**Analysis of Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposons in rice**

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**ABSTRACT**

We investigated Hopi/Osr27 (gypsy) and Houba/Tos5/Osr13 (copy) retrotransposon movements in 10-day-old roots and leaves of Oryza sativa cvs. Ipsala, Beser and Osmanucik-97. Seeds from these three cultivars were germinated between filter papers in Petri dishes for 10 days. Three biologically independent (nonrelated) seeds were germinated for each cultivar. Then, roots and leaves grown from the same rice plant were harvested and used for genomic DNA isolation. Inter-retrotransposon amplified polymorphism—polymerase chain reaction with suitable primers was performed with each DNA template to analyze the movements of Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposons. Polymorphism ratios were evaluated both among cultivars and among roots and leaves from the same cultivar. The polymorphism ratios ranged from 0% to 17% for Hopi/Osr27 and from 10% to 87% for Houba/Tos5/Osr13. The obtained results at retrotransposon and varietal levels indicated that the retrotransposon type and genotype dependence are responsible for the occurrence of different variations. Transposable elements are very important for understanding the relationship between cultivars and evolution. Our findings are expected to contribute to the understanding of spontaneous genomic insertion events and their effects on the genetic and epigenetic changes during rice development.

**Introduction**

Rice is the third important crop plant produced after wheat and maize. It is an important food and at the same time is used as a model organism for genetic studies (genome evolution, etc.). The diploid chromosome number of rice is 24 and this plant’s genome project is one of the first to be finished. A large part of the eukaryotic genomes consists of transposable elements (TEs).[1] Previous studies identified that maize has 75% retrotransposons in a 2800 Mb genome,[2–4] barley has 75% retrotransposons in a 5300 Mb genome,[5] cabbage has 28% retrotransposons in a 600 Mb genome[6] and Vicia species have 45% retrotransposons in a 1300 Mb genome.[7] Depending on the order of the genes encoded, retrotransposons are further classified into Ty1-copy and Ty3-gypsy retrotransposons. The gene order of Ty1-copy retrotransposons is PR-INT-RT-RH (protease, integrase, reverse transcriptase, RNase H, respectively) whereas that of Ty3-gypsy retrotransposons is PR-RT-INT-RH.[8] Based on a unified classification system for eukaryotic TEs,[1] 32,370 elements were classified into 510 distinct families, including 353 gypsy-like families (19,052 elements) and 157 copy-like families (13,318 elements), of which, approximately 95% were reported.[9,10] The ratio of gypsy-like to copy-like elements in soybean is 1.4:1 which is slightly lower than that in maize (1.6:1)[4,11] and much lower than that in rice (4.9:1).[12,13]

The transposition of TEs can generate genome plasticity by inducing various chromosomal mutations, allelic diversity and genome expansion.[14–21] For instance, the genome size of Oryza australiensis, a wild relative of rice, was doubled within the last 3 million years (3 Myr) by aggressive proliferation of long terminal repeat-retrotransposons (LTR-RTs) belonging to three families.[22] Moreover, approximately one-quarter of the rice genome is composed of LTR-RTs.[6,11,12,23–25] Due to their variation capacity between species, retrotransposons are usually studied for the detection of genetic relationships between varieties and related species, and even between different plant organs in the same plants.[26–35]

TEs have been used for genetic markers because of their genome-wide distribution.[36–38] One of these markers is the inter-retrotransposon amplified polymorphism (IRAP). In this method, sequences between two adjacent LTR-RTs are amplified by primers that are complementary to the 3’-end of the LTR sequence.[39] The LTR sequences between adjacent retrotransposons can
be arranged as (1) head-to-head, (2) tail-to-tail or (3) head-to-tail.[38] If the arrangement between two identical tandem duplicate LTR-RTs is either head-to-head or tail-to-tail, a single primer can amplify the spacer. If the adjacent retrotransposons are from different lineages (which is usually the case), two different primers, derived from each LTR sequence, are needed to amplify the IRAP. Each IRAP reaction produces multiple amplicons, ranging in size from 300 to 3000 bp.[40,41] Hopi/Osr27 is a gypsy retrotransposon with a size of 12,892 bp, LTR sequence of 968 bp and copy number of 563, in the rice genome.[42] The objective of this study was to compare Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposons’ movements in 10-day-old roots and leaves in Oryza sativa cvs. Osmancik-97, Beser and Ipsala, which were obtained from the same seed, by using IRAP molecular marker technique to find out the possible differences in transposon-mediated polymorphisms.

