

**ASSOCIATION ANALYSIS FOR YIELD RELATED TRAITS
AND FOLIAR DISEASE RESISTANCE USING
TRANSPOSON SPECIFIC MARKERS IN A MUTANT
POPULATION OF GROUNDNUT**

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INTRODUCION

Groundnut is one of the most important cultivated grain legumes in the world. It is mostly cultivated in tropical and sub-tropical countries located between 40° N to 50° S with worldwide production of 24.1mt from an area of 37.7 mha with an average productivity of 1564 kg/ha (FAO, 2010). In India, it is grown over an area of 5.31 mha with a production and productivity of 6.93 mt and 1305 kg/ha, respectively (IndiaStat, 2013).

Due to its high oil and protein content, groundnut is considered as one of the most nourishing foods, and hence popularly known as poor man's almond. It supplies about 5.6 calories per grain when consumed raw and 5.8 calories per grain when consumed in roasted form. It is a rich source of essential amino acids, minerals, and vitamins and has good digestibility in both raw and roasted forms (Nagaraj, 1988).

Cultivated groundnut (*Arachis hypogaea*) is a tetraploid with an AB-genome ($2n=4x=40$) of recent origin, arising from hybridization of two wild species and spontaneous chromosome duplication. Comparisons of the karyotypes of diploid wild species with *A. hypogaea*, together with molecular data and phylogeographic considerations suggest that *A. duranensis* and *A. ipaensis* (both $2n=2x=20$) are the donors of A and B genome, respectively (Kochert *et al.*, 1996; Seijo *et al.*, 2004; Seijo *et al.*, 2007; Burow *et al.*, 2009; Moretzsohn *et al.*, 2013). A and B genomes got diverged about 3.5 million years ago, while *A. hypogaea* originated about 3500 years ago (Singh and Simpson, 1994). Homoeologous A- and B-chromosomes rarely pair during meiosis (Smartt, 1990), thus the peanut genome can be characterized as being genetically diploid.

Based on several morphological differences cultivated groundnut is divided into two subspecies viz., *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata*. Subspecies *hypogaea* is characterized by alternate branching, absence of flowers on main axis, long duration (120-160 days) and presence of seed dormancy, while ssp. *fastigiata* is recognized by sequential branching, presence of flowers on main axis, short duration (85-130 days) and lack of seed dormancy. Based on the morphological differences, the two subspecies are subdivided into botanical varieties. Subspecies *hypogaea* is divided into var. *hypogaea* (Virginia bunch/runner) and var. *hirsuta* (Peruvian runner), whereas subsp. *fastigiata* is classified into var. *fastigiata* (Valencia), var. *vulgaris* (Spanish bunch), var. *aequatoria* and var. *peruviana* (Krapovickas, 1969, 1973).

The two subspecies and six botanical varieties evolved due to adaptation and selection by the cultivators. Also, mutations have played a significant role in the intraspecific differentiation and evolution of groundnut (Singh, 1988). The possible role of mutations in the evolution of abundant morphological variation was evident from various subspecific changes brought by induced mutations in breeding programmes (Mouli *et al.*, 1979; Prasad, 1989; Gowda *et al.*, 1996). Earlier breeding programmes have also observed the role of genetic instability in the creation of variability (Norden, 1973). Such mutations and genetic instabilities were difficult to explain with conventional mutation theory as they revealed high frequency of mutations (Patil, 1966), reversions, nonrandom mutations, segregation distortions, unstable variants in advanced generations, somatic mutations (Nevers *et al.*, 1986), multiple character mutations (Ashri, 1972), multiple alternate forms (Lim, 1979), homozygous mutations (Larkin *et al.*, 1984; Qingshu *et al.*, 1994) and mutation outbursts (Green, 1988). These unusual features of mutations indicate the activation of cryptic transposable elements (TEs) as the possible cause of mutations (Gowda *et al.*, 1996).

Observation that insertion of a miniature inverted-repeat transposable element (MITE), a class II non-autonomous TE in the *AhFAD2B* gene encoding oleoyl-PC desaturase upon exposure to diethyl sulphate (DES) led to two independent high oleate mutations indicated the role of TEs in groundnut mutations (Patel *et al.*, 2004). Subsequently, a MITE from groundnut (*AhMITE1*) was shown to be involved in the origin of late leaf spot resistant mutants (Bhat *et al.*, 2008; Gowda *et al.*, 2010) and implicated in the mutational and evolutionary origin of botanical types in groundnut (Gowda *et al.*, 2011).

