Artificial optimization of bamboo *Ppmar2* transposase and host factors effects on *Ppmar2* transposition in yeast

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**Mariner-like elements (MLEs)** are promising tools for gene cloning, gene expression, and gene tagging. We have characterized two *MLE* transposons from moso bamboo, *Ppmar1* and *Ppmar2*. *Ppmar2*, is smaller in size and has higher natural activities, thus making it a more potential genomic tool compared to *Ppmar1*. Using a two-component system consisting of a transposase expression cassette and a non-autonomous transposon cotransformed in yeast, we investigated the transposition activity of *Ppmar2* and created hyperactive transposases. Five out of 19 amino acid mutations in *Ppmar2* outperformed the wild-type in terms of catalytic activities, especially with the S347R mutant having 6.7-fold higher transposition activity. Moreover, *36 yeast mutants with single-gene deletion were chosen to screen the effects of the host factors on *Ppmar2NA* transposition. Compared to the control strain (*his3Δ*), the mobility of *Ppmar2* was greatly increased in 9 mutants and dramatically decreased in 7 mutants. The transposition ability in the *efm1Δ* mutant was 15-fold higher than in the control, while it was lowered to 1/66 in the *rtt10Δ* mutant. Transcriptomic analysis exhibited that *EFM1* defect led to the significantly impaired *DDR2*, *HSP70* expression and dramatically boosted *JEN1* expression, whereas *RTT10* defect resulted in significantly suppressed expression of *UTP20*, *RPA190* and *RRP5*. Protein methylation, chromatin and RNA transcription may affect the *Ppmar2NA* transposition efficiency in yeast. Overall, the findings provided evidence for transposition regulation and offered an alternative genomic tool for moso bamboo and other plants.

**KEYWORDS**

mariner-like element (MLE), artificial optimization, transposase, host factor, bamboo, yeast
Introduction

Transposons are found in a wide variety of species and migrate across the genome. There are two types of transposons: DNA transposons and RNA transposons. Mariner-like elements (MLEs) belong to the DNA transposon family. Mariner transposon was first characterized in the Drosophila mauritiania mutant with white eyes, and later homologous elements were discovered in abundance in plant and animal genomes (Hartl et al., 1997). MLEs are typically comprised of an Open Reading Frame (ORF) that encodes the transposase (Tase), which locates between two paired Terminal Inverted Repeats (TIRs) and Target Site Duplications (TSDs) (Plasterk et al., 1999). Transposase has three well-defined domains. The N-terminal domain contains helix-turn-helix (HTH) motifs that recognize and bind the TIRs. The C-terminalus contains a catalytic domain with a DDE/D triad (Yuan and Wessler, 2011). The two aspartic acid residues (D) and a glutamic acid (E) or another aspartic acid (D) are commonly located 34 or 39 amino acids apart in animal and plant transposases (DD34E/D, DD39D, respectively) (Hartl et al., 1997; Feschotte and Wessler, 2002). The Linker region, harboring a conserved WVPHEL motif, connects the HTH motif and catalytic domain (Liu and Chalmers, 2014).

The conservation of the transposase sequence has a significant impact on transposition efficiency. The three most well-studied MLEs to date are Sleeping Beauty (SB) from fish (Ivics et al., 1997), Mos1 from Drosophila mauritiania (Bryan et al., 1990), as well as Himar1 from Haematobia irritans (Robertson and Lampe, 1995). In SB, a single amino acid mutation yielded around 2-3-fold transposition efficiency, and 9 amino acid mutations (K14R, K33A, R115H, RKEN214-217DAVQ, M243H, T314N) by DNA shuffling resulted in a hyperactive mutant SB100X with a 100-fold increase in transposition activity (Mâtes et al., 2009). Germon et al. (2009) used systematic single amino acid substitutions to create two hyperactive Mos1 mutants (FETY and FET) that were 60- and 800-fold more active than the wild-type Mos1 version. The Linker region is also important for transposition frequency. Almost all single mutations of the WVPHEL motif in Himar1 and Hsmar1 transposase led to highly active transposase variants, but which easily produce non-productive DNA double-strand breaks that can induce DNA damage and mutations (Butler et al., 2006; Lampe, 2010; Liu and Chalmers, 2014).