Materials and methods
Inter-retrotransposon amplified polymorphism—polymerase chain reaction (IRAP-PCR) profiles of root and leaf tissues of the same plantlets were compared with each other. In addition, three plantlets of each cultivar were analyzed in terms of PCR lineages (which is usually the case), two different primers, derived from each PCR sequence, are needed to amplify the IRAP. Each IRAP reaction produces multiple amplicons, ranging in size from 300 to 3000 bp.[40,41] Hopi/Osr27 is a gypsy retrotransposon with a size of 12,892 bp, LTR sequence of 968 bp and copy number of 563, in the rice genome.[42] The objective of this study was to compare Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposons’ movements in 10-day-old roots and leaves in Oryza sativa cvs. Osmancik-97, Beser and Ipsala, which were obtained from the same seed, by using IRAP molecular marker technique to find out the possible differences in transposon-mediated polymorphisms.

Plant materials and DNA isolation
Seeds of three cultivars of O. sativa (Ipsala, Beser and Osmancik-97) were used to investigate the retrotransposon polymorphism between root and leaf tissues. The seeds were germinated at 25 °C in Petri dishes that contained moist filter paper. After 10 days of germination, roots and leaves of each plant were harvested individually. Genomic DNAs were isolated from three roots and three leaves of each cultivar, according to the protocol of Pervaiz et al.[43] The quantity and quality of DNAs were measured by NanoDrop 2000c uv-Vis spectrophotometer (Thermo Scientific, 2000c). Before the IRAP analysis, the DNA's concentration was equalized to 10 ng/μL.

IRAP analysis
The IRAP technique was used to investigate the retrotransposon polymorphism. For this purpose, we assigned Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposons as candidates. Hopi/Osr27 sequences of O. sativa cv. japonica were obtained from National Center for Biotechnology Information (NCBI, accession number: AF537364.1) and Houba/Tos5/Osr13 sequences O. sativa cv. japonica were also obtained from NCBI (accession number: AF537365.1).

The IRAP primers were designed based on the 5’ and 3’ LTR sequences of Hopi/Osr27 and Houba/Tos5/Osr13. The primer sequences are given in Table 1. The IRAP-PCR was performed in a total volume of 20 μL, containing 20 ng template DNA, 10 nmol forward and reverse primers designed by Integrated DNA Technologies (IDT) and SaphireAmp Fast PCR Master Mix (Takara, RR350A). Primer dimer or other contaminations were checked by using no template control (negative control). In this control, the PCR contents were the same as in IRAP-PCR, but without template (water was used instead of template). The 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 30 s, 72 °C for 3 min and the reaction was completed with a cycle of final extension at 72 °C for 7 min. The PCR products were resolved in a 8% polyacrylamide (29:1 Acrylamide:Bis) gel electrophoresis (Bio-Rad, Proean) at 150 V for 8 h in 1X TBE buffer (pH 8.0). A molecular weight marker (GeneRulerTM DNA Ladder Mix, SM0331, Thermo Scientific) was also loaded to determine the sizes of the PCR fragments. The gel was stained with ethidium bromide in 1X TBE buffer for 15 min. After staining, the gel was visualized on UV transilluminator, photographed and used for data analyses.

Data analyses
The 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 between root and leaf tissues by Jaccard’s coefficient.[44] The Jaccard’s similarity index was calculated using the formula: NAB/(NAB + NB + NA), where NAB is the number of bands shared by two samples, NA indicates the amplified fragments in sample A and NB represents the amplified fragments in sample B.

