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Development and Utilization of Transposable Element - Based Marker System for Groundnut Improvement

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Abstract

Transposable element (TE) based markers offer a useful genomic resource in groundnut because of their large number and high level of polymorphism. During the last five years, ~3,000 TE markers have been developed using transposon display and genome-wide sequence analysis. These markers were used for constructing the linkage maps and to identify the genomic regions governing various productivity traits and resistance to foliar diseases in groundnut. A few trait-specific markers have been useful in identifying the genes. In addition, the TE markers were used for genetic studies and understanding the genomic instability. This report reviews the development and utilization of TE markers in groundnut.

Keywords : Markers, marker-assisted breeding, QTL, transposable element

Groundnut (*Arachis hypogaea* L. 2n=4x=40) is a major food, oilseed and fodder crop world-wide with a global production of 45.22 mt from an area of 25.44 mha (Faostat, 2016). Groundnut is known as Poor Man's Nut due to its nutritive values in terms of protein (20-40%), carbohydrate (10-20%) and several other components such as vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium.

Breeding in groundnut mainly focusses on plant morphological traits (Kayam *et al.*, 2017), productivity traits (Faye *et al.*, 2015; Huang *et al.*, 2015 and Luo *et al.*, 2017), resistance to diseases and insects (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011; Shoba *et al.*, 2012; Sujay *et al.*, 2012; Shoba *et al.*, 2013; Kolekar *et al.*, 2016; Leal-Bertioli *et al.*, 2016; Zhou *et al.*, 2016b and Pandey *et al.*, 2017), tolerance to abiotic stress (Ravi *et al.*, 2011 and Gautami *et al.*, 2012) and nutritional quality traits (Sarvamangala *et al.*, 2011; Pandey *et al.*, 2014 and Mondal *et al.*, 2015). Several improved varieties have been developed with better phenotypes for these traits, and these improved varieties alone have contributed to 30 per cent yield increase in India since, 1967 (Reddy and Basu, 1989).

Currently the conventional breeding methods are complemented with the molecular breeding tools to enhance the overall efficiency (Varshney, 2016). But the use of the molecular breeding methods requires the identification of genomic regions and genes responsible for the important traits so that the plant selection can be exercised based on the genotype rather than the phenotype. This can be best achieved when appropriate genotypes are available, and a large number of molecular markers displaying high polymorphism are developed.

Though groundnut displays considerably high morphological variations for plant habit, seed color, and resistance to biotic and abiotic factors, the variation at DNA level has been very low (Kochert *et al.*, 1991; Halward *et al.*, 1992; Paik-Ro *et al.*, 1992). Low genetic polymorphism has been attributed to several bottlenecks (Mallikarjuna *et al.*, 2011) leading to narrow genetic base of groundnut (Kochert *et al.*, 1991), indicating neutral selection for genetic polymorphism, while intense selection for morphological traits (Gepts, 1993). This feature of groundnut demands a large number of DNA markers which can display very high polymorphism among the genotypes for genetic and genomic studies.

Considerable efforts have been made to develop and employ various marker systems in groundnut. They include RFLP (Halward *et al.*, 1991, 1992), DAF and AFLP (He and Prakash, 1997), SSR (Hopkins *et al.*, 1999; Palmieri *et al.*, 2002; He *et al.*, 2003; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2004; Moretzsohn *et al.*, 2005; Palmieri *et al.*, 2005 and Hong *et al.*,

2008). Subsequently, a large number of SSR markers were developed and /or used in groundnut (Zhang *et al.*, 2012; Huang *et al.*, 2016; Peng *et al.*, 2016; Zhou *et al.*, 2016a). CAPS markers were developed for detecting the mutations at *AhFAD2A* and *AhFAD2B* (Chu *et al.*, 2009). Recently, Diversity Array Technology (DArT) marker platform has also been developed for groundnut (Kilian, 2008). Currently, the advent of next-generation sequencing and genotyping technologies have enabled the detection of Single Nucleotide Polymorphisms (SNPs), which have emerged as the marker of choice in crop breeding (Varshney *et al.*, 2009). SNPs have been detected from diploids (Alves *et al.*, 2008 and Nagy *et al.*, 2012) as well as cultivated tetraploids (Wang *et al.*, 2013; Zhou *et al.*, 2014; Hong *et al.*, 2015; Shirasawa *et al.*, 2016 and Clevenger *et al.*, 2017).