Besides the sequences of transposases, DNA methylation, chromatin status, over-production inhibition (OPI) and host proteins may influence the transposition frequency. It was shown that the methylated SB was at least 100-fold more active than the unmethylated version. CpG methylation of the SB region and heterochromatin formation facilitated the transposition reaction (Yusa et al., 2004). The MLE transposase activity was also inhibited by OPI (Lohe and Hartl, 1996). Overproduction of wild-type transposase enhanced the attachment of the transposase dimer and competition for free transposon ends, ultimately lowering the transposase activity. OPI started to occur when the number of transposase dimers was superior to the number of available TIRs (Claeys et al., 2013). High Mobility Group B1 (HMGB1) was a host-encoded cofactor of SB transposition and was involved in the formation of transposase-transposon complexes. Transposition of SB was severely suppressed in the HMGB1-deficient mouse cells (Zayed et al., 2003). In yeast (Saccharomyces cerevisiae), more than 200 host factors have been found to be associated with Ty1 and Ty3 retrotransposon (Irwin et al., 2005; Curcio et al., 2015). These factors were hypothesized of being involved in chromatin and transcript elongation, translation and cytoplasmic RNA processing, vesicular trafficking, nuclear transport, and DNA maintenance.

Two full-length MLEs named Ppmar1 and Ppmar2 were previously identified from the genome of moso bamboo (Phyllostachys edulis) (Zhou et al., 2015). Ppmar1 and Ppmar2 were shown to be transposable in Arabidopsis thaliana and yeast (Zhou et al., 2016; Zhou et al., 2017; Ramakrishnan et al., 2019a; Ramakrishnan et al., 2019b). Site-directed mutation boosted the activity of the Ppmar1 mutant (S171A) by more than 10-fold (Zhou et al., 2017). Ppmar2 is smaller in size (Zhou et al., 2016) and has a higher natural activity (Zhou et al., 2017), thus making it a more potential genomic tool compared to Ppmar1. Moso bamboo has a very lengthy vegetative growth cycle (~60 years), and hence rarely reproduces sexually, but reproduces via rhizomes during the vegetative period (Janzen, 1976; Watanabe et al., 1982). Breeding of moso bamboo via hybridization is extremely difficult due to its occasional sexual reproduction. So, a bamboo mutant library via an efficient transformation system will be a preference for future breeding. Currently, the most used genomic tools are T-DNA insertion and gene targeting (e.g., CRISPR/Cas). Insertional mutagenesis based on active transposases may be a promising tool to manipulate the genome of moso bamboo.

In the present study, we focused on the Ppmar2 transposon to develop hyperactive Ppmar2 mutants by rational mutagenesis. The host factors in yeast which regulated Ppmar2 transposition were also screened. The hyperactive Ppmar2 transposon system reported in this study with its outstanding features including its compact size and non-linked insertion sites could provide an alternative genomic tool for moso bamboo and other plants.

Methods

Construction of the Ppmar2 transposition system in yeast

The Ppmar2 transposition system was constructed using the transposon donor vector, pWL89A, and the transposase
expression vector, pAG415gal-ccdB, as described by Ramakrishnan et al. (2019a; 2019b). Ppmar2 non-autonomous transposon (Ppmar2NA) was cloned by amplifying its 5’ and 3’ TIRs as well as the adjacent sequence, followed by an overlap PCR. Ppmar2NA was inserted into the Xho I site at the 5’ untranslated region (UTR) of the Ade2 gene in the vector pWL89A possessing two selectable markers, Ura3 and Ade2. The transposon donor vector was named pWL89A-Ppmar2NA. The Ppmar2 transposase sequences were amplified by adding Not I and EcoR V sites at both ends to fit the pAG415gal-ccdB vector. Both restriction enzymes cut the pAG415gal-ccdB vector. The transposase fragment and the backbone of pAG415gal-ccdB were then ligated by T4 DNA ligase to obtain the recombined vectors pAG415gal-transposase with Leu2 selectable marker. The transposase was promoted to be expressed under the gal promoter.