Results and discussion
In the present study, Hopi/Osr27 and Houba/Tos5/Osr13 retrotransposon mediated polymorphism between root
and leaf tissues of three *O. sativa* cultivars (Ipsala, Beser and Osmancik-97) was investigated. For this purpose, *Hopio/Osr27* band profiles produced 10 homomorphic IRAP bands, which were observed in each sample (roots and leaves), with a variable length between 250 and 3000 bp (Figure 1). In Beser cultivar, the root and leaf tissues of all three plants were homomorphic (Figure 1; lanes 13–18). This result showed that there may be no transposition events of *Hopio/Osr27* retrotransposon during germination. Because all individuals had common band profiles, it may be concluded that Beser cultivar does not have any naturally occurring polymorphisms in terms of this retrotransposon. IRAP-PCR analyses of Ipsala cultivar root and leaf also showed the same band profile, similar to Beser cultivar. However, the third individual (Figure 1; lanes 11 and 12) of Ipsala had one polymorphic band that was not observed in the other two individuals. This result might prove that Ipsala cultivar has a natural polymorphism, with respect to *Hopio/Osr27* retrotransposon, although there are no transposition events during or following germination. The third cultivar, Osmancik-97, had a different profile than Ipsala and Beser because it had both homomorphic and polymorphic profiles between root and leaf tissues of individuals. The first individual of Osmancik-97 was homomorphic based on its root and leaf tissues’ IRAP profiles. Also, it had the same band profile as Beser’s and as the first two samples of Ipsala. However, the second individual of Osmancik-97 had 8% polymorphism between root and leaf tissues (Table 2). While the root profile of the second individual was the same as Ipsala’s third plant sample, the leaf profile was same as the leaf profile of the third individual of Osmancik-97. The last sample of Osmancik-97 (Figure 1; lanes 5 and 6) had the highest polymorphism rate between root and leaf tissues (17%). *Hopio/Osr27* is one of the *gypsy* type LTR-RTs that is represented with high copy number (1332 copies) in the *O. sativa*’s genome. This might show that *Hopio/Osr27* is an active retrotransposon through the evolutionary processes.[42] However, it was epigenetically silenced as other retrotransposons. Epigenetic mechanisms provide a control system to retrotransposon burst.[21] There are various explanations about the somatic activities of TEs, such as stress and developmental stages.[45,46]

*Houba/Tos5/Osr13* retrotransposon bands showed different profiles among cultivars with length between 250 and 2500 bp (Figure 2). IRAP analyses of *Houba/Tos5/Osr13* retrotransposon resulted in higher polymorphism ratio (10%–87%) than *Hopio/Osr27* (0%–17%) (Table 2). This showed that *Houba/Tos5/Osr13* might have a more effective role than *Hopio/Osr27* in tissue differentiation. However, the results from Ipsala and Beser cultivars were not consistent with the Osmancik-97 results. Polymorphism rates of different tissues in the same individuals and of different individuals in the same cultivar were
Table 2. Polymorphism ratios of Hopi/Osr27 and Houba/Tos5/Osr13.

