

Houba retrotransposon-based molecular markers: a tool for variation analysis in rice

Gözde YÜZBAŞIOĞLU, Sibel YILMAZ, Nermin GÖZÜKIRMIZI*

Department of Molecular Biology and Genetics, Faculty of Science, İstanbul University, Vezneciler, İstanbul, Turkey

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Abstract: Rice is one of the most important crop plants as well as a model organism used for genetic studies. Transposons, especially retrotransposons, have a significant impact on the rice genome. A considerable percentage of the rice genome is composed of transposons (about 35%). Thus, transposons are an important internal factor for the genome evolution of rice varieties. In this study, we performed fingerprint analyses of 37 *Oryza sativa* L. cultivars using an IRAP marker. For this purpose, we designed IRAP primers specific to *Houba* (*Tos5/Osr13*), *RIRE1* and *Hopi* (*Osr27*), and *Osr30* retrotransposons, the most abundant retrotransposons in the rice genome. Polymorphism ratios were calculated with Jaccard similarity index. Our results showed that 37 cultivars of rice have a variable range of polymorphisms. The maximum polymorphisms were 75% for *Hopi*, 57% for *Osr30*, 52% for *Houba*, and 45% for *RIRE1*. Although these are high percentages of polymorphisms, all cultivars had a certain number of identical bands in all tested retrotransposons. These results prove that retrotransposon-based marker techniques could be useful for identification of cultivars in rice. *Houba* in particular is a good candidate for rice fingerprinting because of its band pattern quality.

Key words: Inter retrotransposon amplified polymorphism (IRAP), variation, *Oryza sativa* L., transposon

1. Introduction

Rice is one of the most important crop plants, providing nutrition for more than half of the world's population. Because of the growing rate of the world population, an increase in rice production will also be needed. In terms of economic significance, thousands of potentially valuable allelic variations of traits remain unutilized (Hossain et al., 2012). Evaluation of the genetic diversity in the improved rice genotypes is thought to be a good approach to overcoming this (Ravi et al., 2003; Kibria et al., 2009; Sivaranjani et al., 2010). New biotechnological techniques provide the opportunity to evaluate genetic variation and relationships among rice varieties. Thus, DNA-based analyses could be a significant part of effective breeding programs (Ashu and Sengar, 2015).

Genome project results in rice showed that 35% of the rice genome consists of transposons (TEs), or the dynamic elements of genomes. The transposition of TEs can generate genome plasticity by inducing various chromosomal mutations, allelic diversity, and genome expansion (Oliver and Greene, 2009; Fedoroff, 2013; Oliver et al., 2013; Lee and Kim, 2014). TEs are classified into two main groups, retrotransposons (Class I) and DNA transposons (Class II) based on their transposition mechanism (Finnegan, 1989). Retrotransposons can be used as genetic markers because

of their genome-wide distribution (Kalendar et al., 2011; Bonchev and Parisod, 2013; Poccai et al., 2013). One of these markers is the inter-retrotransposon amplified polymorphism (IRAP).

Hopi (*Osr27*), *Houba* (*Tos5/Osr13*), *RIRE1*, and *Osr30* are abundant retrotransposons in the rice genome. *Hopi* and *Osr30* belong to the *gypsy* retrotransposon family and have 1332 and 565 copy numbers in the rice genome, respectively. *Houba* and *RIRE1* are *copia* retrotransposons and have 563 and 262 copy numbers in the rice genome, respectively (Vitte et al., 2007).

The objective of this study was to compare *Hopi*, *Houba*, *Osr30*, and *RIRE1* retrotransposon movements in 37 *Oryza sativa* L. cultivars by using the IRAP molecular marker technique to find possible differences in retrotransposon-mediated polymorphisms that could be useful for rice fingerprinting.

2. Materials and methods

2.1. Plant material and DNA isolation

To investigate retrotransposon polymorphisms among individuals, 37 *Oryza sativa* L. cultivars were used (Table 1). These cultivars were obtained from Trakya Agronomic Research Institute. They included all the major cultivated varieties in Turkey. All cultivars are tolerant or resistant to

* Correspondence: nermin@istanbul.edu.tr

Table 1. *Oryza sativa* L. cultivars and parents.