Yeast transposition assay

The two prepared plasmids (pWL89a-Ppmar2NA and pAG415gal-transposase) were co-transformed into S. cerevisiae strain DG2523. Transformed yeast strains were grown on medium lacking Leucine and uracil (SD-his-ura) but with 2% galactose at 30°C in the dark for 3 days, followed by the suspension of single colonies in 150 μl water and plating onto medium lacking adenine, Leucine, and uracil (SD-ade-his-ura) with 2% galactose as the carbon sources. The plates were incubated at 30°C in the dark for about 3 days to allow the growth of ADE2 revertant colonies.

Transposition footprints analysis

The fragments covered Ppmar2NA excision spots on pWL89a were amplified using primers of yeast T-5 (5’-CAC CCC AGG CTT TAC ACT TTA TG-3’) and T-3 (5’-GTT GCT TAT TTG TTT GCC AGG AG-3’). PCR products were cloned into the pUC18-T vector for sequencing.

Insertion sites and insertion bias analysis

Genomic DNA was extracted from the single ADE2 revertant colonies and then was sheared into 500-bp fragments using Covaris E220 ultrasonicator (Covaris, UK). The libraries were normalized, pooled, and sequenced via Illumina high-throughput sequencing platform NovaSeq 6000 (2×250 bp paired-end run). Low-quality sequences were filtered by Sickle (v1.33) with Q30 and 125bp minimal length (Joshi and Fass, 2011). Filtered reads were aligned to the Ppmar2NA sequence through the local blast. Subsequently, the reads containing the Ppmar2NA sequence and adjacent sequence were aligned to the S. cerevisiae reference genome (https://yeastgenome.org/) to identify the insertion sites. The 20bp upstream and downstream of the insertion sites were aligned to verify the nucleotide distribution characteristics of the Ppmar2NA insertion sites.

Site-directed mutagenesis of Ppmar2 transposase

To identify Ppmar2 non-conserved transposase sites, we downloaded 22 MLE transposase sequences from GenBank and aligned them with the Ppmar2 transposase using MEGA11.0.10 (Tamura et al., 2021). Mutagenesis was performed with the QuikChange Lightning Site-Directed Mutagenesis Kit using the primers listed in Supplementary Table S1. The selected sites of the Ppmar2 transposase were mutated into corresponding amino acids (Supplementary Table S2). The mutated Ppmar2 transposases then replaced the template on pAG415gal-transposase to examine the transposition activities. All plasmids were sequenced to confirm the presence of the targeted mutation. Homologous hyperactive mutation sites in the Mos1 and Himar1 transposase were mutated into the corresponding amino acid in the Ppmar2 transposase.

Transposition frequency of Ppmar2 mutants

The transposition frequencies of Ppmar2 mutants were evaluated by ADE2 revertant frequencies. Each galactose-induced colony was suspended in 50 μl of water and plated on media without adenine. The cell suspension was equally diluted to 1×10^5 volume and was plated on SD media lacking adenine, Leucine and uracil (SD-ade-his-ura) to obtain the total number of galactose-induced colonies. The assay of each mutant was performed with six biological replicates.

Transposition frequency analysis of Ppmar2 in yeast mutants

To examine the host factors’ effects on Ppmar2 transposition in yeast, 36 mutants with single-gene deletions were selected (Table S3) which were kindly provided by Charles Boone (Toronto University, Toronto, in Canada), and His3Δ was used as the control strain. These genes are involved in methylation, DNA replication, transcription, translation, as well as the regulation of retrotransposon (Ty1 and Ty3) (Irwin et al., 2005; Curcio et al., 2015).
Gene expression via RNA-seq