When compared to afore-mentioned marker systems, *Arachis hypogaea* transposable element (AhTE) marker system has recorded the highest level of polymorphism in groundnut (Shirasawa *et al.*, 2012a), and also it has been user-friendly (Kolekar *et al.*, 2016). AhTE marker system was proposed (Bhat *et al.*, 2008; Gowda *et al.*, 2010 and Gowda *et al.*, 2011), and subsequently a large number of such markers was developed in groundnut (Shirasawa *et al.*, 2012a and Shirasawa *et al.*, 2012b). AhTE markers were successfully used to construct linkage maps (Shirasawa *et al.*, 2013 and Kolekar *et al.*, 2016), identify QTL for resistance to late leaf spot (LLS) and rust (Kolekar *et al.*, 2016) and marker-assisted backcrossing in groundnut. This review focuses on the development and utilization of AhTE markers in groundnut.

Development of AhTE Markers

Induced mutation study in groundnut identified a miniature inverted-repeat transposable elements (MITE, subsequently called *AhMITE1*), and demonstrated its role in the functional disruption of the fatty-acid desaturase-encoding gene *AhFAD2B* resulting into high oleic acid mutations (Patel *et al.*, 2004). Development of a marker based on *AhMITE1* was proposed (Bhat *et al.*, 2008) and that marker showed strong association with LLS resistance (Gowda *et al.*, 2010) and a possible role in the mutation and evolution of botanical types of groundnut (Gowda *et al.*, 2011). Later, Shirasawa *et al.* (2012a) developed 504 AhTE markers using *AhMITE1*-enriched libraries. The representative *AhMITE1* exhibited a mean length of 205.5 bp and a GC content of 30.1 per cent, with AT-rich, 9 bp target site duplications and 25 bp terminal inverted repeats. Shirasawa *et al.* (2012b) developed additional 535 AhTE markers using transposon-enriched libraries of other cultivars.

Current NGS methods allow generating the whole genome sequence with less cost and time, which can be used to identify novel markers. They provide an opportunity to capture all structural differences at DNA sequence between the genotypes when compared to other methods of marker discovery which use genomic sequences (Hong *et al.*, 2010), genomic libraries (Ferguson *et al.*, 2004), ESTs (Liang *et al.*, 2009 and Song *et al.*, 2010), RNA-Seq (Zhang *et al.*, 2012), transposon display (Shirasawa *et al.*, 2012a), SSR-enriched libraries (Moretzsohn *et al.*, 2005) etc. Also, the bioinformatics tools are now available to identify the genomic polymorphisms and to develop markers (Kang *et al.*, 2016).

Recently, the next generation sequencing reads obtained from whole genome re-sequencing of diverse genotypes were screened for *AhMITE1* insertion polymorphisms using polymorphic TEs and their movement detection (PTEMD) (Kang *et al.*, 2016) to identify 1,632 AhTE markers (Gayathri, 2017) of which 1,125 were new and 507 were already reported (Shirasawa *et al.*, 2012a and Shirasawa *et al.*, 2012b). Many sites allowed designing of the primers for amplification considering several parameters of primer designing. Thus, the primers could be designed for 1,079 sites. The expected PCR product ranged from 101-405 bp among the 1,079 AhTE markers, which was within the range that could be resolved on agarose gels. Unlike in the case of reported AhTE markers (Shirasawa *et al.*, 2012a and Shirasawa *et al.*, 2012b), the newly developed AhTE markers showed nearly equal number of AhTE markers between A and B sub-genome. B genome is known to have more number of transposons than A genome Bertioli *et al.* (2016), therefore, the *A. ipaensis* pseudomolecules were larger than their *A. duranensis* counter parts. Further, unequal distribution of the AhTE markers was

observed across the chromosomes of both the genomes. In rice, such a unequal distribution of CACTA and MITE, the two of the major classes of DNA transposons was observed (Kwon *et al.*, 2006).