No.	Cultivar	P1 XP2	No.	Cultivar	P1XP2
1	Altınyazı	BaldoxRibe	20	Beşer	İpsala mutations
2	Trakya	BaldoXKomsomolsky	21	Kızıltan	VeneriaXThainato
3	Ergene	DeltaXZoria	22	Durağan	PandaXBaldo
4	Meriç	DeltaXAkçeltik	23	Aromatik-1	-
5	İpsala	RodinaXDelta	24	Gala	-
6	Serhat-92	RoccaXKrasnodarsky	25	Tunca	RoccaXThainato
7	Sürek-95	RoccaXRodina	26	Efe	BaldoXDemir
8	Osmancık97	RoccaxEuropa	27	Hamzadere	DemirX83013-TR631-4-1-2
9	Kıral	GritnaXBalilla-28	28	Çakmak	TrakyaXN1-41T-1T-0T
10	Yavuz	RoccaX1979-70-1	29	Paşalı	Osmancık-97X82070-TR480-1-1-1-1
11	Neğış	VialoneXSequal	30	TosyaGüneşi	SavioXBaldo
12	Demir	PlovdivXLido	31	Manyasyıldızı	IR66160-5-2-3-2XVeneria
13	Gönen	BonniXShinei	32	Bigaincisi	BaldoXKoral
14	Kargı	BaldoXBalilla	33	Küplü	BaldoXIR2557-3-1
15	Edirne	BaldoXCalendal	34	Mis-2013	YRF-204XOsmancık-97
16	Halilbey	İpsalaXVeneria	35	Kale	DemirX82079-TR-489
17	Ece	8203-TR413-6-1-1 X 8260-TR470-6-1-1	36	Yatkın	Sürek-95X82079-TR-489
18	Kırkpınar	İpsala X 80110-TR253-4-1-1	37	SürekM711	-
19	Şumnu	RialtoXKoral			

root collar rot disease and paddy blight disease. Seeds were germinated in petri dishes containing moist filter paper at 25 °C under a long day regime (16 h light and 8 h dark). After 10 days of germination, plants were harvested, and genomic DNAs were isolated from leaves of individual plants of each cultivar, according to Pervaiz et al. (2011). The quantities of DNA were measured by Nanodrop (Thermo Scientific, 2000c), and qualities were controlled by 1% agarose gel electrophoresis.

2.2. IRAP analysis

The IRAP technique was used to investigate retrotransposon polymorphism. For this purpose, we chose *Hopi*, *Houba*, *RIRE1*, and *Osr30* retrotransposons. These retrotransposons have not been used for IRAP analyses before. Therefore, primers were designed for the first time in this study. Retrotransposon sequences of *O. sativa* 'Japonica' were obtained from the NCBI database. Using these sequences, IRAP primers were designed manually for each retrotransposon. IRAP primers and the accession numbers of reference sequences are given in Table 2.

IRAP-PCR was performed in a total volume of 20 µL containing 20 ng of template DNA, 10 nmol of forward and reverse primers, and SapphireAmp Fast PCR Master Mix (Takara, RR350A). PCR conditions were as follows: initial denaturation at 94 °C for 2.5 min followed by 30 cycles at 94 °C for 30 s, 55 °C (for *Hopi* and *Osr30*) or 51 °C (for *Houba* and *RIRE1*) for 30 s, 72 °C for 3 min. The reaction was completed with a final extension at 72 °C for 7 min. PCR products were resolved by 3% agarose gel electrophoresis at 120 V for 5 h in 1X TBE buffer. A molecular weight marker (Thermo, 1 Kb #SM0311) was also loaded to determine the sizes of the PCR fragments and gel was visualized by UV transilluminator and photographed. Gel photos were used for data analyses.

2.3. Data analysis

Polymorphism ratios between each cultivar were calculated manually. For this purpose, well-resolved bands were scored as a binary value: "1" for presence and "0" for absence of bands. The binary matrix (1/0) was used to calculate the similarity among 37 *Oryza sativa* L. cultivars by Jaccard coefficient (Jaccard, 1908). The

Table 2. Primer sequences used for IRAP analyses.

