Transposable Element Insertions Influence Plant Traits

Transposons are DNA sequences that are autonomously duplicated and can “jump” out from one location on the genome and be inserted at another place. Transposons randomly shift to different loci in the genome, which can alter the original gene structure and lead to gene silencing or inactivation, consequently changing the traits under the regulation of the functional genes. For example, Zhang et al found a 27.2 kb transposable element insertion in the promoter of the *stiff1* gene of maize that represses the transcription of *stiff1* and, consequently, enhances stalk strength by increasing the cellulose and lignin contents in the cell wall. Sometimes, when a transposon is inserted into the coding region of a gene, it modifies an important functional domain or splice site, consequently changing the transcript. Although the gene could still be translated into a protein, the original function may become weakened or lost. Moreover, some transposable elements (TEs) directly regulate a functional gene as a promoter.

Recent studies have also suggested that plant transposons have important biological functions in gene expression regulation through the generation of noncoding RNAs. Most long non-coding RNA (lncRNAs) originated from intergenic regions where transposons were abundantly distributed, and some microRNA (miRNAs) and short interfering RNAs (siRNAs) were directly or indirectly derived from transposons. These noncoding RNAs are known to regulate genes in plants. Because TE insertions could influence gene expression and function and eventually cause phenotypic variances between individuals, they have received attention for their potential value in the study of genetics.

Transposable Elements Participate in Genome Evolution

In addition to genetic association studies, TEs have also been used the study of evolutionary processes. For example, miniature inverted-repeat elements (MITEs) were considered the driving force of genomic evolution, and the number of MITEs was significantly and positively correlated with genome size. Because of their abundant variety of copy numbers and insert sites and highly species-specific characteristics, transposons have great potential in the study of gene function and genome evolution. Tang et al identified an active MITE "*mfing*" that is associated with the high-tillering dwarf mutant in rice; moreover, they also found that the copy number of *mfing* dramatically varies among *Oryza sativa* (Asian cultivated rice), *Oryza rufipogon* (wild ancestor of Asian cultivated rice), and *Oryza glaberrima* (African cultivated rice), suggesting that the amplification or contraction of *mfing* elements may play an important role in genome evolution and species diversification in rice.

Interestingly, when plants experience biotic and abiotic stresses, many TEs in the plant genome are specifically activated and probably transposed under the regulation of methylation or transposons themselves, which has been found in tobacco, tomato, and other plants. Some researchers have suggested that the activation of TEs under stress may accelerate genome recombination, which may also reflect the environmental adaptation process of plants during the particular lengthy evolutionary history of species.

Potential Application of TEs in Conifer Breeding

Conifers (Coniferales) are the largest and most important group of gymnosperms, as well as the most archaic branch. They have...
a total of 6 families, 69 genera, and 605 species. Although the number of species is limited, most of the important tree species belonging to terrestrial ecosystems evolved from conifers, placing conifers in an important taxonomic status and were usually used for the study of plant evolution history. Moreover, conifers have been regarded as dominant species in the forest for more than 200 million years, not only for supporting large industries by providing wood, fiber, and energy but also for making great contributions to the global ecosystem. Due to their high ecological significance and economic importance, the genetic breeding of conifers has become an important research subject worldwide. With the rapid development of genome sequencing technology, an increasing number of high-quality plant genomes have been reported, from which researchers could get genetic data that could be used for the study of genetic mechanisms and for identifying key genes associated with important economic traits. These genomic resources could be a great resource when making breeding plans and, in addition, accelerate the process of genetic breeding in plants. Although several plant whole-genome sequences have been reported, the sequences available from conifers are limited due to their extremely large genomes and assembly difficulty. Examples of such conifers include Pinus lambertiana (~31 Gb), Pinus taeda (~22 Gb), and Picea abies (~20 Gb). Their genomes contain considerable amounts of repetitive DNA, which contributes to the large size of conifers’ genomes and, especially, a high number of TEs, which provide vast potential genetic resources for further study.

