The Potential Role of Transposable Elements as Molecular Markers

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Abstract
Molecular markers emerged as very important biotechnological tools in plant biotechnology. Molecular markers represent any gene region or DNA fragment related to the gene region in the genome. Numerous molecular marker techniques have been developed especially after the discovery of the Polymerase Chain Reaction. In agricultural biotechnology studies, they are used effectively in many areas such as physical mapping, gene discovery, labeling and genetic diversity with molecular marker technologies. Transposons can also be used as genetic markers because they cause insertional polymorphism. Transposons are DNA sequences that displace within the genome, causing permanent mutations and responsible for genome size changes. With the development of technologies, new techniques and the completion of genome studies in many species, transposon has been observed that it is found in almost every living species and a large part of the genome of many species consists of transposons. Plants are the living things with the highest percentage of transposons. Transposons are grouped as DNA transposons and retrotransposons according to their working principles. Studies are conducted to determine the relationship between retrotransposon markers and species. For this purpose, many marker methods have been developed; based on IRAP and REMAP retrotransposon insertion polymorphisms.

1-Introduction
Genetic variation analysis has become an integral part of plant genetics, breeding and plant ecology...
from past to present time. Gregor Mendel in his studies on pea plant, revealed that genetic inheritance was transferred by analyzing seven morphologically polymorphic characteristics but, morphological variation is not enough to study genetics. Limited distinctive morphological features and environmental effects on morphological diversity limit the use of morphological features in modern genetic analysis. For these reasons, genetic markers are needed in plant breeding studies. Genetic markers are associated with nonrandom variations in natural or wild populations. With the advancement of molecular genetic methodologies, protein and DNA-based molecular markers associated with genetic variation have been developed[1].

Sequences found in more than one location in the genomes of living organisms are called "repetitive sequences". The term “repetitive sequences” (DNA repeats, repetitive DNA) refers to DNA fragments that are present in multiple copies in the genome [2]. These sequences are different DNA fragments consisting of different structures and origins [3]. Repeated sequences are grouped under two classes as "Tandem" sequences and "Transposons". Tandem sequences are sequential, similar sequences in the genome from top to bottom. Transposons are mobile DNA sequences that cause mutations by displacing on the genome [4, 5, 6]. Since transposon elements cause permanent genomic mutations such as duplication, deletion and insertion, they are transferred to the next generations by fertilization and cause the emergence of new alleles. However, transposons are thought to be involved in processes such as cell differentiation, continuity of differentiated cells and organ development in embryonic development processes [7, 8]. Polymorphisms caused by transposons are mostly used as a determinant genetic marker in areas such as DNA fingerprinting, genetic mapping and molecular phylogeny [9]. Especially studies are carried out to determine the relationship between retrotransposon markers and species. For this purpose, many marker methods have been developed to detect new allele formation caused by retrotransposon insertion polymorphisms by using genetic variation analysis [10] and molecular marker techniques [11,12, 13]. In this review, the potential role of transposon elements as molecular markers and studies using them are discussed here.

2- Transposons and the importance of their application in the plant

Transposons are DNA sequences that displace within the genome and cause mutations and changes in the amount of DNA in the genome [14]. Before 1970 little work has been done on transposons, which were initially thought to be insignificant. Later, in 1970 Drosophila melanogaster and Saccharomyces cerevisiae [15, 16], in 1980 Caenorhabditis elegans [17], in 1990s Transposons in filamentous fungi have been described [18]. A large part of all eukaryotic genomes, primarily plant genomes, are composed of retrotransposons. For example, more than 75% of the maize genome sequence [19], about 45% of the human and rice genome segment [20], and 80% of wheat and barley genome consists of transposons. With some exceptions, transposons are found in all genomes [21,22, 23]. Transposons are grouped into 2 classes according to different structure and transposition mechanism. They are classified as RNA transposons (Class I, also called "retrotransposons") and DNA transposons (Class II) [24, 25]. Retrotransposons can reproduce by RNA mediated copy-paste mechanism and cause an increase in the genome size (Fig 1).
Fig 1. Types of transposons in eukaryotes. This figure was drawn by the authors using Allison (2012) [26]

Transposon research in plants have been focused on studies such as genetic diversity [24, 27, 28]. Most of those studies are mostly carried out using PCR-based marker systems. There are many methods developed to determine the retrotransposons in plants. Those widely used molecular marker methods are: Inter Retrotransposon Amplified Polymorphism (IRAP) [29], Retrotransposon Microsatellite Amplified Polymorphism (REMAP), Retrotransposon Based Insertional Polymorphism (RBIP), Sequence Specific Amplified Polymorphism (SSAP), Random Amplified Polymorphism DNA (RAPD), Inter Primer Binding Site Amplification (iPBS) (Table 1) [30, 31].