No.	Primer name	Direction (5'-3')	Accession number	Ta (°C)
1	<i>Houba-F</i> <i>Houba-R</i>	CTTCGAGTGGGCTAAGGCCC GTTTCGACCAAGCAGCCGGTC	AF537365.1	51
2	<i>RIRE1-F</i> <i>RIRE1-R</i>	GCAAGTGTTCCTGGTTTGCGCGG CGTGATATCCAACATCTCCATGTTGCC	D85597.1	51
3	<i>Hopi-F</i> <i>Hopi-R</i>	CGCGAACCTTCCACACACAGACTAG GGCCACGTGGGTGATCGTGTCTGCC	AF537364.1	55
4	<i>Osr30-F</i> <i>Osr30-R</i>	GCAGCCGATTCTCGCTCTGTTTCCG GGCACGCTCACACATCCGAAGGCGA	AC078891.2	55

Jaccard similarity index formula is $N_{AB}/(N_{AB} + N_B + N_A)$. N_{AB} is the number of bands presenting in both samples, N_A represents amplified fragments in sample A, and N_B represents amplified fragments in sample B.

3. Results

Retrotransposition analysis by IRAP marker in all tested cultivars resulted in different percentages of polymorphisms. Polymorphism percentages were 0%–75% for *Hopi*, 0%–57% for *Osr30*, 0%–52% for *Houba*, and 0%–45% for *RIRE1*.

3.1. IRAP results of *gypsy* retrotransposons *Hopi* and *Osr30*

IRAP-PCR of *Hopi* resulted in 12 PCR fragment lines ranging from 1000 to 250 bp (Figure 1). All tested cultivars had variable band patterns. However, 2 of 12 PCR fragments were common in all 37 cultivars. Another fragment about 750 bp in length was also common, with the exception of the 6th cultivar. According to presence

or absence of IRAP bands, polymorphism ratios were calculated between all cultivars (Table 3, upper part). The highest polymorphism ratio was calculated as 75% and the lowest 0% (*Hopi*).

IRAP results of another *gypsy* retrotransposon, *Osr30*, were similar to *Hopi*. However, lower numbers of PCR fragments were observed (ranging from 1000 to 250 bp) (Figure 2). In all 37 rice cultivars, 2 of 7 PCR lines were common. In addition, a very clear PCR fragment that is about 700 bp was also common with the exception of the 23rd cultivar. Likewise, a PCR fragment under 250 bp was present in all species with the exception of the 16th cultivar. Based on the IRAP result in *Osr30*, polymorphism ratios were calculated ranging from 0% to 57% (Table 3, bottom part).

3.2. IRAP results of *copied* retrotransposons *Houba* and *RIRE1*

IRAP-PCR results of *copied* retrotransposons were different than those of *gypsy*. Both *Houba* and *RIRE1* band sizes

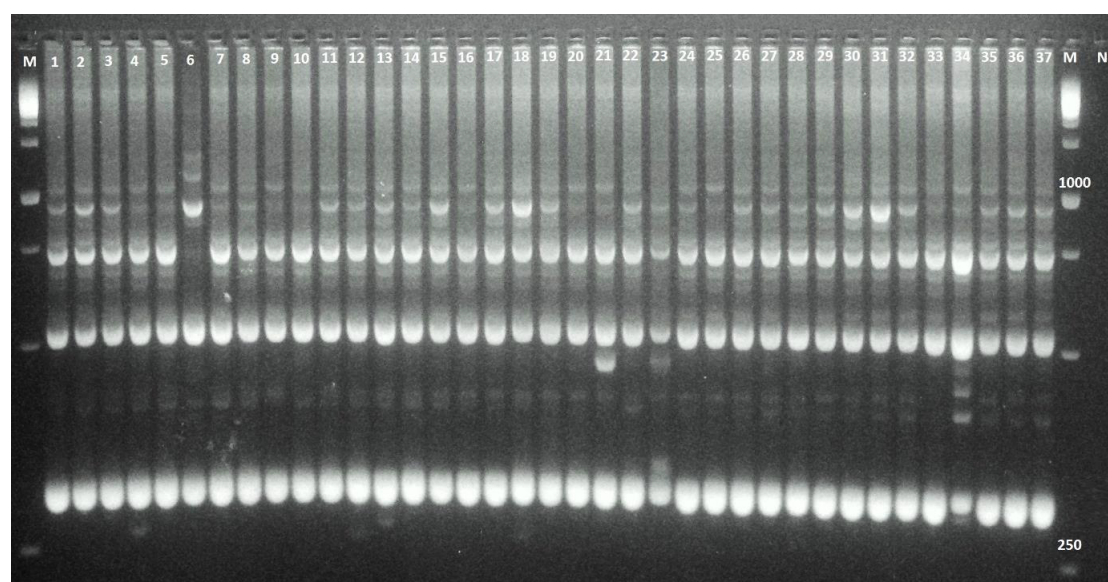
**Figure 1.** *Hopi* IRAP-PCR results: 1–37, rice cultivars in Table 1; M, marker; N, PCR negative control.