The efm1�, rtt10� mutants and the wild type yeast His3A were cotransformed with pWL89a-Ppmar2NA and pAG415gal-transposase plasmids with three colonies for each genotype. The mRNA of the single ADE2 revertant colonies was extracted, when strains grew in YPD liquid medium with the OD600 of 0.5, and was synthesized into cDNA strands with dNTPs and DNA polymerase I. The final cDNA library was obtained through the AMPure XP system and was sequenced on an Illumina HiSeq 2000 platform (Tianjin Nuhe Zhiyuan Bioinformatics Co., Ltd.) to generate 125 bp/150 bp paired-end reads. The adapter, ploy-N and low-quality reads from raw data were removed using Illumina PIPELINE software. Index of the yeast genome has been built using STAR (v2.5.1b) and paired-end clean reads were aligned to the yeast reference genome (https://yeastgenome.org/) and low-quality reads from raw data were removed using Illumina PIPELINE software. Index of the yeast genome has been built using STAR (v2.5.1b) and paired-end clean reads were aligned to the yeast reference genome (https://yeastgenome.org/) with TopHat v2.0.12. The reads mapped to each gene were counted by HTSeq v0.6.0 and were normalized to fragments per kilobase of exon per million fragments mapped (FPKM) (Trapnell et al., 2010). The method of Benjamini and Hochberg was used to adjust the P-value to control the error detection rate (Haynes, 2013). In this experiment, the correction of p < 0.05, with | log2foldchange | > 1 was used for screening differentially expressed genes (DEGs).

Quantitative real-time PCR analysis

Eight DEGs were selected for qRT-PCR analysis. One μg total RNA of each sample was reverse-transcribed using PrimeScript™ RT reagent Kit with gDNA Eraser. The qRT-PCR was performed using the SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) on the iQ™5 Multi-channel Real-time PCR Detection System (Bio-Rad). The relative abundance of each gene expression was calculated from the 2^ΔΔCt values between two TIRs, one to four bases were aligned with TopHat v2.0.12. The reads mapped to each gene were counted by HTSeq v0.6.0 and were normalized to fragments per kilobase of exon per million fragments mapped (FPKM) (Trapnell et al., 2010). The method of Benjamini and Hochberg was used to adjust the P-value to control the error detection rate (Haynes, 2013). In this experiment, the correction of p < 0.05, with | log2foldchange | > 1 was used for screening differentially expressed genes (DEGs).

Results

Verification of the Ppmar2 transposition potential in yeast

To determine whether Ppmar2 can transpose in the yeast genome, we performed transposition assays in yeast utilizing the methods described previously (Ramakrishnan et al., 2019a; Ramakrishnan et al., 2019b). The assays used two constructs, one transposase expression vector and one transposon donor vector. In the transposase expression plasmid, the Ppmar2 transposase coding sequence was fused to the inducible GAL1 promoter, and Ure2 served as the selectable marker. In the transposon donor plasmid, the non-autonomous Ppmar2NA element was inserted in the 5'UTR of the ADE2 reporter gene, and Ura3 served as the selectable marker. Transformants containing both plasmids were selected on a medium with 2% galactose but lacking Leucine and uracil. Colonies of the double transformants were picked and regrown on agar plates without adenine for the selection of ADE2 revertants, which represented the excision of the Ppmar2NA. Notably, ADE2 revertant colonies were obtained in the presence of the Ppmar2 transposase, but none when the control plasmid, pAG415-ccdB, was used.

Plasmid DNA was prepared from the independent ADE2 revertants, and the excision products of Ppmar2NA in the ADE2 5'UTR region were PCR amplified using primers of yeast T-5 and T-3. Sequencing results revealed diverse footprints of excision were generated by Ppmar2NA in the donor plasmid. Between two Ppmar2NA TIRs, one to four bases were retained (Figure 1).

Reinsertion preferences of the excised Ppmar2NA

To follow the fate of the excised Ppmar2NA and localize the reinsertion sites in the yeast genome, genomic DNA was extracted from the independent ADE2 revertants, and sequenced (with 30 times coverage). Following alignment, we identified 7 genetic integration events on 6 yeast chromosomes. All insertions occurred within ~500 bp of the coding regions, with one insertion occurring within the gene (Table 1). As expected, Ppmar2NA in all detected events was inserted into a TA dinucleotide where the AT content of 50 bp sequences nearby the insertion sites was more than 60% (Figure 2). To further validate the Ppmar2NA insertion bias, we analysed the 20 bp sequences flanking the TA on both sides. Alignment of the 7 integration sites revealed that the excised Ppmar2NA was preferentially inserted into AT-rich regions (Figure 2 and Table 1).