Table 1. Comparison of different transposon systems

| Marker | Reason | Methods | Inheritance | Detection | Features | Reference |
|--------|--------|---------|-------------|-----------|----------|-----------|
| IRAP   | DNA insertion | PCR     | Dominance (The dominant alleles mask or override the expression of recessive allele) | Multi-locus | Technical simplicity; retrotransposons between two adjacent amplified LTR. | [30] |
| REMAP  | DNA insertion | PCR     | Dominance | Multi-locus | Technical simplicity; similar to IRAP but between amplification retrotransposon and Microsatellite locus | [30] |
| RBIP   | DNA insertion | PCR     | Codominance (Heterozygotes can be distinguished from homozygotes) | Single-locus | Technical simplicity, sequence knowledge required; very useful for genetic diversity | [32] |
-Retrotransposon Based Molecular Markers (IRAP and REMAP)

Retrotransposons are the most common component of plant genomes that show activity at transcription and integration levels. Retrotransposons are simply classified into four groups: LTR region (Long Terminal Repeat), none LTR region, LINE (long intermediate nuclear elements) and SINE (short intermediate nuclear elements). Retrotransposons are found all over the genome in large numbers, and they are used in somaclonal variation studies because they can move on the genome with cut-paste and copy-paste mechanisms [33, 34]. Retrotransposons containing LTR are also known as gypsy and copia-like retrotransposons. Gypsy-like [35] and copia-like [32, 36] retrotransposons have always existed throughout the plant kingdom.

Retrotransposons has a contribution to the development of the molecular marker system due to their long, conserved and defined base sequences [37]. REMAP and IRAP are used successfully in genome mapping [38] and genomic stability studies of allopolyploid species [39]. In addition, these two marker systems can distinguish from one class of the plant group to its species, as well as genetic diversity studies of vegetatively propagated species [40, 41]. IRAP and REMAP are two marker methods based on amplification based on regions that yield LTRs in the genome [37].

Retrotransposons can be used as markers because they create new splice links between genomic DNA and their conserved ends. To detect polymorphism in retrotransposon splices, marker systems generally rely on PCR amplification between these conserved ends and some components of adjacent genomic DNA [42]. REMAP and IRAP does not require restriction enzyme to generate marker bands in the marker system. IRAP marker system products were generated from two near retrotransposons using primers facing outward. REMAP marker system produces marker bands that reproduce between retrotransposons close to simple array repeats (microsatellite) [31].

Retrotransposons can adapt in both directions within the genome. In the head to head or tail to tail direction, PCR products can be produced using a single primer from elements close enough to each other. It is amplified using both 5 and 3’ LTR primers for genomic DNA elements intervening in the head-to-tail direction. The REMAP method is based on an outward facing LTR primer and a second primer from the microsatellite. Primers (GA) /, (CT) /, (CA) /, (CAC) /, (GTG) / and (CAC) / were designed to microsatellites and all but one of the primers were attached to the 3’ end microsatellite by adding a selective base to the 3’ end. In both techniques, polymorphism could be detected in the presence or absence of PCR products. Approximately 30 bands can be displayed following a single PCR reaction. These markers are extremely polymorphic and are used to determine intraspecific kinship [5].

The IRAP technique shows additional polymorphism by completing the segments of DNA between 2 retrotransposons. With this feature, it has been used in many genetic diversity studies [31, 43, 44, 45]. In the study conducted by Tufan et al. (2020), Oryza sativa L. (rice), Brachypodium distachyon L. P. Beauv., Hordeum vulgare L., (barley) and Triticum aestivum L. (wheat) Hopi, Houba, Osr30 and RIRE1 transposons were investigated using the IRAP-PCR molecular marker technique. In these study, although it results different band profiles and polymorphism rates among individuals of each species; it has been stated that significant polymorphism is observed only among the rice types. According to these results, it shows that the 4 retrotransposon varieties used in the study are still active in the rice plant while they are inactive in other species [46].