MITEs are widely distributed in groundnut (Shirasawa *et al.*, 2012a) like in many animal and plant genome. MITEs have the potential role in gene regulation, defining chromatin domains, genome expansion, genome organization and genome evolution (Ma and Bennetzen, 2004). Also, with the observation that the pattern of MITE insertion varies across individuals and germplasm, they were employed to develop markers (Bonin *et al.*, 2008; Grzebelus *et al.*, 2009; Monden *et al.*, 2009). Recently, 504 *AhMITE1*-specific markers were developed (Shirasawa *et al.*, 2012a) where the PCR was performed using primer pairs designed against both the flanking sequences of *AhMITE1* at each

site. These markers showed higher polymorphism (22.0%) than that of the SSR markers (Koilkonda *et al.*, 2012; Pandey *et al.*, 2012). Later through *in silico* analysis, another 535 transposon markers were developed and validated (Shirasawa *et al.*, 2012b). Transposon markers, like SSR markers, represent potent, co-dominant, and PCR-based markers. They basically detect the transposition of *AhMITE1* at various sites, preferably in the genic region in the genome. Hence TE based markers not only help in working out the polymorphism, but also provide a unique opportunity of detecting specific mutations involving transpositions.

BARC, Mumbai has developed and characterized several mutants by physical mutagenesis, and the mutants vary for various agronomical and productivity traits, in addition to a few morphological traits (Badigannavar *et al.*, 2002). An in-depth analysis of phenotypic data produced on these mutants and their parents, and genotypic data with transposon markers should offer identification of trait-specific markers for improving groundnut for agronomic and productivity traits and disease resistance. Also, these markers being transposon specific, can provide a basis to explain the type of mutations and the genes involved. Therefore, the present investigation was undertaken with the following objectives.

1. To phenotype the groundnut mutants and their parents for morphological and agronomic traits.
2. To genotype the mutants and their parents using the transposon-specific markers.
3. To identify markers that are specifically and significantly associated with morphological and agronomic traits.

REVIEW OF LITERATURE

Cultivated groundnut is a very important legume crop, valued both for subsistence and as a cash crop worldwide. However, it suffers from several biotic and abiotic stresses. Groundnut improvement for productivity traits and resistance to stresses can be accelerated by employing genomic resources, especially the markers. Analysis of a mutant population consisting of a large number of mutants and parents for various traits and transposon-specific markers provides a great scope for identifying trait-specific markers which can be used for groundnut breeding. This chapter presents a review on the literature related to

- 2.1 Origin and domestication of groundnut
- 2.2 Role of mutation in groundnut evolution
- 2.3 Induced mutagenesis in groundnut
- 2.4 Role of transposons in genetic instability and mutations
- 2.5 Transposons and transposon markers
- 2.6 Molecular analysis of mutants and identification of trait specific marker

2.1 Origin and domestication of groundnut

The genus *Arachis* includes 80 species out of which 69 are described by Krapovickas and Gregory (1994) and 11 by Valls and Simpson (2005). Also, around 10 more have been collected in the last decade but need to be described (Bertioli *et al.*, 2011). Genus *Arachis* is taxonomically grouped into nine sections: *Trierectoides*, *Erectoides*, *Procumbentes*, *Rhizomatosae*, *Heteranthae*, *Caulorrhizae*, *Extranervosae*, *Triseminatae* and *Arachis* (Fernandez and Krapovickas, 1994; Krapovickas and Gregory, 1994; Valls and Simpson, 2005; Lavia *et al.*, 2009). Section *Arachis* is considered to be the most diverse, harbouring both annual and perennial species with different chromosome numbers, ploidy levels and karyotypes. Some of the members of sections *Rhizomatosae*, *Heteranthae* and *Caulorrhizae* may produce hybrids with the most derived sections (including *Arachis*), but others show a strong genetic isolation.

Section *Arachis* includes 31 diploid species that show variable degrees of affinity to the complementary genomes of *A. hypogaea* and *A. monticola*, the two tetraploid species which also come under the same section. Strong evidences indicate a very close genomic relationship between cultivated groundnut and *A. monticola*, and the latter has been considered as the immediate wild ancestor of *A. hypogaea*.

A. duranensis (Krapov. & W.C. Greg.) and *A. ipaensis* (Krapov. & W.C. Greg.) belonging to section *Arachis* have been considered as the donors of A and B genome, respectively towards the origin of *A. hypogaea* (Kochert *et al.*, 1996). The phylogenetic analysis based on intron sequences and microsatellite markers (Moretzsohn *et al.*, 2013). And the physical mapping of the 5S and 18S–25S rRNA genes by FISH (Seijo *et al.*, 2004) provided evidence for this hypothesis. The evolutionary divergence of these two diploid species is estimated at about 3.5 million years ago, more recent than the divergence of the two genome components of the allotetraploid crops like cotton and soybean, estimated at about 5–7 and 13 million years ago, respectively (Nielen *et al.*, 2012).

According to the current species distribution and the variability studies of the landraces, Southern Bolivia and north-western Argentina has been the center of origin of groundnut (Krapovickas and Gregory, 1994). Archaeological studies indicated the presence of *A. hypogaea* in the Huarmey Valley, near the Peruvian coast, around 5000 year before (Bonavia, 1982).

Groundnut is an allotetraploid of recent origin with an AABB genome of about 2.8 GB consisting of considerably high repetitive regions. Smartt and Gregory (1967) concluded that *A. hypogaea* has A and B genomes which have been contributed by two diploid species. Kochert *et al.* (1996) supported this by restriction fragment length polymorphism (RFLP) analysis. In their results, most gel lanes had two bands in *A. hypogaea* and only one band in diploids species. Husted (1933, 1936) observed the presence of two quite distinct chromosome pairs in the cultivated peanut. Pair A was much smaller than the remaining chromosomes and had different staining behaviour, while pair B had a secondary constriction.