Targeted mutagenesis of the Ppmar2 transposase to enhance Ppmar2NA transposition in yeast

To improve the transposition activity of Ppmar2, the transposase was modified following the strategy as described (Zhou et al., 2017). We selected 22 MLE transposase sequences from animals and plants, including Mos1 from D. mauritiana (Bryan et al., 1990), Himar1 from H. irritans (Robertson and Lampe, 1995), Ppmar1 from Moso bamboo, and three MLE
transposases from rice (Yang et al., 2006; Yang et al., 2009) (Figure 3). Other sequences were filtered in the NCBI database by blast using the Ppmar2 transposase sequence as a query, which showed high homology with the Ppmar2 transposase. We examined amino acid residues that were partially conserved in MLEs but not in the Ppmar2 transposase after alignment. Thirteen such sites scattered across the three conserved domains of the Ppmar1 transposase (the DNA binding domain, the Linker domain, and the catalytic domain) were identified (Figure 3). Moreover, we selected six homology sites in the Ppmar2 transposase that corresponded to the hyperactive mutation sites in the Mos1 and Himar1 transposase (D129A, D129R, G132A, A168R, L174K and K289A) (Figure 3) (Butler et al., 2006; Germon et al., 2009). The 18 candidate sites, including four in the HTH binding domain, three in the Linker region, and eleven in the catalytic domain, were systematically modified one by one, and the transposase variants were evaluated in the yeast excision assay (Table S2).

Among the 19 transposase mutations (two mutations at the 129th amino acid), 5 of them (L235F, K243R, S347R, S292I, and L174K) dramatically enhanced the Ppmar2 excision activity by more than 2-fold (Figure 4). Four amino acid substitutions, including L266P, L309P, D333P, and A168R, suppressed the transposition. The remaining 10 amino acid substitutions had no significant effects (Figure 4). The S347R mutant exhibited the highest transposition activity, which was 6.7-fold that of the wild type (Figure 4).

**Host factors’ effects on Ppmar2 transposition in yeast**

To investigate the effects of host factors on transposition efficiency, 36 yeast mutants with single-gene deletions were screened (Table S2). Of the 36 genes, 8 genes were involved in methylation, 7 genes in DNA replication, transcription, and translation, and 21 genes were host factors of retrotransposons.
The 36 yeast mutants were double transformed with pWL89a-Ppmar2NA and pAG415gal-transposase. The positive colony assay in each yeast mutant revealed that the transposition frequency was significantly higher in 9 yeast mutants (tmt1Δ, efm1Δ, efm5Δ, mgt1Δ, pdr3Δ, rtt102Δ, chd1Δ, exg2Δ, and cur1Δ) than in the control strain (His3Δ), but significantly lower in 7 yeast mutants (set2Δ, rtt106Δ, rtt10Δ, mlh2Δ, vac7Δ, mrpl10Δ, and itr2Δ), with no obvious differences in the other 20 yeast mutants (Figure 5). Ppmar2NA showed the most active transposition in the efm1Δ mutant, which was 15-fold higher than in His3Δ. Ppmar2NA had the lowest transposition frequency in the rtt10Δ mutant, which was only 1/66 that in the His3Δ strain.

