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Molecular screening of groundnut cultivars with validated TE marker for rust resistance

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Abstract
Groundnut is one of the most important oilseed crops worldwide. Rust (*Puccinia arachidis* Speg.) is the major foliar fungal disease of groundnut which causes yield loss up to 57%. Besides traditional breeding, marker-assisted selection has been successfully initiated for the improvement of rust resistance in groundnut due to development of molecular markers, linkage maps and Quantitative Trait Loci (QTL) mapping. Trait specific markers are useful to hasten the selection of resistant genotype. In the present study, molecular screening of six groundnut genotypes (Phule Morna, Phule Unnati, Phule Warna, TPG 41, TAG 24 and GPBD 4) have been done with *Arachis hypogaea* Transposable Element (AhTE) based marker AhTE0498 which is closely linked to the QTL and validated for rust resistance. Allele for rust resistance was observed at high frequency among the four genotypes along with the resistant check GPBD 4. This trait specific marker can be employed in the marker-assisted breeding.

Keywords: Groundnut, rust, Ah TE marker, linkage map, marker-assisted breeding

Introduction
Groundnut (*Arachis hypogaea* L.) is a self-pollinating, indeterminate, annual and herbaceous legume. Worldwide, groundnut accounts at 6th position for edible oil (44-56%) production among the oilseed crops, 3rd for vegetable protein (22-30%) and 13th among the food crops, utilize as food, animal feed and in fertilizer industries. It also provides carbohydrates (10-25%), vitamins (E, K and B complex), minerals (Ca, P, Mg, Zn and Fe) and fibers. Groundnut is grown in more than hundred countries globally with a production of 40.31 million metric tons from an area of 24.75 million hectares during 2015-16 (Anonymous, 2016) [1].

Groundnut production is being affected by various biotic and abiotic factors. Among biotic stresses, fungal foliar diseases like rust, early and late leaf spots are the major constraints for groundnut production. Rust caused by *Puccinia arachidis* Speg. (Spegazzini, 1884) [21] is the primary cause for yield loss up to 57% (Subrahmanyam and McDonald, 1987) [22] with the adverse effect on grain and fodder. Rust disease damage plant by reducing the green leaf area available for photosynthesis and by stimulating leaflet abscission leading to extensive defoliation (McDonald *et al.,* 1985) [11] which results in lower seed quality, reduced seed size and oil content besides affecting the haulm production and quality. Though different agronomical, biological and chemical control measures are available for rust, it is better to develop resistant variety for sustainable agriculture. Better understanding of the genetics of disease resistance will enable breeders to design an efficient breeding strategy. Resistance to rust is complex and polygenic in nature which may be controlled by a few recessive genes (Paramasivam *et al.,* 1990) [15]. The resistance is predominantly controlled by additive, dominance and additive × additive and additive × dominance genetic effects (Reddy *et al.,* 1987; Varman *et al.,* 1991) [17, 25]. It is least progressive for the development of rust resistant genotypes in groundnut using traditional breeding methods due to narrow genetic base, ploidy level, linkage drag, selection based on phenotype etc. In contrast, molecular breeding is more advantageous than the traditional one with an accuracy in the selection for a desirable trait in a crop plant. Due to the advancement in the groundnut genomics along with the development of molecular markers, linkage maps and QTL mapping for rust resistance (Janila *et al.,* 2016) [7] ; marker-assisted selection is being successfully implemented for the selection and development of rust resistant genotypes in groundnut (Varshney *et al.,* 2014; Yeri and Bhat, 2016; Kolekar *et al.,* 2017) [26, 28, 17].

Two mapping populations, TAG 24 × GPBD 4 and TG 26 × GPBD 4 recombinant inbred lines (RILs) were deliberately studied for linkage map and QTL analysis for rust resistance

(Khedikar *et al.,* 2010; Sarvamangala *et al.,* 2011; Sujay *et al.,* 2012; Kolekar *et al.,* 2016; Pandey *et al.,* 2016) [8, 18, 23, 9, 14]. From these studies, few simple sequence repeat (SSR) markers (GM1536, GM1954, GM2301, GM2009, GM2079 and IPAHM103) and *Arachis hypogaea* Miniature Inverted-repeat Transposable Element (*AhMITE1*) based markers (AhTE0498 and AhTE0928) were shown strong association with rust resistance. Validation of these markers was done in different groundnut genotypes (Sukruth *et al.,* 2015, Kolekar *et al.,* 2016) [24, 9]. Mondal *et al.,* (2013) [12] also found strong association between AhTE0498 marker and rust resistance among the RILs of VG 9514 × TAG 24. These trait specific molecular markers are being utilized for the development of rust resistance in groundnut (Varshney *et al.,* 2014; Yeri and Bhat *et al.,* 2016; Kolekar *et al.,* 2017) [26, 28, 9]. In the present study, molecular screening of five groundnut cultivars (Phule Morna, Phule Unnati, Phule Warna, TAG 24 and TPG 41) along with GPBD 4, the national check for rust resistance was done with the AhTE0498 marker to check the presence of allele for the rust resistance.

Materials and Methods

The work was done at Vidya Pratishthan College of Agricultural Biotechnology, Baramati, Pune District, Maharashtra State, India. Seeds of five groundnut cultivars (Phule Morna, Phule Unnati, Phule Warna, TAG 24 and TPG 41) were collected from All India Co-ordinated Research Project, on Groundnut, M.P.K.V., Rahuri. Seeds of GPBD 4 cultivar were collected from University of Agricultural Sciences, Dharwad. GPBD 4 is used as a national check variety for resistance against foliar diseases, rust and late leaf spot. TAG 24 is a susceptible variety for rust disease. These six groundnut genotypes were grown in the field of Vidya Pratishthan College of Agricultural Biotechnology, Baramati.