On the basis of several morphological differences species *Arachis* grouped into two subspecies *fastigiata* and *hypogaea*. Subspecies *hypogaea* is characterized by alternate branching, absence of

flowers on main axis, long duration (120-160 days) and presence of seed dormancy, while *fastigiata* is recognized by sequential branching, presence of flowers on main axis, short duration (85-130 days) and lack of seed dormancy. Based on the morphological differences, the two subspecies are subdivided into botanical varieties. Subspecies *hypogaea* is divided into var. *hypogaea* (Virginia bunch/runner) and var. *hirsuta* (Peruvian runner), whereas subsp. *fastigiata* is classified into var. *fastigiata* (Valencia), var. *vulgaris* (Spanish bunch), var. *aequatoriana* and var. *peruviana* (Krapovickas, 1969, 1973).

Analysis of non-coding chloroplast DNA regions and the non-transcribed spacer of the nuclear 5S rDNA of the six botanical varieties of the two subspecies, wild tetraploid *A. monticola* and a few diploid species (so far proposed as the most probable relatives of the peanut) showed complete homology in the sequences of all the peanut and *A. monticola* samples (Grabiele *et al.*, 2012). The results strongly suggested that the six botanical varieties have a single genetic origin and that *A. monticola* should be regarded as the immediate tetraploid ancestor from which *A. hypogaea* has arisen upon domestication. The study provided the sequence-based evidence for *A. duranensis* and *A. ipaensis* being the maternal and paternal wild diploid species of the AABB tetraploids, respectively (Grabiele *et al.*, 2012).

2.2 Role of mutation in groundnut evolution

Considerable morphological variability existing among the subspecies and botanical varieties were attributed to differences in a small number of genes (Wynne and Coffelt, 1982). Such a genetic control of the differentiation in groundnut led to a hypothesis that mutations were involved in the evolution of subspecies. Based on the observation that the two diploid species (*A. duranensis* and *A. batozicoi*) morphologically resemble *A. hypogaea* ssp. *fastigiata* (Valencia), the ssp. *hypogaea* was thought to have evolved subsequently through a mutation in ssp. *Fastigiata* (Singh, 1988). It was also supported by the fact that most of the diploid wild species are sequential in branching (like ssp. *fastigiata*). However, the contrasting theory proposed that ssp. *hypogaea* is more like the original wild form, and ssp. *fastigiata* is a more recent form that was derived from ssp. *hypogaea* progenitors (Krapovickas, 1969).

The possible role of spontaneous mutations in the evolution of groundnut was also evident from the various subspecific changes brought by induced mutations in breeding programmes (Mouli *et al.*, 1979; Prasad, 1989). Appearance of different botanical forms from a single genotype, Dharwad Early Runner (DER) which resembles *A. monticola* in morphology and further confirmed the importance of mutations in creating diversity and the differentiation and evolution of groundnut (Gowda *et al.*, 1996).

Various subspecies level changes have been observed among induced mutations (Perry, 1968). Origin of Virginia type mutant from a Spanish parent was observed but no sequential types were found among the vast M₂ populations of Virginia parents (Prasad *et al.*, 1984), which also supported the view that ssp. *hypogaea* originated from ssp. *fastigiata* through a mutation. These observations based on induced mutations (Mouli *et al.*, 1979; Mouli and Kale, 1982; Prasad *et al.*, 1984; Mouli *et al.*, 1986, 1989; Prasad, 1989; Mathur *et al.*, 1999; Mathur *et al.*, 2001) suggest that spontaneous mutations resulting in changes at a few loci might have contributed to the evolution of abundant morphological variation in groundnut.

2.3 Induced mutagenesis in groundnut

Groundnut has been exposed extensively to mutagenic treatments for induction of variability. The crop is suited for mutagenesis for several reasons: it is practically a self-pollinated species: the embryos are easily exposed to mutagens and have 6-8 primordia with buds in their axils making several potential targets available (Ashri, 1976). Physical mutagens such as, X ray (Patil, 1966), gamma ray (Lodha *et al.*, 1985), β -rays (Jiang and Zhou, 1987) and fast neutrons (Shivaraj and Rao, 1962) and chemical mutagens like, ethyl methane sulphonate (EMS), ethidium bromide, acryflavine (Levy, 1976), diethyl methane sulphonate (DES), N-nitroso-N-methyl-urea, Nethyl-N-nitroso-urea, ethylene imine (Prasad *et al.*, 1984) and sodium azide (Venkatachalam and Jayabalan, 1997) have been used to create genetic variability in groundnut. However, most popular method employed for creating genetic variability in groundnut is through gamma and EMS mutagenesis (Reddy *et al.*, 1977). Induced variability has been obtained for various traits including those governed by polygenes (Gregory, 1968).