The loci for these 1,079 markers represented genic and non-genic regions at almost equal proportions. However, the number within the genic region differed. MITE location within genic and non-genic region has been well documented in rice (Lu *et al.*, 2012). Irrespective of their location, MITEs influence gene expression directly or indirectly through small RNAs (Lu *et al.*, 2012), thereby alter the phenotypes. Using sample of 343 AhTE markers, validation was attempted using the same genotypes that were used for *in silico* marker identification. A large number of markers (82.22%) could be validated, indicating the efficiency of the pipeline used for marker development.

Utilization of AhTE Markers

Genetic and Genomic Studies

AhTE markers were used in groundnut for various purposes. Utility of AhTE markers for the genetic and genomic studies to enhance the groundnut productivity was checked using the Recombinant Inbred Line (RIL) and mutant populations (Hake and Bhat, 2017). Of the two alleles (with and without *AhMITE1* insertion) at each locus, the latter was more frequent (0.56) across 79 AhTE markers among the genotypes. Observed heterozygosity (H_O) was higher for intergenic markers when compared to genic AhTE markers. Similarly, the AhTE markers from the A genome showed higher H_O when compared to those from B genome. Mutant population in general showed the higher heterozygosity over the RIL population. AhTE markers displayed as high as 65.82 per cent polymorphism, based on which the genotypes could be classified into two groups. Thus, the study indicated the usefulness of the AhTE markers in the genetic and genomic studies in groundnut.

Similarly, the markers developed by Gayathri (2017) showed very high level of polymorphism. VL 1 and 110, which are the parents of a RIL mapping population, showed 35.5 per cent polymorphism. Similarly, the parents of two extensively used mapping populations (TG 26 × GPBD 4 and TAG 24 × GPBD 4) (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011; Sujay *et al.*, 2012 and Kolekar *et al.*, 2016) showed 22.3 and 16.7 per cent polymorphism, respectively. Also, a mapping population derived from TMV 2 and TMV 2-NLM, a mutant of TMV 2 showed 26.2 per cent polymorphism for parents. Parents of the backcross populations (TMV 2 × ICGV 86699 and TMV 2 × ICGV 99005) also showed high polymorphism (~22%). These AhTE markers can be used for enriching the linkage maps of VL 1 × 110, TG 26 × GPBD 4, TAG 24 × GPBD 4 and TMV 2 × TMV 2-NLM. Also, they can be employed for selecting the backcross lines in marker-assisted breeding.

Since, the AhTE markers showed very level of polymorphism, they were employed for mapping. Using 91 AhTE markers, a partial linkage map of 1,205.66 cM was constructed in the RIL mapping population (TMV 2 × TMV 2-NLM) in groundnut. The marker order was grossly comparable to the maps for SKF2 (Satonoka × Kintoki) and NYF2 (YI-0311 × Nakateyutaka), which were also constructed with AhTE markers (Shirasawa *et al.*, 2012b). The linkage map of TAG 24 × GPBD 4 was improved by adding 91 AhTE markers. Similarly, the map of TG 26 × GPBD 4 was improved by mapping 49 AhTE markers. A consensus map was developed using the markers common to the linkage maps of TAG 24 × GPBD 4 and TG 26 × GPBD 4, where the former map was used as the framework map. The consensus map carried 348 marker loci (239 SSRs and 109 AhTE) on 20 LGs with a total map distance of 1727.39 cM and average inter–marker distance of 4.96 cM.

