DNA fingerprinting of cultivated rice with rice retrotransposon probes

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ABSTRACT

Cloned sequences of three retrotransposons of rice, Tos1-1, Tos2-1 and Tos3-1, were used as molecular genetic markers to distinguish the cultivars of Asian and African rice, Oryza sativa and Oryza glaberrima. DNAs of six cultivars each of Indica and Japonica types of O. sativa were analyzed after digestion with four different restriction enzymes. Indica cultivars could be distinguished from each other by any of three types of one probe-one restriction enzyme combination. Although the hybridization patterns were similar among Japonica cultivars, these cultivars could be distinguished from each other by one type of one probe-one enzyme combination. Five cultivars of O. glaberrima examined were also distinguished from each other by using one probe-one enzyme combination. The results shown here indicate that retrotransposon-mediated fingerprinting is an efficient method to distinguish or identify the cultivars of rice. Retrotransposon-mediated fingerprinting should become a general method, because retrotransposons are ubiquitous in plant species and retrotransposon probes can easily be obtained from any plant species.

1. INTRODUCTION

Single copy genomic DNAs or cDNAs have been used as molecular genetic markers to construct restriction fragment length polymorphism (RFLP) maps of crop plants, such as rice (McCouch et al., 1988; Saito et al., 1991), maize (Helentjaris, 1987), lettuce (Landry et al., 1987), soybean (Apuya et al., 1988), oilseed (Figidore et al., 1988), tomato (Young and Tanksley, 1989), potato (Gebhardt et al., 1989) and wheat (Liu et al., 1990). These probes can be used to distinguish or identify cultivars as well as species. For example, potato cultivars are distinguished from each other by using RFLP markers and a sensitive separation technique for restriction fragments (Gebhardt et al., 1989). However, RFLP markers are not always effective, especially, for distinguishing cultivars

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having close genetic backgrounds, for example cultivars of Japonica rice (Kawase et al., 1989).

Most RFLP probes give the information on one locus and are not informative enough in distinguishing cultivars. This problem may be solved by combining multiple probes and restriction enzymes. Another, more effective way is to use probes which detect highly polymorphic, multiple loci. Typical examples are a probe for human minisatellite region (Jeffreys et al., 1985a, b) and a M13 probe (Vassart et al., 1987). These probes, primarily used in animals to detect fingerprints, were also used in plants (Dallas, 1988; Rogstad et al., 1988; Ryskov et al., 1988; Nybom et al., 1990). In addition to those probes, other middle repetitive sequence probes should be used to detect fingerprints. In Drosophila, a major part of middle repetitive sequences consists of one class of transposable element, retrotansposons (Bingham and Zachar, 1989). Recent results suggest that this may be also true in plants (Hirochika et al., 1992; Hirochika and Fukuchi, 1992). Because retrotansposons undergo the replicative transposition through RNA as an intermediate, the transposed sequences are stable enough for genetic analysis. In contrast, transposable elements well studied in maize (Zea mays) and snap dragon (Antirrhinum majus) undergo the non-replicative transposition and the transposed sequences are excised frequently. Probes for retrotansposons and endogenous retroviruses, which are closely related to retrotansposons, were used for the pathotype identification of the rice blast fungus (Hamer et al., 1989; Levy et al., 1991) and RFLP mapping of the mouse genome (Frankel et al., 1990), respectively. Many retrotansposons were identified in yeast and animals, but not in plants. Recently, we have reported two general methods to clone plant retrotansposons (Hirochika et al., 1992). By using these two methods, five families (Tos1-Tos5) of retrotansposons of rice were cloned. Furthermore, the evidence indicating that the rice genome may carry 1000 copies of retrotansposons was obtained. We have also shown that the Tos1-Tos3 probes detect genetic differences among different species of rice and even between ecotypes of cultivated rice (O. sativa). In this report we used retrotansposon probes as molecular genetic markers to detect DNA fingerprints of rice cultivars.