DEGs in the mutants with dramatically distinct transposition ability Ppmar2NA

To explore the host factors’ effects and investigate the DEGs involved in the transposition regulation, we performed the transcriptome analyses for the strains with distinct
transposition competence *Ppmar2NA* (*efm1Δ* vs. *His3Δ* and *rtt10Δ* vs. *His3Δ*). Sixty-seven DEGs were validated with a *P*-value lower than 0.0001 (Figure 6) and enriched via GO and KEGG. These DEGs were related to ribosome biogenesis, RNA modification and DNA modifications which might be involved in the regulation the *Ppmar2* transposition (Figures 1S, 2S). In the mutant *efm1Δ* with highly active *Ppmar2NA*, three genes linked with DNA repair and RNA transcription (*DNA Damage Responsive 2* (*DDR2*), *Heat Shock Protein 70* (*HSP70*) and *Monocarboxylate/proton symporter of the plasma membrane* (*JEN1*)), might be likely related to regulation of the *Ppmar2NA* transposition. *DDR2* and *HSP70* were highly expressed in the *efm1Δ* strain, while the expression of *JEN1* was repressed. In the *rtt10Δ* null mutant, three genes (*U3 snoRNA-associated protein 20* (*UTP20*), *DNA-directed RNA polymerase I subunit* (*RPA190*), *U3 small nucleolar RNA-associated protein* (*RRP5*)), involved in ribosome assembly and RNA polymerase synthesis, were markedly downregulated.

qRT-PCR was performed to validate the RNA-seq data by checking the expression levels of *Elongation Factor Methyltransferase* (*EFM1*), *Regulator of Ty1 Transposition* (*RTT10*), *DDR2*, *HSP70*, *JEN1*, *UTP20*, *RPA190* and *RRP5*. No expression of *EFM1* was detected in *efm1Δ*, and a low-level expression of *RTT10* in *rtt10Δ*, as predicted. The other gene expression patterns displayed via qRT-PCR were completely consistent with the RNA-seq data-derived gene expression pattern (Supplementary Figure 3).

**Discussion**

Yeast has been widely used as a heterologous host to evaluate the transposition activity of plant DNA transposons, such as *Osmar5* and *Stowaways MITE* in rice (Yang et al., 2006; Yang et al., 2009), and *Ac/Ds* in maize (Lazarow et al., 2012). We have identified two MLE transposons in moso bamboo, *Ppmar1* and
Ppmar2, which both could jump in *Arabidopsis thaliana* and yeast genome (Zhou et al., 2016; Zhou et al., 2017; Ramakrishnan et al., 2019a; Ramakrishnan et al., 2019b). Ppmar2 has a relatively smaller size and naturally higher activity than Ppmar1, thus making it a more potential genomic tool. To improve the transposition efficiency and develop an alternative gene-tagging tool, we create a hyperactive Ppmar2 via mutagenesis and screen the host factors influencing transposition of Ppmar2 in yeast.

**Ppmar2NA’s transposition footprints and insertion preferences resembled the other MLEs**

Using a two-component system consisting of a transposase expression cassette and a non-autonomous transposon cotransformed in yeast, we examined the transposition activity of Ppmar2NA. The footprints of Ppmar2NA after excision

![Heat map of DEGs expression in HisΔ, efm1Δ, rtt10Δ strains. The expression patterns of DDR2, HSP70, JEN1, UTP20, RPA190 and RRPS genes were marked. The color scale indicates the relative expression level.](image)
revealed that Ppmar2NA was preferentially cut between the two TIRs, leaving 1-4 staggered nucleotides (Figure 1). Similarly, the Osmar5 transposase generated a staggered cut with one to four nucleotides at both ends of the transposon element (Yang et al., 2006), whereas the animal transposases Mos1, Sleeping Beauty, and Frog Prince were cleaved but left no more than three nucleotides (Luo et al., 1998; Dawson and Finnegan, 2003; Miskey et al., 2003). On the other hand, Ppmar2NA tended to insert into the TA-rich regions and generated AT TSDs (Figure 2). In short, as a member of the MLEs family transposons, Ppmar2NA shared a similar cleavage footprint and insertion preference.

Some amino acids in key sites are important for the catalytic activity of Ppmar2 transposase

Eighteen non-conserved amino acids in Ppmar2 transposase, including 4 in the HTH binding domain, 3 in the linker junction region, and 11 in the DDE catalytic domain, were selected for mutagenesis to improve the transposition activity (Figure 3). Another 6 homology sites in Ppmar2 corresponding to hyperactive mutation sites in Mos1 and Himar1 were also chosen (D129A, D129R, G132A, A168R, L174K and K289A) (Butler et al., 2006; Germon et al., 2009). The catalytic activities in five of the six mutants were improved, which was consistent with the mutation effect in Mos1 and Himar1 except for A168R (Figure 4) (Butler et al., 2006; Germon et al., 2009). Strangely, the five hyperactive mutation sites are not conserved in the sequences of Ppmar2 transposase (Figure 4).