Young leaves (100 mg) of groundnut genotypes were collected and genomic DNA was isolated by using the modified CTAB method (Cuc *et al.,* 2008) [3]. RNase A treatment is used for the removal of RNA from genomic DNA samples. DNA quantification was done with 0.8% agarose gel electrophoresis and Nanodrop spectrophotometer (Thermo Scientific™ NanoDrop 2000c, USA). Six groundnut genotypes (Phule Morna, Phule Unnati, Phule Warna, TAG 24, TPG 41 and GPBD 4) were screened with a molecular marker AhTE0498. PCR reaction was carried out for all six groundnut genotypes by using Eppendorf thermal cycler using primer pairs for the AhTE0498 marker. Primers were synthesized for marker AhTE0498_Forward 5' ATGACTTACATGTAGCAATTG 3' and AhTE0498_Reverse 5' TGAAAGGAGTCAAAGGTCATG 3' (Shirasawa *et al.,* 2012) [19]. Each reaction was carried out with 20 µl reaction volume containing 5 ng of genomic DNA, 10 pmol of each primer, 2 mM of dNTPs, 2 mM $MgCl_2$, 1 X amplification buffer and 1 U of Taq DNA polymerase. The thermal cycling conditions were as follows: 4 min initial denaturation at 94 °C; 35 cycles of 30 s denaturation at 94 °C, 30 s of annealing at 58 °C, 2 min extension at 72 °C; and a final 10 min extension at 72 °C. PCR amplified products were checked on the 2% agarose gel.

Results and Discussion

A quantity of genomic DNA was at good yield for all six groundnut genotypes. DNA concentration was in the range of 50-109 ng/µl with the negligible contamination of protein, RNA and other cell debris. Modified CTAB method was efficient for the isolation of genomic DNA from groundnut leaf tissues. Molecular marker AhTE0498 could amplify larger DNA fragment (342 bp) only from the rust susceptible genotype TAG 24 whereas the unique band of a fragment with short length (140 bp) was observed among other four genotypes (Phule Morna, Phule Unnati, Phule Warna and TPG 41) which was as similar as from the rust resistant genotype GPBD 4 (Figure 1).

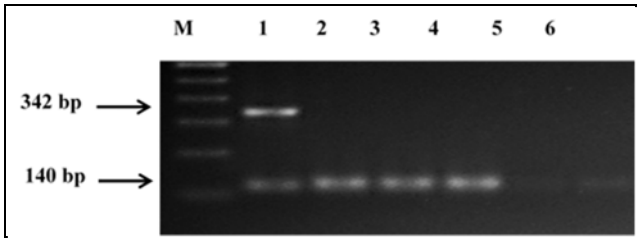

Fig 1: Molecular screening of groundnut cultivars with rustresistance linked marker AhTE0498 (M:100 bp ladder; 1: TAG 24; 2: GPBD 4; 3: Phule Morna; 4: Phule Unnati; 5: Phule Warna; 6: TPG 41)

AhTE markers are developed from non-autonomous Miniature inverted-repeat transposable elements (MITEs) (Shirasawa *et al.,* 2012) [19]. Here, the larger size of a PCR product will be the size of the MITE insertion with flanking sequences, i. e. ''full site'' and for "empty site" the size of the PCR product will be shorter because of lack of MITE insertion and only with the flanking sequences. AhTE markers are dominant and/or co-dominant in nature with high level of polymorphism which have been successfully used to study genetic diversity (Wang *et al.,* 2013) [27] and mapping in groundnut (Shirasawa *et al.,* 2013, Kolekar *et al.,* 2016) [20, 9]. From TAG 24 × GPBD 4 RILs population in groundnut, AhTE0498 marker was found to be closely linked to the QTL for rust resistance with highest Phenotypic Variance Explained (PVE) value of 70.4% and also validation study in the RILs of TG 26 × GPBD 4 population could identified strongest association (R^2 of 49.4-52.3%) between rust and AhTE0498 (Kolekar *et al.,* 2016) [9]. Mondal *et al.* (2013) [12] also observed a strong association between AhTE0498 and rust resistance gene from the RILs of VG 9514 × TAG 24. Phule Morna, Phule Unnati, Phule Warna and TPG 41 possess allele for rust resistance at the locus AhTE0498. These genotypes can be used as a donor for rust resistance in the groundnut breeding programme.

MITEs are considered among the major factors for eukaryotic evolution (Naito *et al.,* 2009) [13]. MITEs possess transposition activity into the genic region or in the near vicinity of genic region from its site of origin which may affect the gene expression (Feschotte *et al.,* 2002) [4]. It was found that transposable elements contributed 61.7% and 68.5% to the groundnut genomes of diploid species *A. duranensis* and *A. ipaensis*, respectively (Bertioli *et al.,* 2016) [2]. Induced mutation study in the groundnut emerged out with the identification of non-autonomous class II type miniature inverted repeat transposable element (MITE) and its role in the functional disruption of the fatty-acid desaturase-encoding gene ahFAD2B (Patel *et al.,* 2004) [16]. Association of a *AhMITE1*-specific marker with LLS resistance, role of *AhMITE* in the mutation and evolution of botanical types of groundnut was thoroughly studied (Gowda *et al.,* 2010; Gowda *et al.,* 2011) [5, 6].

Conclusion

Trait-specific AhTE markers have the advantage over the other molecular markers with respect to their reliability and

use in the marker-assisted breeding for the reason that their PCR products differ by 205 bp, which can easily be resolved on 2% agarose gel. These markers can be employed for marker-assisted breeding.

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