Table 3. Polymorphism ratios of *Hopi* and *Osr30*.

Hopi																																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
Qst	1	-	12	12	12	67	12	12	12	12	22	22	22	22	22	22	22	22	22	12	12	12	30	22	22	22	22	30	30	30	22	0	30	0	0	0		
	2	14	-	0	0	70	0	0	22	0	11	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	3	14	0	-	0	70	0	0	22	0	11	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	4	14	0	0	-	70	0	0	22	0	11	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	5	0	14	14	-	70	0	0	22	0	11	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	6	14	0	0	0	14	-	70	70	70	73	73	73	73	73	73	73	73	73	70	73	70	75	73	73	73	73	64	64	64	64	73	67	75	67	67	67	
	7	14	0	0	0	14	0	-	0	22	0	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	8	0	14	14	14	0	14	14	-	22	0	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	9	14	0	0	0	14	0	0	14	-	22	11	11	11	11	11	11	11	11	0	11	22	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	10	14	0	0	0	14	0	0	14	0	-	11	11	11	11	11	11	11	11	22	30	0	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	11	14	0	0	0	14	0	0	14	0	0	-	0	0	0	0	0	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	12	14	0	0	0	14	0	0	14	0	0	-	0	0	0	0	0	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	13	14	0	0	0	14	0	0	14	0	0	0	-	0	0	0	0	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	14	14	0	0	0	14	0	0	14	0	0	0	0	0	0	0	0	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	15	14	0	0	0	14	0	0	14	0	0	0	0	0	0	-	0	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	16	29	14	14	14	29	14	14	29	14	14	14	14	14	14	14	-	0	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	17	0	14	14	14	0	14	14	0	14	14	14	14	14	14	14	29	-	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	18	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	-	0	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	19	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	-	11	20	11	10	0	0	0	0	10	10	10	10	0	22	22	22	22	22	
	20	17	29	29	29	17	29	29	17	29	29	29	29	29	29	43	17	29	29	-	11	22	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	21	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	-	30	10	20	20	20	20	27	27	27	27	20	22	10	22	22	22	
	22	17	29	29	29	17	29	29	17	29	29	29	29	29	29	43	17	29	29	0	29	-	20	11	11	11	11	20	20	20	20	11	12	20	12	12	12	
	23	33	43	43	33	43	43	33	43	43	43	43	43	43	43	57	33	43	43	20	43	20	-	10	10	10	10	18	18	18	18	10	30	0	30	30	30	
	24	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	-	0	0	0	10	10	10	10	0	22	22	22	22	22	
	25	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	-	0	0	10	10	10	10	0	22	22	22	22	22	
	26	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	-	0	10	10	10	10	0	22	22	22	22	22	
	27	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	0	14	14	-	10	10	10	10	0	22	22	22	22	22	
	28	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	0	14	-	0	0	0	10	30	18	30	30	30	
	29	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	0	14	14	0	14	-	0	0	10	30	18	30	30	30	
	30	17	29	29	29	17	29	29	17	29	29	29	29	29	29	43	17	29	29	33	29	33	50	17	29	29	17	29	17	-	0	10	30	18	30	30	30	
	31	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	0	14	14	0	14	0	17	-	10	30	18	30	30	30	
	32	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	0	14	0	14	0	14	29	14	-	22	30	22	22
	33	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	0	14	14	0	14	0	17	0	14	-	30	0	0	0	
	34	0	14	14	14	0	14	14	0	14	14	14	14	14	14	29	0	14	14	17	14	17	33	0	14	14	0	14	0	17	0	14	0	-	30	30	30	
	35	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	0	14	0	14	0	14	29	14	0	14	-	0	0
	36	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	0	14	0	14	0	14	29	14	0	14	0	-	0
	37	14	0	0	0	14	0	0	14	0	0	0	0	0	0	14	14	0	0	29	0	29	43	14	0	0	14	0	14	0	14	29	14	0	14	0	0	-