QTL Mapping

QTL analysis in the RILs of TMV 2 × TMV 2-NLM detected seven major QTL regions for the important agronomic and productivity traits across the years (Hake, 2017). QTL region flanked between AhTE0357 and AhTE0050 on the LG A03 governed days to 50 per cent flowering (PVE 18.5-22.0%) and the number of secondary branches (PVE 10.8-28.4%). QTL region flanked by AhTE0391 and AhTE0572 on the LGA09 governed oleic acid (PVE 12.4-15.1%), linoleic acid, palmitic acid and O/L ratio. A candidate genomic region (39.5 cM long) on LG AhXV governed

LLS and rust resistance (maximum PVE of 44.5% and 53.7%, respectively). This region was mapped with four AhTE markers (AhTE0621, AhTE0360, AhTE0498 and AhTE0928) in addition to already mapped SSR markers (Kolekar, 2017).

Marker-assisted breeding

Marker-assisted breeding for improving resistance to LLS and rust is being attempted using elite varieties (JL 24, TMV 2, Dh 86 and ICGS 76) as the recurrent parent and various resistant genotypes as donor. A large number of backcross populations have been developed using the markers (AhTE and SSR) linked to LLS and rust resistance. Currently, such backcross lines are undergoing multi-location testing for variety development and commercial release.

Genomic instability

AhTE markers are useful in studying genetic instability. An unstable genotype (VL 6) (Gowda *et al.*, 1996) was checked for somatic mutations. From a single plant of VL 6, genomic DNA was isolated from five different leaves on different branches. They were tested for the differential activity of *AhMITE1* at 20 loci. Interestingly, differential activity of *AhMITE1* was observed at 2 loci (10%), indicating the somatic mutations. Such somatic mutations were not observed among the stable varieties of groundnut like GPBD 4, TMV 2, TAG 24 and TG 26 when checked at 110 loci. Genome-wide analysis for *AhMITE1*among the genetically unstable mutants showed both the gain and loss of copies of *AhMITE1*. A gain of 179 copies and a loss of 137 copies was observed for *AhMITE1*. No major differences were observed for the number of such sites across the chromosomes and also for the genic and intergenic sites. Some of the transpositional sites being the part of genic regions, *AhMITE1* activity co-segregated with the phenotypes of a few traits. For example, three independent LLS resistant mutants from VL 1 had *AhMITE1* insertion at *Aradu. R2B96* (*AraduV14167.a1*.M1) predicted on A10 chromosome. LLS susceptible spontaneous revertants were devoid of *AhMITE1* insertion at *Aradu. R2B96.* Detailed analysis showed that *AhMITE1* was inserted at 84 bp upstream of the gene *Aradu. R2B96* among the resistant genotypes (Hake, 2017).

Gene Identification

AhTE markers offer an advantage of identifying the gene(s) governing a trait since the transposable element acts as a DNA tag. In groundnut, AhTE0391 was associated with linoleic acid and oleic acid contents. AhTE0357 was associated with branching pattern, days to 50 per cent flowering, number of primary and secondary branches. Similarly, AhTE0025 was associated with test weight and pod width. The marker locus corresponding to AhTE0391 coincided with the gene *Aradu.7N61X*. The *AhMITE1* transposition site was at 2,129 bp of the second intronic region of *Aradu.7N61X*. Function prediction revealed that *Aradu.7N61X* codes for alpha glucosidase. At AhTE0357 locus, *AhMITE1* was inserted at 79 bp downstream region of the gene *Araip.TG1BL*. Functional prediction revealed that *Araip.TG1BL* codes for galactose oxidase/keltch, beta-propeller protein. At AhTE0025 locus, *AhMITE1* was inserted in the intronic region of *Aradu.7065G* on A07 chromosome coding for aldo/keto reductase family oxidoreductase (Hake, 2017).

Since, a large portion of the genome is made up of transposable elements, numerous TE markers can be developed in groundnut in future. Not much efforts have been made to develop retrotransposon markers in groundnut till date. Considering the polymorphism exhibited by these TE markers, it looks promising to develop TE markers in large numbers using genome-wide analysis. They can be used for genetic as well as genomics studies to find their association with the important traits. Insertion of TEs both in genic and intergenic regions is known to regulate gene expression. Therefore, TE markers can be useful in identifying marker-trait associations as well as identifying the gene(s).

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