2. MATERIALS AND METHODS

Plant materials

Rice cultivars were derived from the collection of University of Tsukuba (Tsukuba, Japan) and grown in the paddy field. Mature leaves of about ten individuals of each cultivar were sampled for DNA isolation.

Plant DNA extraction and Southern hybridization

Total DNA was isolated from leaves as described by Murray and Thompson (1980). About 5 µg of DNA was digested with four different restriction endonuc-
leases (EcoRI, BamHI, HindIII, and XbaI), electrophoresed in a 0.8% agarose gel and transferred to a nylon membrane (Nytran, Schleicher & Schuell) as described by Sambrook et al. (1989).

Tos1- and Tos2-specific probes were LTR-containing fragments of 0.3 and 0.7 kb, respectively, derived from Tos1-1 and Tos2-1 retrotransposons (Fig. 1a, b). Tos3-specific probe was a 1.8-kb fragment derived from the internal region of Tos3-1 (Fig. 1c). Preparation of $^{32}$P-labelled probes, hybridization and washing were carried out as described by Hirochika et al. (1992).

3. RESULTS AND DISCUSSION

To examine the effectiveness of retrotransposon probes in fingerprinting rice cultivars, DNAs of six cultivars each from Indica and Japonica ecotypes of rice (O. sativa) (Table 1) were examined by Southern hybridization after digestion with four different restriction enzymes. Three retrotransposon probes used are shown in Fig. 1. Fig. 2a shows the hybridization patterns of EcoRI-digested DNAs by using Tos1 probe. Although the copy number of Tos1 was estimated to be about 30 (Hirochika et al., 1992), less than 20 hybridizing bands were detected, probably due to overlapping of the bands. In Japonica cultivars (lanes 1–6), Norin 10 and Koshihikari (lanes 2, 4) were distinguished from Aikoku, Norin 29, Fujisaka 5 and Nipponbare (lanes 1, 3, 5, 6) by the presence of a band 1 (the lower intensities in the high molecular weight region in lanes 1 and 6 were simply due to unequal loading in these two lanes and partial degradation of the samples). Aikoku (lane 1) and Nipponbare (lane 6) were distinguished from other Japonica cultivars by the presence of bands 3 and 2, respectively. In Indica cultivars (lanes 7–12), all of the cultivars were distinguished from each other by the

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Ecotype</th>
<th>Type of cultivar</th>
<th>Origin</th>
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<tbody>
<tr>
<td>Aikoku</td>
<td>Japonica</td>
<td>Native</td>
<td>Japan</td>
</tr>
<tr>
<td>Norin 10</td>
<td>Japonica</td>
<td>Improved</td>
<td>Japan</td>
</tr>
<tr>
<td>Norin 29</td>
<td>Japonica</td>
<td>Improved</td>
<td>Japan</td>
</tr>
<tr>
<td>Fujisaka 5</td>
<td>Japonica</td>
<td>Improved</td>
<td>Japan</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>Japonica</td>
<td>Improved</td>
<td>Japan</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>Japonica</td>
<td>Improved</td>
<td>Japan</td>
</tr>
<tr>
<td>Dee-geo-woo-gen</td>
<td>Indica</td>
<td>Native</td>
<td>China</td>
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<tr>
<td>Taichung Native 1</td>
<td>Indica</td>
<td>Improved</td>
<td>China</td>
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<tr>
<td>Tsai-yuan-chung</td>
<td>Indica</td>
<td>Native</td>
<td>China</td>
</tr>
<tr>
<td>IR 8</td>
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<td>Improved</td>
<td>Philippines</td>
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<td>Culture 340</td>
<td>Indica</td>
<td>Native</td>
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<td>Peku</td>
<td>Indica</td>
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Fig. 1. Restriction maps of Tos1-1 (a), Tos2-1 (b) and Tos3-1 (c) retrotransposon clones. Tos1- and Tos2-specific probes are LTR-containing fragments of 0.36 and 0.7 kb, respectively. Tos3-specific probe is an internal fragment of 1.8 kb generated by digestion with XhoI and SphI. LTR: Long terminal repeat, PBS: Primer binding site.
presence or absence of bands 4 to 12. With other three enzymes, similar results were obtained (data not shown). Although some differences were detected among cultivars of Japonica rice, six Japonica cultivars could not be distinguished from each other with any of these enzymes used. Japonica and Indica ecotypes were clearly distinguished from each other by the presence of ecotype-specific