Osr30

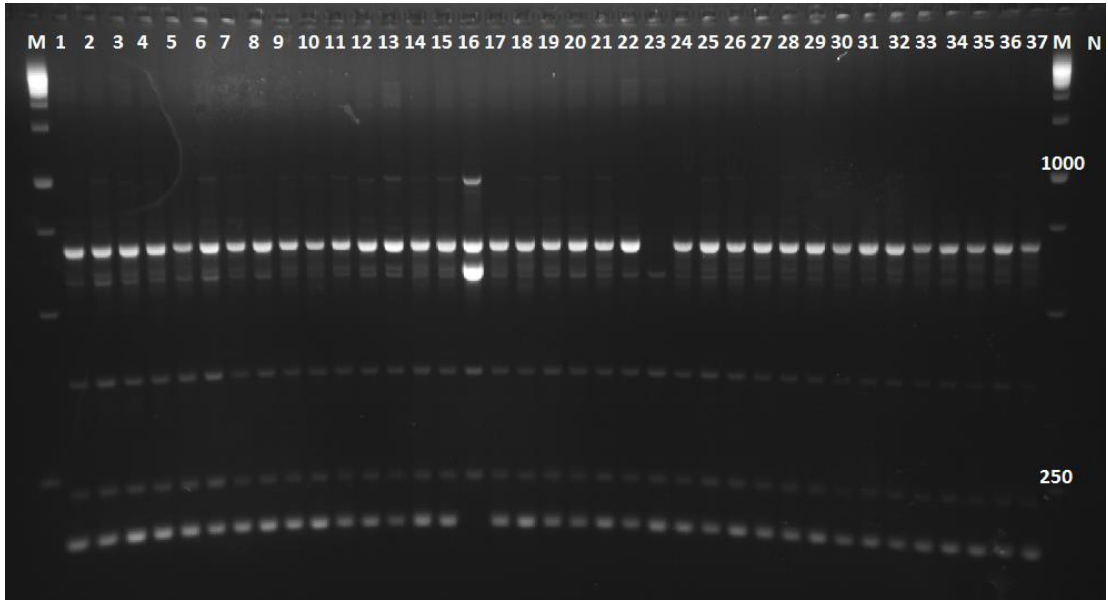


Figure 2. *Osr30* IRAP-PCR results: 1–37, rice cultivars in Table 1; M, marker; N, PCR negative control.

ranged from ~10,000 to ~400 bp. This band distribution of *copia* retrotransposons was much higher than *gypsy*'s. *Houba* resulted in the best IRAP profile when compared to the other 3 (Figure 3). It gave 29 PCR band lines, and 10 of them were present in all plant samples. The polymorphism ratios of *Houba* were calculated at variable percentages from 0% to 52% (Table 4, bottom part).

IRAP results in *RIRE1* were similar to *Houba*, in a band range from ~10,000 to ~400 bp. However, it provided only 12 PCR fragment lines, and 6 of them were common in all 37 test samples (Figure 4). In addition, polymorphism ratios were variable (0%–45%) (Table 4, upper part).

4. Discussion

In this study, we analyzed 37 rice cultivars in terms of retrotransposition events by IRAP marker for 4

retrotransposons (*Hopi*, *Osr30*, *Houba*, and *RIRE1*). All tested cultivars showed different degrees of polymorphism in each of the tested retrotransposons. This study is the first research to generate fingerprinting with the IRAP marker system in rice cultivars in Turkey.

Despite the importance of retrotransposons for genome dynamics and gene activity, our understanding of their biology is still in a primitive state. The studies that search for polymorphism in individual retrotransposons are limited, although the genome project (International Rice Genome Sequencing Project, 2005) and 3000 genome project (Li et al., 2014) of rice were completed.

In rice breeding, DNA fingerprinting is required for the precise identification of cultivars. Many families of retrotransposons are represented by multiple copies in the eukaryotic, especially cereal, genomes. Integrated copies of