In a previous study, we reported our work on the identification and classification of TEs in 3 important conifers, P. abies, P. glauca, and P. taeda, at the whole-genome level. In total, 413 423 TEs were identified from the 3 conifers, and further classification suggested a great variety among the 3 species (Table 1). Further identification of the genetic functions or association with the economic characteristics will facilitate breeding. Identifying functional genes associated with interesting traits through the construction of transposon-based mutation libraries and the development of TEs as molecular markers that could be used for assisted breeding are 2 main methods by which TEs could be used in plant breeding. Related research has been carried out in other economic plants. Transposon-based mutation libraries are important tools for the study of functional genomics in economic crops, including Oryza sativa and Zea maize. Currently, studies have been conducted in which exogenous TEs have been inserted into the plant genome to create mutants using genome walking. Tnt1, Activator/Dissociation (Ac/Ds), and other TEs are traditional transposon systems used in the construction of mutation libraries. For example, Tnt1 was originally found in Nicotiana tabacum and is usually not active in common plant tissues; however, it can be activated in other plant species via tissue culture. Moreover, it can be stably inherited and re-transpose during tissue culture. In a recent study, Sun et al found that Tnt1 insertions are basically randomly distributed throughout the genome and can be identified on both arms of chromosomes in a Tnt1-based Medicago truncatula mutation library. Moreover, they found that the Tnt1 insertion is positively correlated with gene methylation and negatively correlated with CG content in M truncatula, suggesting a special tendency of Tnt1 insertions. Zhou et al identified 2 mariner-like elements (MLEs), Ppmar1 and Ppmar2, from moso bamboo that contain terminal inverted repeats. Furthermore, heterologous transfer of the 2 MLEs to Arabidopsis italiana suggested that they could also transpose in other plants. However, Tnt1 must be activated in vitro tissue culture; therefore, a stable and effective tissue culture system in conifers must be constructed first. Another Activator/Dissociation (Ac/Ds) element comes from maize and is a classic maize transposon method that is used for constructing mutation libraries. Both elements can duplicate and transpose throughout the plant genome. Previous studies have suggested that Ac/Ds can insert into gene-rich regions and alter the expression of genes or the genome through the formation of unstable insertion alleles, stable derivatives, or excision alleles. Until now, Ac/Ds transposons have been shown to be effective in approximately 20 plant species and have become the most widely used TEs for gene tagging and functional genomics studies in plants. For example, Carter et al produced activation-tagged and knockout mutants in the processing of tomato cultivar M82 using the Ac/Ds system and agrobacterium-mediated method. Finally, a population of 509 independent transposed lines with only Dt insertions throughout all 12 chromosomes was developed. Wang et al established an Ac-based transposon system with the donor Ac tightly linked with sugary1 (su1) on maize chromosome 4S, and a total of 208 independent long-distance-transposed Ac lines were identified. This Ac-based system combined with 2 other Ac-based regional mutagenesis systems in maize could be integrated into a new maize mutation library with a more even and high-density distribution of Ac elements throughout the genome for functional genomics studies. Molecular markers are an important tool for genetic studies and breeding, the construction of linkage maps, and population genetics analysis. Transposable elements constitute most of the repetitive sequences and are distributed throughout the genome in plants; thus, TE could have great potential to be explored as molecular markers. Liang et al identified 336 long terminal repeats (LTR) effective primer pairs that could amplify DNA from P. virginiana, 117 of which could produce amplification in other Prunus species; in addition, 59 of the LTR markers were used to construct a linkage map after qualification. Sequence-characterized amplified region (SCAR) markers derived from transposons are another TE-based molecular markers that are notorious for being low time consuming and highly efficient. Roy et al developed 108 CACTA transposable element-derived SCAR markers in maize, 32 of which could be integrated into the genetic map of a Recombinant Inbred Lines (RIL) population; in addition, 76 markers could
be used for diversity analysis of different corn lines. Inter-
retrotransposon amplified polymorphisms (IRAP) are another
important type of molecular marker that were developed from
polymorphisms in the conserved region of retrotransposon
sequences from the same or allied species. Cui et al. developed
stable and polymorphic amplifications in Pinus massoniana,
according to the conservation region of the reverse transcriptase
sequences of Ty1-copia and Ty-gypsy-type retrotransposons.
These IRAP markers were effectively used in the identification
of germplasm and genetic relationships, as well as further
genetic breeding in P. massoniana. Moreover, because LTRs are
usually conserved and widely distributed in the plant genomes,
they can be used for developing TE junction-based markers.
The study of LTRs has provided a great example of molecular
marker development in conifers.

**Conclusions**

TEs have great potential to be used in conifer breeding; how-
ever, a limited number of related studies have been reported. In
future studies, mutant libraries of conifers could be constructed

| CLASS | ORDER | SUPERFAMILY | P. ABIES MEMBERS/FAMILIES | P. GLAUCÉ MEMBERS/FAMILIES | P. TAEDA MEMBERS/FAMILIES |
|-------|-------|-------------|--------------------------|---------------------------|--------------------------|
| Retrotransposons | LTR | Caulimovirus | 51/17 | 196/43 | 315/35 |
| | | Copia | 7304/78 | 35 826/89 | 40 645/92 |
| | | Copia(Xen1) | | 26/5 | |
| | | DIRS | 76/51 | 226/113 | 287/143 |
| | | ERV | | 7/3 | |
| | | ERV1 | 299/124 | 749/231 | 1392/182 |
| | | ERV4 | 25/21 | 44/39 | 145/46 |
| | | ERVK | 153/88 | 563/187 | 584/169 |
| | | ERVL | 28/22 | 105/71 | 115/75 |
| | | Gypsy | 12 267/129 | 64 831/396 | 58 349/113 |
| | | Ngaro | 29/24 | 63/41 | 126/63 |
| | | Pao | 241/84 | 832/229 | 874/179 |
| | | RUUnknown | 9225/941 | 42 750/3435 | 43 951/1147 |
| LINE (long interspersed nuclear element) | L1 | 5230/83 | 11 150/40 | 24 553/27 |
| | | PTE-X | 4/4 | |
| | | Tad1 | 4/4 | |
| Subtotal | | 34 936/1670 | 157 335/4914 | 171 369/2279 |
| DNA transposons | TIR (terminal inverted repeat) | hAT | 8/8 | 12/12 | 7/7 |
| | | TcMar | 3/3 | ||
| | | PIF-Harbinger | 3/3 | 4/4 | |
| | | CMC | 3/3 | ||
| | | DUUnknown | 7/7 | 12/12 | 2/2 |
| | MITE | MITE | 378/277 | 287/261 | 390/297 |
| Helitron | Helitron | 6575/609 | 21 869/359 | 20 220/403 |
| Subtotal | | 6971/904 | 22 183/647 | 20 629/719 |
| Total | | 41 907/2574 | 179 518/5561 | 191 998/2998 |

Abbreviations: LINE, long interspersed nuclear element; LTR, long terminal repeats; MITE, miniature inverted-repeat element; TE, transposable elements; TIR, terminal inverted repeat.
to identify essential genes associated with target traits, according to the genotype and phenotype variations in the library. Moreover, TE-based molecular markers could be developed for use in molecular breeding in conifers. It is important to mention that the massive genome sizes and relatively long life cycles of conifers make the use of TEs in conifer breeding more challenging than in annual crop breeding and much foundational work is needed.

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Author Contributions

JW, NL, FY, and YX composed, reviewed, and approved the final article.

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