The linker region connects the HTH motif and catalytic domain, which are not conserved as WVPHEL motif in Ppmar2 transposase. Three mutations (A168R, C169R, and L174K) located in the Linker region of the Ppmar2 transposase, which regulated the spatial structure of the transposase, exhibited divergent transposition activities (Butler et al., 2006; Liu and Chalmers, 2014). The catalytic activity of the C169R mutation did not change significantly, but the A168R mutant totally lost the catalytic activity. The L174K transposase mutant, on the other hand, showed considerably higher catalytic activity than the wild type (Figure 4). The catalytic domain featured by a conserved DDE/D motif played a vital role in transposition activity (Yang et al., 2006). It has been reported that substitution of the DDD to the DDE in the Tc1 family abolished the transposase activity (Lohe et al., 1997). The transposition efficiency of Ppmar2 transposase varied among the targeted mutation sites (L235F, K243R, I259F, L266P, K289A, S292I, V297I, Q306E, L309P, D333P, and S347R). The L235F, K243R, S347R and S292I mutation greatly boosted the transposition.

Especially, the S347R mutation resulted in a 6.7-fold higher transposition frequency (Figure 4). The hyperactive Ppmar2 system would be used in plant genomic engineering as an alternative transposon-based technique.

It should be noted that the high catalytic activity of transposase mutations may not directly lead to high-frequency transposition due to OPI. Construction of transposase mutations which are usually less sensitive to OPI, e.g. low TIR binding affinity, and low stability of the transposase dimers will contribute to the high transposition frequency (Liu and Chalmers, 2014; Tellier and Chalmers, 2020). In the following, mutations that change in Ppmar2 transposition kinetics that shift the OPI equilibrium will be considered.

Host factors, including EFM1 and RTT10 genes, significantly affected the transposition frequency of Ppmar2NA in yeast

The yeast single-gene deletion mutant library was effectively used to investigate the impact of host factors on the activity of retrotransposons Ty1 and Ty3. More than 200 host factors, including those involved in chromatin and transcript elongation, translation and cytoplasmic RNA processing, vesicular trafficking, nuclear transport, and DNA maintenance, regulated the transposon activity of yeast Ty1 (Curcio et al., 2015) and Ty3 (Irwin et al., 2005). The 36 related yeast mutants with single gene deletions were selected in this study. The transposition efficiencies of Ppmar2NA in 9 yeast mutants (mtt1Δ, efm1Δ, efm5Δ, mgt1Δ, chd1Δ, pdr3Δ, cur1Δ, exg2Δ, and rtt102Δ) were 2-fold more active than that in the control yeast. Notably, in the efm1Δ mutant, the transposition efficiency increased by 15-fold. Among the 9 genes, four are related to protein methylation, one to DNA methylation, one to chromatin organization, and the remaining 4 were host factors of retrotransposon. In another 5 yeast mutants (set2Δ, rtt101Δ, mlt2Δ, mrt1Δ, and itv2Δ), the transposition efficiency of Ppmar2NA was at least 1/2 less active than in the control strain His3Δ, especially in rtt101Δ mutant, where the transposition efficiency decreased by more than 65-fold. One of the 5 genes was associated with histone methylation, whereas the other 4 genes were host factors of retrotransposon. These findings indicated that DNA transposon and RNA transposon may share common host factors (Irwin et al., 2005; Curcio et al., 2015).