Fig. 2. Southern blots of 12 cultivars of O. sativa with Tos1-specific (a), Tos2-specific (b), and Tos3-specific (c) probes. DNAs were digested with EcoRI (a), BamHI (b), and HindIII (c). Numbered triangles indicate polymorphic bands. A circle indicates a Japonica-specific band. In Fig. 2a, high-molecular weight regions of lanes 1 to 10 were printed brightly in order to show bands clearly. Lanes 1–6: Japonica cultivars Aikoku, Norin 10, Norin 29, Koshihikari, Fujisaka 5 and Nipponbare, respectively. Lanes 7–12: Indica cultivars Dee-geo-woo-gen, Taichung native 1, Tsai-yuan-chung, IR 8, Culture 340 and Peku, respectively.
bands (for example, Japonica-specific band of 3.5 kb indicated by a circle in Fig. 2a).

The above results show that the Indica cultivars can be distinguished from each other by using one probe (Tos1 probe)-one enzyme combination. Human minisatellite DNA probe has been also successfully used to distinguish cultivars of African rice (*O. glaberrima*) as well as the Indica cultivars (Dallas, 1988). Tos1 probe is as effective in distinguishing those cultivars as the minisatellite DNA probe, because five cultivars of African rice could be distinguished from each other by a combination of Tos1 probe and *Eco*RI digestion (Fig. 3). Because Japonica cultivars are genetically more homogeneous than Indica cultivars (Takahashi, 1984), it seems difficult to distinguish Japonica cultivars by using molecular probes. In fact, only one out of 24 RFLP probes, which detect polymorphism between Indica and Japonica types, detected polymorphism among Japonica

Fig. 3. Southern blot of cultivars of *O. glaberrima*. DNAs were digested with *Eco*RI and hybridized with Tos1-specific probe. Lane 1: cultivar of Asian rice (*O. sativa*) Koshihikari. Lanes 2–6: cultivars of African rice (*O. glaberrima*) Gla 16, Gla 20, Gla 21, Gla 25 and Gla 30, respectively. Arrows with numbers indicate the bands polymorphic among cultivars of African rice.
cultivars (Kawase et al., 1989). By using this probe, six Japonica cultivars were classified only into two groups. On the other hand, the efficient fingerprinting of Japonica cultivars using ten RFLP probes was reported by another group (Wang and Tanksley, 1989). This difference is probably due to the difference in the rice cultivars used: we and Kawase et al. (1989) used the cultivars from Japan, whereas most of the cultivars used by Wang and Tanksley (1989) were derived from the rest of Asian countries.

Fingerprinting of Japonica cultivars is important from two points of view. Firstly, the probes for fingerprinting, if mapped close to the agronomically important genetic loci, will be used as molecular genetic markers for breeding of Japonica cultivars. Secondly, the fingerprinting is used for identification of Japonica cultivars. So, we have next tried to distinguish Japonica cultivars by using other two retrotransposon-specific probes.

The hybridization patterns by using Tos2 probe are shown in Fig. 2b. By combining this probe and BamHI-digestion, twelve cultivars could be distinguished from each other. Japonica cultivars could be distinguished by the presence or absence of six bands. Bands 3 and 5 are Aikoku- and Fujisaka 5-specific, respectively. Norin 10 and Koshihikari were distinguished from others by the presence of a band 4, and Koshihikari was distinguished from Norin 10 by the presence of a band 6. Norin 29 was distinguished from Nipponbare by the presence of a band 1. Much more bands (at least eighteen) were available for distinguishing Indica cultivars. Although only minor differences were found among Japonica cultivars with other restriction enzymes, the band patterns were more variable among Indica cultivars. For example, XbaI digestion did not detect any difference among Japonica cultivars (data not shown). In contrast, each Indica cultivar showed a different pattern. In summary, Tos2 probe can be used for fingerprinting cultivars of Japonica as well as Indica rice. These results were unexpected, because Tos2 probe detected less differences between Indica and Japonica types than Tos1 and Tos3 probes (Hirochika et al., 1992).