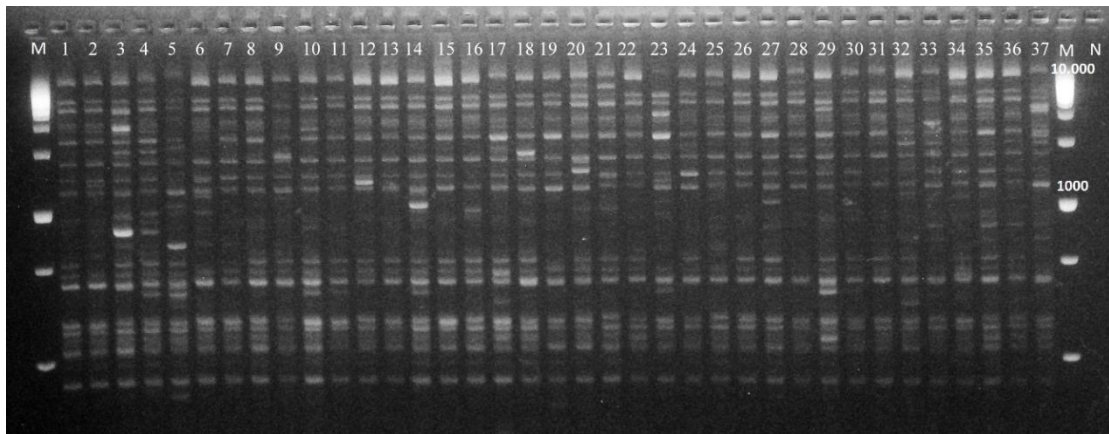


Figure 3. *Houba* IRAP-PCR results: 1–37, rice cultivars in Table 1; M, marker; N, PCR negative control.

Table 4. Polymorphism ratios of *RfREI* and *Houba*.

RREI																																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1	-	0	0	0	18	11	22	22	0	22	0	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
2	17	-	0	0	18	11	22	22	0	22	0	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
3	20	29	-	0	18	11	22	22	0	22	0	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
4	24	39	14	-	18	11	22	22	0	22	0	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
5	36	43	33	29	-	27	36	36	18	36	18	36	18	42	18	36	36	27	10	27	36	27	25	9	36	36	18	45	27	36	36	25	45	18	36	18	
6	32	28	25	29	41	-	12	12	11	12	11	12	11	22	11	12	12	0	20	0	12	0	12	27	12	12	11	25	22	22	22	30	25	11	33	11	
7	12	18	25	29	33	24	-	0	22	0	22	0	22	12	22	0	0	12	30	12	0	12	30	30	0	0	22	14	33	0	25	40	40	14	22	25	
8	17	22	29	32	36	24	6	-	22	0	22	0	22	10	22	0	0	12	30	12	0	12	30	30	0	0	22	14	33	0	25	40	40	14	22	25	
9	28	29	38	41	45	29	19	24	-	22	0	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
10	11	18	24	27	32	32	12	17	28	-	22	0	22	12	22	0	0	12	30	12	0	12	30	30	0	0	22	14	33	0	14	40	40	14	22	14	
11	17	22	29	24	29	28	6	12	24	6	-	22	0	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
12	17	12	29	32	36	28	6	12	24	17	12	-	22	12	22	0	0	12	30	12	0	12	30	30	0	0	22	14	33	0	14	40	40	14	22	14	
13	12	18	25	29	33	24	0	6	19	12	6	6	-	30	0	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
14	26	32	36	32	29	28	18	22	24	17	12	22	18	-	30	12	12	36	22	12	22	20	36	12	12	30	25	40	12	33	45	25	30	33	30		
15	18	24	30	33	38	19	7	12	13	18	12	12	7	12	-	22	22	11	10	11	22	11	10	10	22	22	0	33	11	22	22	20	33	0	22	0	
16	28	33	30	33	38	19	19	24	25	28	24	24	19	24	13	-	0	12	30	12	0	12	30	30	0	0	22	14	33	0	14	40	40	14	22	14	
17	30	35	32	35	32	32	22	17	28	30	26	26	22	26	18	26	-	12	30	12	0	12	30	30	0	0	22	14	33	0	14	40	40	14	22	14	
18	25	32	36	39	46	28	18	12	12	26	22	26	18	22	12	24	17	-	20	0	12	0	12	27	12	12	11	25	22	22	22	30	25	11	33	11	