2.4 Role of transposons in genetic instability and mutations

Genetic instability has been observed in a number of groundnut breeding programmes (Norden, 1973). In Africa, the wide genetic diversity representing different subspecies and botanical types probably originated from a single source mainly consisting of *fastigiata* types (Gibbons *et al.*, 1972). In northern Rhodesia, a true breeding strain with self-colored red testa was selected from Mani Pintar, an unusual variety possessing variegated red and white testa (Smartt, 1960). The variant showed occasional reversions to the parental type and the reversible change was ascribed to the chromosomal rearrangements. Apart from these reports of genetic instabilities, several studies indicated unusual features which could not be explained through conventional mutation theory. The features included high frequency of mutations and reversions, nonrandom mutations, segregation distortions, unstable variants in advanced generations, somatic mutations (Nevers *et al.*, 1986), multiple character mutations, multiple alternate forms (Lim, 1979), homozygous mutations (Larkin *et al.*, 1984), mutation outbursts (Green, 1988), high frequency of multiple mutations (Ashri, 1972), homozygous mutations (Qingshu *et al.*, 1994) and a large number of mutants from a single aberrant XI cytological variant (Patil, 1966).

These unusual features of mutation and genetic instabilities indicated the activation of cryptic transposable elements (TEs) as the possible cause of mutations. Various stresses to the genome are known to activate the cryptic TEs (McClintock, 1978; Wessler, 1988). TEs have been indicated as the cause of genetic instability in a number of crop species (Benjamin, 1981). TEs, which are widely known to cause insertion mutations and chromosomal rearrangements are the most often evoked macro evolutionary agents in the organismic evolution (Charlesworth *et al.*, 1994). They are considered as the possible motors of the evolutionary changes in *A. hypogaea*.

Patel *et al.* (2004) reported insertion of a transposon (MITE) in the *ahFAD2B* gene in a chemically induced mutant. The mutant had a frame shift mutation resulting in a putatively truncated protein which resulted in high-oleate phenotype. Further investigations on the same groundnut MITE (*AhMITE1*) showed a strong association between its transposition and the high-frequency origin of late leaf spot (LLS)-resistant mutants (Gowda *et al.*, 2011). Analysis of South American landraces revealed the differential activity of *AhMITE1* between the genotypes belonging to spp. *fastigiata* and spp. *hypogaea*. Four accessions of the primitive allotetraploid, *A. monticola* were devoid of *AhMITE1* at a pre-determined site, indicating only recent activation of the element, possibly because of the “genomic shock” resulting from hybridization followed by allopolyploidization (Gowda *et al.*, 2011).

2.5 Transposons and transposon markers

Transposons are discrete segments of DNA that is distinguished by their ability to move and replicate within the genome and have been found to be ubiquitous in most living organisms. Based on the transposition mechanism, two classes of transposable elements are recognized. Class I type elements transpose via RNA and cDNA, whereas class II type elements transpose through DNA (Kumar and Bennetzen, 1999; Bennetzen, 2000). Transposable elements in each class can be subdivided as autonomous and non-autonomous. The former produces transposase enzyme required for transposition and the latter does not (Bennetzen, 2000).

Plant transposons are extremely powerful mutagens, inserting into gene and resulting into chromosomal inversion, duplication and deletions (Chénais *et al.*, 2012). This feature of the TEs has made them extremely useful tools to gain genetic understanding of plant genome (Nielen *et al.*, 2010; Lu *et al.*, 2012; Nielen *et al.*, 2012). Britten and Davidson (1969) hypothesized that novel gene structure and function is regulated by repetitive fraction of the genome, which was controversial earlier, but now it is accepted that some TEs play a role as important regulators of gene expression leading to alterations in the developmental patterns (Lu *et al.*, 2012; Yu *et al.*, 2013).

Miniature inverted-repeat transposable elements (MITEs) are the non-autonomous elements belonging to class II TEs. They are found in the genomes of plants and animals. Probably they are derived from the large element by an internal deletion (Wessler *et al.*, 1995) thus lacking transposase-coding ability, but retaining both insertion and excision property (Jiang *et al.*, 2004). They have very high copy number in a genome, which can range from 3,000 to 10,000. MITEs are structurally characterized by relatively small (generally 80-500 bp) size, AT rich and non-coding interspersed elements with a terminal inverted repeat of ~25 bp. They have insertion preference for genic region (mostly AT rich).

The genome-wide examination of MITE-derived sequences could elucidate the contribution of MITEs to gene evolution in rice (Lu *et al.*, 2012). MITEs have contributed to the emergence of novel genes (Oki *et al.*, 2008). Approximately 6000 MITE- derived sequences were found in gene regions (exons and introns) and 67,000 in intergenic regions in rice. In gene regions, most MITEs were located in introns rather than exons. For over 300 protein-coding genes, coding sequences, transcription start sites, and splicing sites overlap with MITEs. These sequence alterations via MITE insertions potentially affect the biological functions of gene products (Lu *et al.*, 2012). MITEs that were closely associated with gene sequences (Wessler, 1998; Mao *et al.*, 2000; Feng *et al.*, 2002; Santiago *et al.*, 2002; Wright *et al.*, 2003; Oki *et al.*, 2008) were frequently transcribed along with the plant genes (Oki *et al.*, 2008; Kuang *et al.*, 2009).