The hybridization patterns with Tos3 probe were less variable among Indica as well as Japonica cultivars. Only minor differences were detected with HindIII digestion at the high molecular weight region (bands 1-6; Fig. 2c). This difference between Tos3 probe and other two probes may not be due to the characters of retrotransposons themselves. Tos3 probe is an internal fragment of Tos3-1 retrotransposon, whereas Tos1 and Tos2 probes are the fragments corresponding to the terminal region of retrotransposons (LTR: long terminal repeat). Retrotransposons are flanked by LTRs as in the case of Tos3-1. Tos1-1 and Tos2-1 clones contain only one LTR, suggesting these clone may not cover the entire sequences of retrotransposons. LTR probes detect the fragments containing the sequences flanking the retrotransposons in addition to the retrotransposon sequence itself. So, this difference may be responsible to that in the efficiency of detecting the fingerprints. In fact, more fingerprints were detected with Tos3-1
LTR probe (data not shown).

The molecular bases for the difference in the band patterns detected with Tos probes are not known. At least three factors change the band patterns. These are mutations, methylation of DNA and transposition of retrotransposons. Mutations include point, deletion or insertion mutations. The cytosine residues in the CpG sequence are known to be frequently methylated and the methylated cytosine residues are hotspots for mutation in mammalian DNA (Barker et al., 1984). In plants, the cytosine residues in the CpNpG sequence are also methylated (Vanyushin, 1984). Because Tos1, Tos2 and Tos3 sequences are highly methylated in the rice genome (Fukuchi et al., unpublished results), point mutations in the methylated cytosine residues may induce the fingerprints. Among the restriction enzymes used here, EcoRI and BamHI are sensitive to cytosine methylation. So, a part of the fingerprints detected with these enzymes may be explained by the methylation. Most members of Tos1 and Tos2 retrotransposons seem to be defective (Hirochika et al., 1992). The organization of Tos3-1 is close to that of the intact retrotransposon, but Tos3-1 seems to be also inactive due to point mutations. However, we cannot exclude the possibility that some of the fingerprints may have been induced by transposition. Although most of retrotransposons of yeast and Drosophila are active, only a few retrotransposons of plants have been shown to be active (Grandbastien et al., 1989; Varagona et al., 1992; Hirochika, 1993). Because Tos3-1 transposed after Indica-Japonica differentiation (Hirochika et al., 1992), some of the fingerprints detected between Japonica and Indica cultivars should have been induced by transposition.

The results described here show that the retrotransposon probes are effective in detecting fingerprints even among the Japonica cultivars having close genetic backgrounds. This may also be true for the human minisatellite DNA and M13 probes. In contrast to these probes, a number of probes are available from retrotransposon sequences, because we have estimated that about 30 families of retrotransposons exist in the rice genome (Hirochika et al., 1992) and have recently cloned sequences of at least twelve families of retrotransposons of rice (Hirochika and Fukuchi, 1992). Retrotransposon-mediated fingerprinting should become a general method, because retrotransposons are ubiquitous in plant species (Flavell et al., 1992; Voytas et al., 1992; Hirochika and Hirochika, 1993) and retrotransposon probes can easily be obtained from any plant species by using two general cloning methods as described (Hirochika et al., 1992).

In addition to their use for fingerprinting, retrotransposon probes can be used as markers for linkage analysis (Stoyle et al., 1988; Frankel et al., 1990). In fact, we have identified one Tos1-hybridizing band linked to the agronomically important gene which has been introduced from Indica to Japonica rice (Fukuchi et al., 1992). Retrotransposon probes may be also used to fill the gaps in the RFLP maps of rice (McCouch et al., 1988; Saito et al., 1991).
REFERENCES