19	18	12	30	41	45	19	19	24	25	18	24	24	19	24	13	25	28	24	-	20	30	20	18	18	30	30	10	40	20	30	30	10	45	10	30	10	
20	17	26	24	35	46	24	22	26	28	21	26	26	22	26	18	30	26	18	-	12	0	12	27	12	12	11	25	22	22	22	30	25	11	33	11		
21	25	30	27	37	48	26	26	21	32	25	30	30	26	30	22	22	25	21	22	6	-	12	30	30	0	0	22	14	33	0	14	40	40	14	22	14	
22	26	18	36	32	36	28	18	12	24	17	12	22	18	12	12	24	17	12	24	26	21	-	12	27	12	12	11	25	22	22	22	30	25	11	33	11	
23	17	12	36	39	52	28	28	32	33	26	32	22	28	32	24	33	35	32	12	26	30	32	-	18	30	10	40	20	30	30	30	40	10	30	10		
24	22	28	25	29	29	24	12	18	19	22	18	12	18	7	19	12	18	19	22	30	18	28	-	30	30	10	40	20	30	30	27	40	10	30	10		
25	32	28	33	36	41	12	24	28	29	32	28	24	28	19	29	32	28	19	22	26	28	28	24	-	0	22	14	33	0	14	40	14	22	14	22		
26	18	24	30	33	38	19	7	12	13	18	12	12	7	12	0	13	18	12	13	18	22	12	24	7	19	-	22	14	33	0	14	40	14	22	14		
27	17	22	29	39	36	28	18	22	24	17	22	22	18	12	12	24	26	22	12	17	21	22	22	18	28	12	-	33	11	22	22	20	33	0	22	0	
28	38	35	39	42	46	40	40	43	37	30	35	43	40	35	37	45	38	35	28	30	33	35	35	32	32	37	35	-	25	14	14	33	33	0	33	14	
29	21	26	24	35	39	32	22	17	28	21	26	26	22	26	18	28	11	17	18	21	16	17	26	12	32	18	17	30	-	33	12	11	25	11	12	11	
30	12	19	25	36	41	24	12	19	19	12	18	12	18	7	19	22	18	7	12	17	18	18	12	24	7	6	32	12	-	14	40	14	22	14	22		
31	18	24	30	41	38	29	19	24	25	18	24	24	19	24	13	13	28	24	13	18	22	24	24	19	29	13	12	37	18	7	-	22	22	14	22	25	
32	30	35	32	35	24	32	22	26	28	30	26	26	22	17	18	28	11	26	28	30	33	26	35	12	32	18	17	38	21	22	28	-	33	20	22	20	
33	42	39	35	38	50	35	35	39	31	42	39	35	35	39	31	41	25	35	31	42	47	39	39	25	35	31	39	33	33	39	41	33	-	33	20	22	20
34	30	35	32	35	32	32	22	26	28	30	26	26	22	17	18	28	21	26	28	21	25	26	35	12	24	18	17	30	21	22	28	11	33	-	33	14	
35	21	32	19	30	42	26	26	21	32	25	30	30	26	30	22	32	25	21	22	25	20	21	30	26	26	22	41	16	17	22	33	37	33	-	22	0	
36	30	35	24	27	39	32	22	17	28	30	26	26	22	26	18	28	11	17	28	30	25	17	35	12	32	18	26	41	11	22	28	21	24	17	16	-	
37	33	38	19	30	39	35	35	30	32	33	38	35	38	32	40	25	30	32	33	29	30	38	26	35	32	30	41	16	26	32	33	28	33	11	16	-	