In yeast, Elongation Factor Methyltransferase (EFM1), which encoded lysine methyltransferase SET8, may characteristically methylate H4K20 (Zhang and Bruice, 2008) and function in
protein modification, chromosome-protein binding regulation, and gene transcription and translation. Studies have shown that EFM1 methylates H4K20 into three proteins H4K20me1, H4K20me2 and H4K20me3 (Yang and Mizzen, 2009). H4K20me1 could further promote chromatin condensation with the help of Condensin II (Weirich et al., 2015). Also, H4K20me1 and H4K20me2 inhibited gene transcription and chromosomal shrinkage by binding to the Lethal (3) Malignant Brain Tumor-Like Protein 1 (L3MBTL1) (Li et al., 2007). H4K20me3 was generally regarded as a marker of transcriptional inhibition in heterochromatin (Schotta et al., 2004). H4K20 was not methylated when the EFM1 gene was knocked out, which might be related to the high transposition frequency of the Ppmar2 in the efm1Δ mutant yeast.

The DEGs identified with the comparison of efm1Δ and His3Δ revealed that the efm1Δ cell conditions were changed significantly. The DDR2 gene encoded a stress protein involved in DNA repair (Kobayashi et al., 1996). The expression of this gene was dramatically elevated in the efm1Δ mutant, suggesting that it may efficiently repair the damaged DNA. The HSP70, which encoded heat shock factor (Abrams et al., 2014), was also obviously upregulated in the efm1Δ mutant. Jardim et al. (2015) found evidence that mos1 may be co-activated with HSP70 genes. JEN1 mediated high-affinity uptake of carbon sources lactate, pyruvate, acetate, and micronutrient selenite, JEN1 expression and localization are tightly regulated, with transcription repression, mRNA degradation, and protein endocytosis and degradation all occurring in the presence of glucose (Haurie et al., 2001).

The RTT10 gene, which encoded a member of the WD40 protein family, was a retrotransposition-related gene. Ty1 was more active in the rtt10Δ mutant than in the His3Δ strain, and the RTT10 gene might prevent Ty1 RNA from being reversely transcribed into cDNA (Scholes et al., 2001). Moreover, RTT10 contributed to the synthesis of transcription factor TFIIID, regulating the ribosome small subunit rRNA synthesis (Dragon et al., 2002). Three DEGs (UTP20, RPA190 and RRPS5) in the rtt10Δ mutant might be related to the lowered transposition activity. The UTP20 protein was a component of the small-subunit (SSU) processome, which modulated the 18S rRNA synthesis (Gallagher et al., 2004). The expression level of this gene in the rtt10Δ mutant was significantly lowered. RRPS5 was another component of the SSU processome and 90S preribosome related to the synthesis of 18S and 5.8S rRNAs (Venema and Tollervey, 1996). The expression level was much lower in the rtt10Δ mutant than in the His3Δ RPA190, encoding RNA polymerase A (Ménet et al., 1988), was also downregulated in the rtt10Δ mutant. The discovery of host factors associated with Ppmar2 mobility lays an important foundation for understanding the mechanism of Ppmar2 transposition in the heterologous host.

In the present study, we created hyperactive Ppmar2 transposons via site-direct mutagenesis and screened the heterologous host factors that influenced the transposition activity. Ppmar2 was proved to efficient to able to excision and reinserted into the TA-rich region, which was the same as the other MLEs in plants. EFM1 and RTT10 related to protein methylation, chromatin and RNA transcription greatly impact the heterologous transposition efficiency of Ppmar2 in yeast. The Ppmar2 transposon system is a promising tool for insertion mutagenesis in moso bamboo and might be used as an alternative to the existing transposon tagging systems in the other plants.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

MZ designed the experiments; XZ and JX performed the research; CX, XC and L-HZ participated in the research; MZ and XZ wrote the manuscript. All authors have read and approved the manuscript.

Funding

This work was funded by grants from the Zhejiang Provincial Natural Science Foundation of China (No. LZ19C160001 and LQ21C160003), the National Natural Science Foundation of China (No. 31870656, 31470615 and 32001326), the Talent Research Foundation of Zhejiang A&F University (No. 2019FR055) and Independent research project of state key laboratory of subtropical silviculture (ZY20200301).

Acknowledgments

We would like to extend our sincere gratitude and appreciation to all reviewers for their valuable comments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1004732/full#supplementary-material
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