In groundnut, the activity of MITE in causing mutations was reported by Patel *et al.* (2004). Further, the same MITE (*AhMITE1*) was shown to be activated by environmental or induced stresses such as ethyl methane sulfonate (EMS), gamma irradiation, environmental condition and tissue culture (Gowda *et al.*, 2011). Southern blot analysis revealed multiple *AhMITE1* copies in the genomes of *A. magna* (BB), *A. monticola* (AABB) and *A. hypogaea* (AABB), but not in the genome of *A. duranensis* (AA). This result suggested that *AhMITE1* elements had amplified in diploid species of the B genome and then transferred to tetraploid species. The tetraploid *A. hypogaea* was generated by hybridization between A and B diploids. Since differences in *AhMITE1* insertion positions were observed not only among the four *Arachis* species but also between the four *A. hypogaea* lines, it is likely that *AhMITE1* transposition occurred both before and after the generation of *A. hypogaea*. These MITE elements exhibited a mean length of 205.5 bp and a GC content of 30.1%, with AT-rich, 9 bp target site duplications and 25 bp terminal inverted repeats.

Examination of the repetitive component of groundnut genome revealed the presence of several types of TEs. A substantial proportion of the highly repetitive component of the peanut A genome appears to be accounted for by relatively few LTR retrotransposons and their truncated copies or solo LTRs. The most abundant of the retrotransposons are non-autonomous. The activity of these retrotransposons has been a very significant driver of genome evolution since the evolutionary divergence of the A and B genomes (Bertioli *et al.*, 2013).

Recently, *FIDEL* and *Matita* retroelements have been identified in groundnut (Nielen *et al.*, 2010; Nielen *et al.*, 2012). It was demonstrated that *Matita* is not randomly distributed in the genome but exhibits a significant tendency of being more abundant near resistance gene homologues than near single-copy genes (Ratnaparkhe *et al.*, 2011). The peanut allergen genes are also interspersed with low-copy genes and transposable elements (Ratnaparkhe, 2012).

With the observation that the pattern of MITE insertion varies among germplasms or individuals, TEs were employed to develop markers (Bonin *et al.*, 2008; Grzebelus *et al.*, 2009; Monden *et al.*, 2009). In groundnut, a PCR was developed (Gowda *et al.*, 2010) to detect the presence or absence of *AhMITE1* at a pre-determined site in the genome (Bhat *et al.*, 2008). For this PCR, a forward primer (5' GGGAGAAGAAAAGGATGAGA 3') was designed based on the *AhMITE1* flanking sequence tag (FST1) recovered from TMV 2 genotype of groundnut (Bhat *et al.*, 2008), and the reverse primer (5' TCTCATGAAGATGCTTTGGT 3') was specific to *AhMITE1* (Patel *et al.*, 2004). Amplification of a 242 base pair (bp) product indicated the presence of *AhMITE1* at FST1-linked site in the genome.

Subsequently, Shirasawa *et al.*, (2012a) identified 504 sites within groundnut genome that had MITE insertion. Based on the flanking sequences at 504 sites, primers were designed in an attempt to convert them as markers. PCR analysis detected 13.4% and 30.6% polymorphism among Virginia lines and between Virginia and Spanish types, respectively. Compared to the polymorphism obtained with SSR markers, MITE-specific markers displayed very high level of polymorphism. Further, *in silico* polymorphism analysis could increase the efficiency of development of transposon markers (Shirasawa *et al.*, 2012b). A total of 535 such markers were developed, which also showed very high level of polymorphism.

2.6 Molecular analysis of mutants and identification of trait specific marker

In groundnut, only a few studies embarked on using the markers to detect the variability and find trait-marker association using mutant population (Bhagwat *et al.*, 1997) used RAPD markers to study the variability among 12 mutants obtained from Spanish Improved. Another study with 21 mutants belonging to different botanical types showed limited molecular diversity when 27 RAPD markers were

employed (Varshakumari *et al.*, 2009). However, these studies could not detect any trait-specific marker.

Trait-specific markers are tightly linked markers to economically and agronomically important traits. Trait-marker mapping can be done through linkage/association mapping. Association mapping is a powerful approach to search for genotype (marker)–phenotype (trait) correlations within a diverse collection of germplasm or breeding materials (Myles *et al.*, 2009). Markers that have statistically significant association with any trait can be used in marker assisted selection (MAS), where a trait of interest is selected, not based on the trait itself, but on a marker linked to it (Ribaut *et al.*, 2001). Marker loci that are tightly linked to major genes can be used for selection and are sometimes more efficient than direct selection for the target gene (Van *et al.*, 2011).