| Houba/Tos5/Osr13 | OL1 | OR1 | OL2 | OR2 | OL3 | OR3 | IL1 | IR1 | IL2 | IR2 | IL3 | IR3 | BL1 | BR1 | BL2 | BR2 | BL3 | BR3 |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| OL1              | 0   | 17  | 9   | 17  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 9   | 9   | 0   | 0   | 0   | 0   | 0   | 0   |
| OR1              | 68  | 17  | 9   | 17  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 9   | 9   | 0   | 0   | 0   | 0   | 0   | 0   |
| OL2              | 50  | 59  | 0   | 8   | 0   | 17  | 17  | 17  | 17  | 8   | 8   | 8   | 17  | 17  | 17  | 17  | 17  | 17  | 17  |
| OR2              | 68  | 10  | 59  | 0   | 8   | 9   | 9   | 9   | 9   | 0   | 0   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   |
| OL3              | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  |
| OR3              | 64  | 21  | 59  | 21  | 43  | 0   | 0   | 0   | 0   | 9   | 9   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| IL1              | 55  | 62  | 36  | 62  | 43  | 64  | 0   | 0   | 0   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   |
| IR1              | 70  | 30  | 70  | 37  | 55  | 46  | 56  | 0   | 0   | 0   | 9   | 9   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| IL2              | 40  | 62  | 36  | 62  | 50  | 64  | 34  | 62  | 0   | 9   | 9   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| IR2              | 67  | 22  | 65  | 29  | 58  | 32  | 60  | 27  | 60  | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   |
| IL3              | 65  | 40  | 62  | 40  | 38  | 29  | 58  | 46  | 58  | 23  | 0   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   |
| IR3              | 69  | 75  | 54  | 75  | 47  | 65  | 45  | 65  | 45  | 74  | 60  | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   | 9   |
| BL1              | 75  | 68  | 75  | 72  | 44  | 58  | 67  | 57  | 57  | 67  | 52  | 38  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| BR1              | 70  | 44  | 67  | 50  | 48  | 46  | 56  | 29  | 56  | 46  | 58  | 42  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| BL2              | 54  | 67  | 69  | 67  | 53  | 62  | 46  | 55  | 54  | 52  | 50  | 57  | 56  | 55  | 0   | 0   | 0   | 0   | 0   |
| BR2              | 82  | 50  | 76  | 50  | 70  | 58  | 75  | 57  | 75  | 55  | 65  | 83  | 74  | 57  | 57  | 0   | 0   | 0   | 0   |
| BL3              | 83  | 71  | 67  | 71  | 75  | 73  | 73  | 80  | 73  | 81  | 75  | 75  | 71  | 74  | 79  | 71  | 0   | 0   | 0   |
| BR3              | 86  | 57  | 80  | 68  | 71  | 70  | 77  | 63  | 79  | 60  | 65  | 87  | 75  | 63  | 64  | 14  | 73  | 73  | 73  |

Note: Osmancik-97 (O); Ipsala (I); Beser (B); leaf (L); root (R)
*Numbers (%) refer to each individual of each cultivar.

Figure 2. IRAP-PCR results of Houba/Tos5/Osr13 in O. sativa cvs.
Note: cv. Osmancik-97 (lanes 1–6), (lanes 1, 3, 5: leaves; lanes 2, 4, 6: roots); cv. Ipsala (lanes 7–12) (lanes 7, 9, 11: leaves; lanes 8, 10, 12: roots); cv. Beser (lanes 13–18) (lanes 13, 15, 17: leaves; lanes 14, 16, 18: roots). Negative control (NC); marker (M); some polymorphic bands are marked with arrows.
variable. As opposed to Hopi/Osr27, there was a 14%—79% polymorphism rate in Beser cultivar (Figure 2; lanes 13–18) and 23%—74% in Ipsala cultivar. Retrotransposons are commonly studied with different plant species and even with different plant organs in the same individual. Vukich et al. [47] examined the expression differences between copy and gypsy elements in sunflower (Helianthus annuus L.) root, leaf and flower tissues. In another study, Marakli et al. [28] concluded that movements of the BAGY2 retrotransposon were more stable compared to those of BARE1 in roots and leaves derived from the same barley embryo. Cakmak et al. [48] reported that some SIRE1 retrotransposition events occurred not only in different 10-day-old roots and leaves, but also in roots and leaves derived from the same embryo in barley. There are two common perspectives about the effects of retrotransposons’ movements. Some researchers believe that the role of TEs for the germline differentiation is insignificant, whereas others think that transposition events can be beneficial or harmful for the organisms.[49]

Conclusions

In our study, we compared three different rice cultivars (Osmancik-97, Beser and Ipsala) with two different rice-specific retrotransposons (Hopi/Osr27 and Houba/Tos5/Osr13). We observed differences in retrotransposon movements not only among cultivars, but also among different plant organs in the same individual. Despite the importance of retrotransposons for the genome dynamics and gene activity, our understanding of their biology is still in a primitive state. Our results with rice retrotransposons could be helpful for the understanding of the mechanisms responsible for polymorphism.

Disclosure statement

No potential conflict of interest was reported by the authors.

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