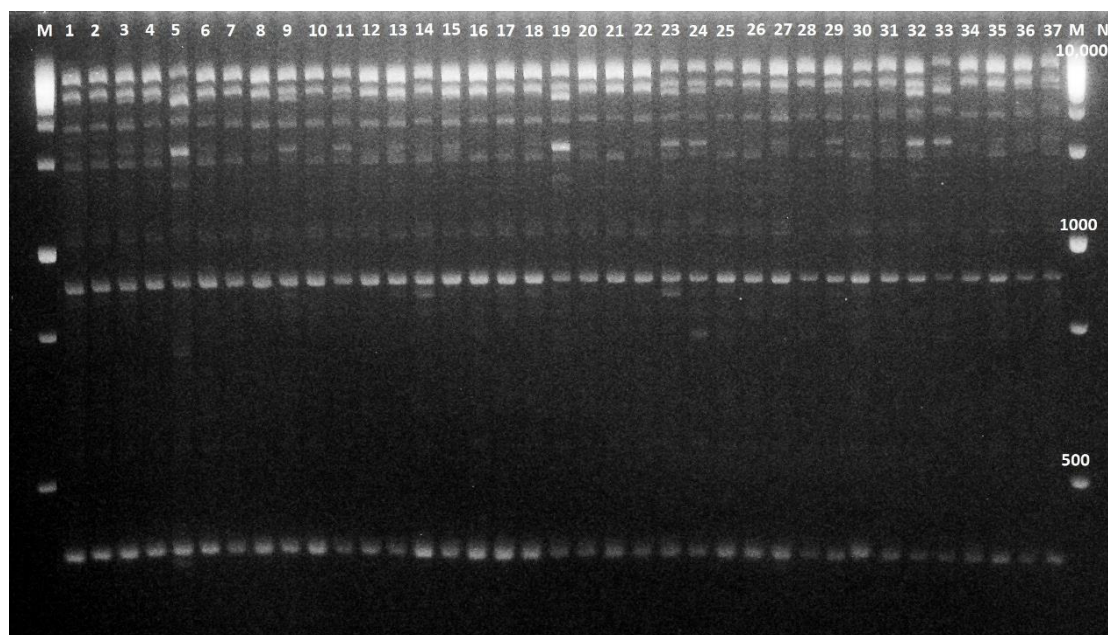


Figure 4. *RIRE1* IRAP-PCR results: 1–37, rice cultivars in Table 1; M, marker; N, PCR negative control.

retrotransposons are inherited genetically. Despite their high copy numbers in the genome, most of the retrotransposons were inactivated through evolutionary processes. However, they can be activated by biotic and abiotic stress conditions (Hamad et al., 2012). Because of this, retrotransposon insertion polymorphisms among cultivars are useful for DNA fingerprinting (Monden et al., 2014).

Molecular markers are useful tools for identification of genetic variation among cultivars. There are many studies for cultivar-specific DNA fingerprint analyses in rice that are performed with molecular markers such as microsatellite, SSR, ISSR, and RAPD (Muhammad et al., 2009; Zhu et al., 2012; Subudhi et al., 2013; Ashu and Sengar, 2015; Vemireddy et al., 2015). SSR, RAPD, and ISSR PCR-based marker systems were used in 30 rice varieties to generate a DNA fingerprint database (Ashu and Sengar, 2015). The SSR marker system was used in analysis for fingerprinting 14 varieties of rice cultivated in Punjab State, India (Sarao et al., 2009). Moreover, SSR markers were used for demonstrating the relationship between the 48 main commercial rice cultivars grown in China; however, SSR markers have limited application in fingerprinting due to the high cost and intense research effort involved (Zhu et al., 2012).

The IRAP marker system can be a useful tool for investigating rice breeding (Kalendar et al., 1999). A retrotransposon-based marker, like IRAP, has not been used for the identification of rice cultivars until now. However, ISSR, IRAP, and SSR marker systems were used for indicating the phylogenetic relationships of organisms

other than rice, such as *Secale* (Achrem et al., 2014).

The IRAP technique is sufficient for primer binding to a special region of retrotransposons. IRAP results are reproducible, unlike the results produced by the RAPD method. Moreover, IRAP is cheaper and more easily applicable than AFLP. IRAP is a useful technique for revealing large changes in the genome compared to other marker techniques such as RFLP, SNPs, AFLP, and microsatellites (Schulman et al., 2012).

However, all retrotransposons are not useful for rice-cultivar-specific DNA fingerprinting by IRAP marker. In our experiments, we indicated that only *Houba* is a good candidate for fingerprinting analyses in rice because of its band profile quality. Although polymorphism rates were lower in *Houba* (52%), compared with *gypsy* retrotransposons *Hopi* and *Osr30*, its band length rates (between 10,000 and 400 bp) and total band numbers (29 PCR bands) were higher. However, there are studies using different primer pairs in IRAP-PCR, one of which was carried out in *Secale*. In that study, 73 amplification products which were generated with IRAP primers and 86.8% were polymorphic; however, the PCR product was in a narrow range (109 bp to 3374 bp) (Achrem et al., 2014).

5. Conclusion

Molecular markers based on retrotransposons have potential value for biodiversity, phylogenetic, and evolutionary analyses in rice (Kwon et al., 2005; Kang and Kang, 2008). One of the most important steps is

finding suitable retrotransposons to analyze in specific plant species. DNA fingerprinting between rice cultivars by means of IRAP marker provided meaningful data that can be extended with additional rice retrotransposons. During analyses, we observed that these retrotransposons, especially *Houba*, are suitable for DNA fingerprinting. The data obtained from this study can be used for a variety of information and contribute to the construction

of a database of rice varieties. Our studies with this retrotransposon are continuing, and include analyzing its movements in different plant parts.

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