In groundnut, very limited studies on marker–trait associations have been made. Using the peanut minicore which is a subset of samples representing the diversity in the U.S. peanut germplasm collection, the functional SNP from the *FAD2A* gene was identified as significantly associated with oleic acid (C18:1), linoleic acid (C18:2), and oleic-to-linoleic (O/L) ratio (Wang *et al.*, 2011). Association study between SSR markers and agronomic traits like pod width, seed length, 100-pod weight and the 100-seed weight was conducted using Chinese peanut minicore (Jiang *et al.*, 2013). Availability of large number of mutants differing for morphological and productivity traits can also make a useful mutant population, and when used along with a large number of transposon markers, might not only allow identifying trait specific marker, but also enable unravelling the basis of mutation leading to novel phenotypes.

MATERIAL AND METHODS

Identification of molecular markers and genes for specific morphological and agronomical traits would assist in molecular breeding. Since transposons especially MITEs have preference for genic region and their transpositions are frequently associated with groundnut mutations, markers detecting such transpositions play offer a great scope in identifying the trait-specific markers. Markers governing important traits can be identified by single marker analysis. Further, the markers showing significant association can be checked across several independent mutants with common mutant phenotype to find out in how many cases they are polymorphic between the mutant and parent. Also, if the polymorphism involves common allelic pattern across the mutants, then the marker provides substantial confidence. Material and methods used in the field evaluation of the mutants and their genotyping with transposon markers to identify the trait-specific markers are described in this chapter.

3.1 Mutant population

The study employed a mutant population consisting of thirty five mutants and ten parents (Table 1) obtained from BARC, Mumbai. Although, out of all mutants used in the study some are primary mutants and some are secondary and tertiary mutants derived from primary and secondary mutants, respectively, interrelationship between parents and mutants derived from them was given in Fig. 1. These stabilised mutants were developed by mutagenesis using gamma and X rays.

3.2 Experimental site

The field experiment was conducted during *kharif* 2012 at IABT Garden of Main Agricultural Research Station, UAS, Dharwad belonging to transitional tract of Karnataka with 15° 13' north latitude, 75°07' east longitude and an altitude of 678 m above mean sea level. The experimental block consisted of vertisol soil with pH of 7.0-7.5.

3.3 Experimental layout

The mutants and parents of the mutant population were sown on 3rd July 2012 in a Randomized Complete Block Design (RCBD) with a spacing of 45 cm between rows and 10 cm between plants in two replications. Standard package of practice was followed to raise the crop. The crop was harvested on 14th October 2012.

3.3 Experimental layout

The mutants and parents of the mutant population were sown on 3rd July 2012 in a Randomized Complete Block Design (RCBD) with a spacing of 45 cm between rows and 10 cm between plants in two replications. Standard package of practice was followed to raise the crop. The crop was harvested on 14th October 2012.

3.4 Observations

Mutants and their parents were evaluated for growth, morphological, productivity and nutritional traits, and their reaction to late leaf spot and rust diseases. Most of the field observations were recorded on five randomly selected plants as per the groundnut descriptor (IBPGR/ICRISAT, 1992) and the mean value was computed for each genotype.

3.4.1 Growth characters

- 1) Main stem length (cm)

The height of the plant was measured in centimeters (cm) from the ground level to the tip of the main stem at the time of harvest.

- 2) Number of primary branches

The number of n+1 branches on the main stem was counted at the time of harvest.

- 3) Number of secondary branches

Number of branches borne on primary branches was counted.

- 4) Length of primary branches (cm)

Table 1: Parents and induced groundnut mutants used in the study

Sl. No.	Parent	Mutant	Mutagen
1	Spanish Improved	TG 1	X ray
		TG 2	X ray
		TGM 8	X ray
		TGM 14	X ray
		TGM 50	X ray
		TGM 51	X ray
		TGM 58	X ray
2	TG 2	TGM 19	γ ray
3	TAG 24	TGM 46	Spontaneous (natural) mutant
		TGM 96	γ ray
		TGM 101	γ ray
		TGM 111	γ ray
		TGM 112	γ ray
		TGM 113	γ ray
		TGM 117	γ ray
		TGM 119	γ ray
		TGM 138	γ ray
		TGM 156	γ ray
4	TGM 8	TGM 43	Spontaneous (natural) mutant
5	TG 9	TGM 36	γ ray
6	TG 16	TGM 44	Spontaneous (natural) mutant
7	TG 18 A	TGM 20	γ ray
		TGM 37	γ ray
		TGM 54	γ ray
8	Somnath	TGM 47	Spontaneous (natural) mutant
9	TG 66	TGM 165	γ ray
		TGM 167	γ ray + sodium azide
		TGM 178	γ ray
		TGM 189	γ ray
		TGM 193	γ ray
		TGM 196	γ ray
		TGM 198	γ ray
		TGM 213	γ ray
		TGM 214	γ ray
		TGM 215	γ ray
		TGM 218	γ ray
10	TG 51	TGM 223	γ ray
		TGM 225	γ ray