The IRAP and REMAP markers have been used to determine the similarities between barley, rice, wheat, banana, olive and many different species [27]. In the study done by Haji (2019), Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like retrotransposon movements were investigated using
IRAP molecular marker technique on traditionally produced "Şencan 9" Solanum lycopersicum L. Varieties in Turkey. In that study, tomato plants were regenerated from seed tissue culture. Next, the first leaf or cotyledon was removed from the regenerated plants and cultured in MS medium supplemented with 2, 4-D hormone to induce callus formation. Polymorphism was determined for Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like retrotransposons. Conventionally grown tomatoes are highly polymorphic compared to organically grown ones. These results show that herbicide, insecticide and fungicide application can enhance the action of Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like retrotransposons in the tomato genome. Also, according to the results obtained, it is stated that tomato plant contributes to polymorphism in flowering stage compared to mature seedling and fruiting stage [47].

In the study by Yetgin (2019), she examined the movements of Houba, Osr30, RIRE1, Hopi, Sukkula and Nikita retrotransposons in Turkish upland rice and Bafra Yildiz rice cultivars grown under different boron concentrations using the IRAP molecular marker technique. Polymorphism rates were determined as 0-37%, 0-87%, 0-100%, 0-60%, 0-57% and 0-100% for Houba, Osr30, RIRE1, Hopi, Sukkula and Nikita, respectively. Fewer band formation was observed at low boron concentration. However, more bands were observed in Bafra Yildiz rice samples grown under high boron concentration application. These results showed that different boron concentrations of rice plant can increase retrotransposon movements in Turkish upland rice and Bafra Yildiz rice varieties. In addition, it is thought that the physiological changes observed in rice samples grown under different boron concentrations may be caused by these retrotransposon movements [48].

In the study by Abed (2019), the movements of Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like retrotransposons were examined using the IRAP-PCR molecular marker technique with samples taken from tuber and leaf tissues of 19 cultivars of potato (Solanum tuberosum L.). The polymorphism between the cultivars was determined using the Jaccard similarity coefficient formula. Polymorphism rates among varieties for Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like transposons in PCR products obtained from leaf samples were in the range of 0-20%, 0-92%, 0-100%, 0-83%, 0-60% and 0-88% respectively. Polymorphism rates between varieties for Sukkula, Nikita, P-Tst-1, P-Tst-3, P-Tst-6 and Copia like transposons in PCR products obtained from tuber samples were reported that in the range of 0-20%, 0-64%, 0%, of 0-63, 0-57%, 0-40% and 0-20% respectively [49].

Carpentier et al. (2019) conducted a study in which they classified the retrotransposon relationships with rice varieties by using 3000 genomes of rice (Indica, Japonica, Aus / Boro) which cultivated in the Asian continent. They stated that; they detected polymorphisms of more than 50,000 transposable elements in the study carried out using 32 retrotransposon families in 3000 rice genomes. Additionally, they stated that; 7 transposon elements revealed a high rate of polymorphism [50].

3- Future Prospects of Transposon Based Molecular Markers in Plant

In the light of this information, it is understood that there will be progress and development in transposon studies as new analysis tools will be developed [45]. As today’s limited numbers of genome studies completed will increases, the working principles of transposons and their effects on the gene and genome will be better understood. Because; there is an increasing evidence that transposable elements (TE) play a key role in regulating gene expression. In the future with a full-scale transcriptional profiling, whether TE’s will increase gene expression or not will be better understood.

Similarly, it remains to be revealed whether TE’s affect methylation of DNA in the genome or not. In a comprehensive study to be conducted, a conclusion can be reached by looking at the prevalence of each TE in different populations. Thus, the importance of elucidating epigenetic mechanisms will be better understood. Additionally today’s increase of complicated effects of stress factors on plants will cause new results. It is thought that these changes will also affect the active movements of
transposons. All these results show that the movements of transposons are the tools that need to be carefully analyzed in the future [51].

It is thought that the movement mechanisms of transposons at transcriptional, translational and insertional levels will be elucidated by adding or removing processes using the Crispir / cas method, which will have a direct effects on studies such as cancer, yield improvement and resistance to various stress factors. As a result, since arabidopsis thaliana, rice and tomato plants can be used as model plants, it can be said that they will lead to a clearer understanding of mechanisms action for transposons [52].

In recent studies, understanding the effect of transposons on yield and seed size requires understanding the effects of smaller-sized transposons, and it is understood that this will change depending on the development of omic and marker systems [53].

4- Conclusion

Generally, transposon elements have a great role as molecular markers to detect genetic variation among species. Genetical variation (polymorphism) can be created by transposable elements through insertional mutations, regulation of gene expression, deletion, duplication and inversion, the formation of new genomes and new functions. Therefore to study transposon movements several molecular markers had been developed and used in molecular biology. These molecular markers include inter-retro transposon amplified polymorphism (IRAP), retro-transposon microsatellite amplified polymorphism (REMAP), and retro-transposon based insertional polymorphism (RBIP).

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