

: Paleovirology; Endogenous pararetrovirus; R
Genealogy; Recombination

Pararetroviruses (*C* and *H*) have double-stranded DNA genome and resemble retroviruses in that they employ reverse transcription for genome replication, but they lack the processes and molecular machinery for integration into the host genome (Temin, 1985). However, with the increasing decryption of plant genomes, a growing number of endogenous pararetrovirus (EPRV) sequences have been discovered as ancient integrated analogs of most members of the *C* family. Integration of pararetroviruses was thought to involve illegitimate recombination or double-strand break repair via non-homologous end joining between the pararetrovirus and host genomes. Rice tungro disease is a significant constraint on rice (*Oryza sativa* L.) yields in Southeast Asia. The disease is caused primarily by infection with rice tungro bacilliform virus (*C*, RTBV), a known extant rice pararetrovirus. The evolutionary history of rice has been studied deeply and described. Cultivated rice, which includes subspecies *indica* and *javanica*, was domesticated from the wild species *Oryza rufipolyploid*. *O. rufipolyploid* is thought to have diverged into at least two ecotypes, perennial and annual (the annual type is also called *O. rufipolyploid*), 0.16 million years ago (Mya), as estimated by the molecular clock. Here, we compared the eRTBVL sequences from the three rice genome databases by comprehensive analyses and screened orthologous sequences in diverse cultivated and wild rice accessions to understand the evolutionary route of the eRTBVL sequences.

To investigate the origin of the endogenous pararetrovirus elements in the rice genome, we performed a strict phylogenetic analysis of RTBV sequences during rice speciation, and inferred the geographical origin of the RTBV sequences.

blue, W1943). Sequences sorted into the same family are indicated by

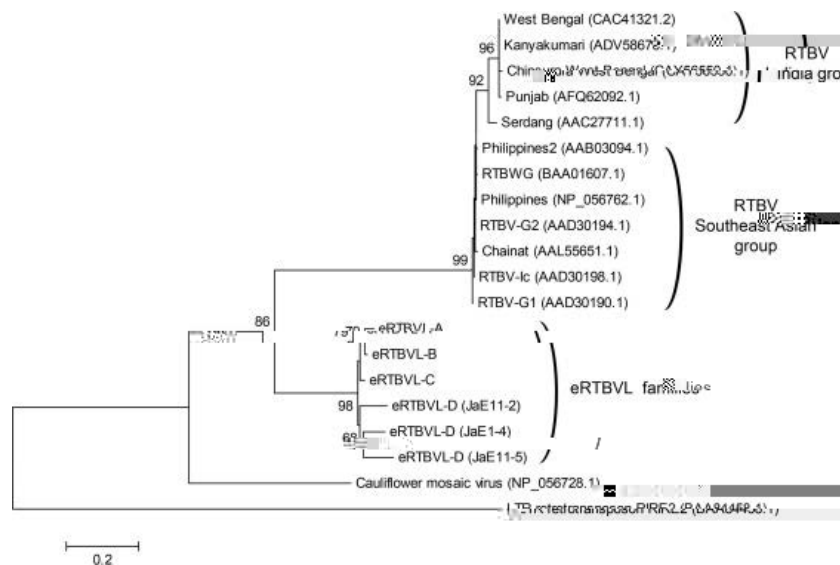


Figure 1: Distribution of eRTBAL in Aisa.

the eRTBVL families and RTBV strains was based on the amino acid sequences of the RT/RH region using the WAG+G model, which was chosen after model testing. Support for the ML trees was evaluated by 1000 bootstrap replicates. All the sequence alignments

C
 Total DNAs were extracted from the leaf samples of the cultivated and wild rice accessions by Plant DNAzol (Invitrogen, Carlsbad, CA, USA). The DNA concentration in each sample was measured by NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) and all were adjusted to a similar level. Primer pairs were generally designed and used as follows: for amplification 1, a forward primer for the left flank (P1) and a reverse primer for the interior of the eRTBVL locus (P2) were used; for amplification 2, a forward primer for the interior of the eRTBVL locus (P3) and a reverse primer for the right flank (P4) were used; for amplification 3, a primer pair for left and right flanks (P1, P4) were used to amplify the whole length of the eRTBVL locus or the empty donor site. Polymerase chain reactions (PCR) were performed using Ex Taq or LA Taq polymerase (Takara, Shiga, Japan) in a PTC-200 thermal cycling system (GMI, Ramsey, MN, USA). When the whole length of some long eRTBVLs failed to amplify, the reaction was adjusted to amplify the possible empty donor sites in the corresponding rice accessions. The PCR products were resolved on a 1–2% agarose gel and analyzed with a Typhoon 8600 PhosphorImager (GE Healthcare, Little Chalfont, U.K.). Consensus sequences of the eRTBVL-A1, -A2, -B, -C and -X families were acquired by aligning the nucleotide sequences from the defined regions for each family (the chimeric sequences that possibly resulted from intra-recombination after integration were excluded). The eRTBVL sequences that spanned at least 80% of the length of each region in the three rice genomes were used in the alignments. The consensus sequences of each region were combined, and ambiguous regions at the terminals were trimmed to produce the final whole length consensus sequences. These consensus sequences were then aligned by ClustalW and manually edited for use in the recombination analysis. We employed the RDP,

GENECONV and BOOTSCAN methods in the RDP3 package. eRTBVL sequences from the Nipponbare genome DDBJ accession numbers: BR000029-BR000031 as queries. The following BLAST parameters were used: word size, 11; gap open, 5; gap extend, 2; penalty, -3; and reward, 2. The contigs spanning high-identity matches (e-values < 1e-10, alignment length > 100 bp) were extracted, and filtered so that only the contigs with > 50 bp flanking at least one end of the eRTBVL loci.

References

- Zhou Z, Robards K (2002) Composition and functional properties of rice. Int J
- Yoshida H, Tomiyama Y, Mizushima Y (2010) Lipid components, fatty acids and triacylglycerol molecular species of black and red rices. Food Chem 123: 210-215.
- Kim NH, Kwak J, Baik JY, Yoon MR, Lee JS, et al. (2015) Changes in lipid substances in rice during grain development.
- Heinemann RJB, Fagundes PDL (2005) Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. J Food
- Chen HH, Hung CL (2015) storage characteristics of brown rice. Food Bioprocess Technol 8: 471–477.
- Stout MJ, Riggio MR, Zou L, Roberts R (2002) and feeding behaviour of the rice water weevil, Lissorhoptrus oryzophilus (Coleoptera: Curculionidae). J Econ Entomol 95: 715-721.
- Zou L, Stout MJ (2004) Degree-day models to predict the emergence and development of rice water weevil (Coleoptera: Curculionidae) in southwest Louisiana. Environ Entomol 33: 1541-1548.
- Zou L, Stout MJ (2004) Lissorhoptrus oryzophilus Kuschel on the growth and yield components of rice, Oryza Sativa
- Stout MJ, Hamm JC (2011) dermacorX-100 against the rice water weevil at three planting dates. Annu Res Report 103: 331-332.
- Blackman B, Autin T (2014) Management practices of Louisiana and Texas rice growers. La Agric Mag 57: 14-15.