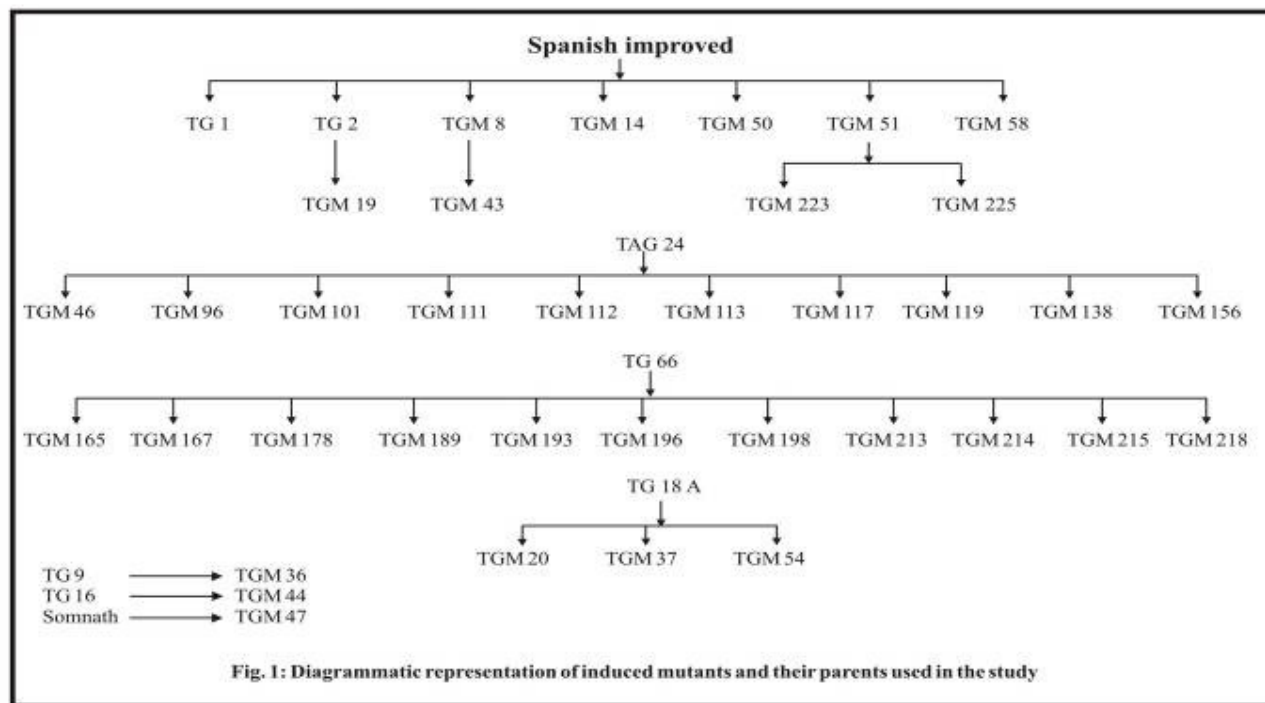


Fig. 1: Diagrammatic representation of induced mutants and their parents used in the study

The length of primary branches was measured in centimeters (cm) from the main stem to the tip of the branch at the time of harvest.

5) Leaf length (mm)

The length of leaf was measured on the third leaf, apical leaflet of the main stem in centimeters (cm) when fully expanded.

6) Leaf width (mm)

The width of leaf was measured on the third leaf, fully expanded apical leaflet on the main stem in centimeters (cm) at its widest point.

3.4.2 Productivity parameters

1) Pod length (cm)

The length of five dried and cleaned pods were measured using a vernier caliper.

2) Pod width (cm)

The width of five dried and cleaned pods were measured using a vernier caliper.

3) Number of pods per plant

Number of pods per plant was counted at the time of harvest.

4) Pod yield per plant (g/plant)

Pod yield per plant was calculated by dividing total pod yield per plot by number of plants in the plot, and expressed as g/plant.

5) Sound mature kernel weight (%)

Number of well matured kernels from a sample (100 g) and expressed in percentage.

6) Test weight (gm)

The well dried and cleaned pods from each genotype were shelled, 100 kernels at random were counted and weight was recorded in grams.

7) Shelling percentage

Shelling percentage was calculated by weighing the kernels shelled from a unit weight of pods and expressed in percentage.

3.4.3 Nutritional traits

1) Oil content (%)

Per cent oil content of each genotype was estimated by near infrared (NIR) spectroscopy at Seed Quality Testing and Research Laboratory, Seed Unit, UAS, Dharwad.

2) Protein content (%)

Per cent protein content of each genotype was estimated by near infrared (NIR) spectroscopy at Seed Quality Testing and Research Laboratory, Seed Unit, UAS, Dharwad.

4) Oleic acid content

Oleic acid content was estimated from well matured equal sized kernels using near infrared (NIR) spectroscopy at Seed Quality Testing and Research Laboratory, Seed Unit, UAS, Dharwad.

