% I2-KI. For gene mapping, 56 highly sterile (pollen fertility below 10%) BC1 progenies were phenotyped as 1, and 48 highly fertile (fertility above 75%) progenies were as 2. The intermediaries were scored as missing data.

2.3. DNA extraction

Total genomic DNA was extracted using the method described by McCouch et al.12

2.4. Bulked line analysis (BLA)

Equivalent amounts of DNA of the 15 R lines were bulked into three R pools, each of which consisted of five R lines. DNA of the six B lines were bulked into a B pool. Since two pools possessed opposite target phenotypes, but random genetic background, the RFLP markers closely linked to the target gene would show bands distinct from the B and R pools.

2.5. RFLP analysis

The DNA was digested with six restriction endonucleases (BamHI, BglII, Dral, EcoRI, EcoRV, HindIII) for polymorphic marker screening. RFLP probe labeling, DNA hybridization and chemiluminescent detection were carried out with the DIG system (Boehringer Mannheim) according to the manufacturer’s recommendations.

2.6. Gene mapping

Mapping was carried out with the Mapmaker (Lander ES, 1993, Mapmaker/exe 3.0). Square of correlation coefficient (Rsq), phenotypic means of marker classes, and T-test for the significance of difference between the phenotypic means was conducted with statistic function in Microsoft Excel.

3. Results

3.1. RFLP marker identification by BLA method

A total of 180 RFLP clones covering 12 rice chromosomes spanning approximately 10-cM intervals were screened against DNA from the B and R pools. Of these, two markers located on the long arm of chromosome 10 revealed the expected polymorphism between the B and R pools. The R pools had a band not detected in the B pool. One marker, RG134, gave a 1.9-kb band on the DNA of the R pools digested with HindIII, but not on the DNA of the B pool (Fig. 1). Another marker, RG561, showed that R pools had a 11.2-kb and a 5.2-kb band on the digestion with EcoRV and HindIII, respectively, but the B pool did not have those two bands (Fig. not shown).

3.2. BLA method verification by progeny analysis

The two positive markers were screened on the two segregating populations having fertility scoring from 0.0% to 98.5%. No polymorphism between the parents of the two populations was detected with the marker RG561. The RG134 clone only showed polymorphism between the parents of the BC1 population. Further investigation

Figure 1. Identification of RFLP marker associated with rice Rf gene by BLA method. Lane M is lambda DNA digested by HindIII. Bulked DNA were digested with two restriction enzymes. Hybridization of RG134 with the DNA digested by HindIII revealed that all three R bulks (lanes 1,2,3) have a 1.9 kb band, while that band is missing in B pool (lane 4). Lanes 5, 6, 7, and 8 are EcoRV-digested DNA, which are the three R bulks and the B pool, respectively.
with this population and this marker revealed that sterile progenies were homologous, showing the same banding pattern as the CMS line (recurrent female parent), while fertile progenies were heterozygous, containing two RFLP loci from both parents (Fig. 2). Pollen fertility means of marker classes were 15.04% for the homozygous genotype (rr), and 71.53% for the heterozygous genotype (Rr). The difference between the two phenotypic means was highly significant (P < 0.01). The percentage of recombination between marker RG134 and the Rf gene was 8.8%. This result confirmed that the target gene was located on the long arm of chromosome 10.

In this chromosome region, further analysis was conducted with more RFLP clones. Five markers, namely, S11014, S11148, R716, C1361, G37, showed polymorphism and linkage in one or two populations. Using the BC1 population and these six markers, a partial linkage map of rice chromosome 10 was constructed. An Rf gene was mapped in the C1361 and S11148 interval, 2.1 cM from C1361.

Chromosome 7 was also screened by bulked segregating progenies of the two populations using 30 RFLP markers covering a region of approximately 5 cM. These results are in agreement with that of BLA method; That is, no linkage between an Rf gene and the markers was detected.

4. Discussion

In this study, the Rf gene for rice CMS-WA cytoplasm identified was localized approximately in the same chromosomal region where Rf-1, an Rf gene for rice CMS-BT cytoplasm, is located, since Rf-1 was also linked with RG13413 and was close to C1361.214 The results of this study are also in agreement with the trisomic analysis conducted on chromosome 10 by Bharaj et al.,10 in which an Rf gene was detected on this chromosome.

Although NILs and BSA are effective for the identification of the DNA markers associated with target genes, the BLA method allows the genetic stock to be prepared more quickly. This advantage is more obvious in forest tree possessing a long generation time. In particular, as the BLA method is not based on a segregating population, this method can be useful for asexually propagating organisms where generation of a segregating population is difficult due to sterility.

Because informative marker identification by the BLA method is based on the polymorphism between a pair of bulks, not between a pair of parents, the maximum distance between the target gene and an informative marker which can be detected by this method is expected to be shorter than the maximum distance of approximately 25 cM by the BSA method.6

Although the BLA method can not be used to directly localize genes, it is useful for the identification of DNA markers that are associated with the target gene. Through those markers, the gene can be precisely localized if the markers used have been previously mapped. In this study, the markers used have already been mapped on rice genetic linkage maps.2,3 Therefore, the Rf gene can be localized by the BLA method.

One limitation of the BLA method is that the bulked lines should have the same target phenotype but vary over non-target phenotypes. The origin of the target gene in bulked lines should be the same for the BLA method.
to be effective. In this respect, the Rf gene of rice CMS-WA is a good model for BLA. The Rf genes originate only from the tropical and subtropical indica rice, not from any other types of rice, such as japonica rice.\(^9,15\) For minor genes, this method may not be so effective, because minor genes lack clear expression. In comparison, the BSA method can be used to identify the DNA markers linked with major QTLs.\(^6\)

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**References**