3.4.4 Screening for rust and late leaf spot resistance

LLS and rust inoculums were maintained on TMV 2 (national check variety susceptible to LLS and rust) and mutant 28-2 (resistant to LLS but susceptible to rust) plants grown in growth chamber. The artificial epiphytotic condition for LLS and rust was created in the field using 'Spreader Row Technique' (Subrahmanyam *et al.*, 1995) in which the disease spreader plants (TMV 2 and mutant 28-2) were planted at every 10th row in the experimental plot. The infected leaves from susceptible plants grown in the growth chamber were collected and soaked in water for 30 min. LLS conidia and rust urediniospores were released by rubbing the infected leaves in the water. The inoculum containing 20,000 conidia/urediniospores per ml water was mixed with Tween 80 (0.2

ml/1,000 ml of water) as a mild surfactant. When the plants were 35 days old, they were sprayed in the evening with the inoculum using a Knapsack sprayer for a week. High humidity was maintained by irrigating the field in the night with sprinkler or furrow irrigation. Additional inoculum was provided by placing pots containing diseased plants at every 20 rows.

3.4.5 Disease scoring for rust and late leaf spot

Modified 9 point scale (1-9 score) (Subbarao *et al.*, 1990) was used for scoring rust and LLS disease at different stages of the crop i.e., 70, 80 and 90 DAS.

3.5 Genotyping with markers

3.5.1 Genomic DNA isolation

DNA was isolated from the young leaves of mutants and parents grown in the field by following cetyltrimethyl ammonium bromide (CTAB) method with a few modifications (Cuc *et al.*, 2008).

- 1) Young leaves were ground to a fine powder in liquid nitrogen using a mortar and pestle and transferred to a 1.5 ml microcentrifuge tube.
- 2) The powder was immediately added with preheated 1X CTAB buffer (650 μ l). Contents were mixed vigorously by vortexing and inverting. The tube was incubated at 65°C for 30 min with occasional inversion.
- 3) The samples were cooled on the bench to room temperature and were centrifuged at 13,000 rpm for 20 min to pellet the cellular debris, proteins and polysaccharides.
- 4) An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the extract and mixed by gentle inversion for 5 to 10 min to form a uniform milky white emulsion.
- 5) The mixture was centrifuged at 13,000 rpm for 20 min.
- 6) The aqueous phase was pipetted out gently, avoiding the interface and added with an equal volume of pre chilled isopropanol. Contents were mixed gently by inverting and incubated overnight at -20°C.
- 7) Content was centrifuged at 13,000 rpm for 20 min. Supernatant was discarded.
- 8) The pellet was washed with 70% alcohol by spinning at 9,000 rpm for 10 min. Alcohol was completely discarded to get clear pellet. Pellet was air dried by placing the tube in a hot dry bath at 37°C for 3 hr.
- 9) The pellet was dissolved in 200 μ l of T₁₀E₁.

3.5.2 Quantification of DNA

DNA yield was quantified by using Nano Drop (UV technologies, USA). DNA concentration and purity was also checked by running the samples on 0.8% agarose gel with known concentration of uncut lambda DNA of 50 ng/ μ l, 100 ng/ μ l and 200 ng/ μ l. The DNA stocks of the samples were diluted to a working concentration of 5 ng/ μ l.

3.5.3 Polymerase chain reaction (PCR)

Mutants and parents were genotyped with transposon marker (AhTE markers) (Shirasawa *et al.*, 2012a; Shirasawa *et al.*, 2012b). DNA amplification was performed in a 20 μ l reaction mixture (Table 2) with appropriate PCR profile (Table 3) using Eppendorf Mastercycler® pro and BIO-RAD T100™ Thermal cyclers.

PCR reaction mixture

Component	Quantity (μ l)
Water (HPLC grade)	13.0
PCR buffer (10 X)	2.0
dNTPs (2 mM)	2.0
AhTE primer (F/R) (10 pmol/ μ l)	0.5/0.5
DNA template	1.5
Taq DNA Polymerase	0.5
Total	20.0

PCR profile

Sl. No.	Step	Temperature (°C)	Duration (min)	No. of cycles
1	Denaturation	94	5	1
2	Denaturation	94	2	38
	Annealing	52	1	
	Primer extension	72	2	
3	Final extension	72	10	1
4	Hold	4		

3.5.4 Separation of PCR products

The PCR products were mixed with 2 µl of loading dye (Bromophenol blue) and loaded on 1.5% agarose gel prepared in 1X TAE buffer containing ethidium bromide (5 µl/100 ml). Products were separated at 80 volts till they clearly resolved. The gel was observed and documented with DNA Bio-Imaging system.

3.5.5 Scoring the alleles

Specific PCR product was identified for each marker (Shirasawa *et al.*, 2012a; Shirasawa *et al.*, 2012b) and scored as “1” (with *AhMITE1* insertion), “2” (without *AhMITE1* insertion) or “0” (absence of specific PCR product).

3.6 Statistical analysis

3.6.1 Phenotypic data analysis

Statistical analysis of the data was carried out using statistical package Windostat Version 8.1 and SPSS Version 16.0 available at the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. The following statistical methods were employed for data analysis.

3.6.2 Mean and range

Mean was calculated for each genotype by adding the observed value from the plants sampled in each replication and dividing the sum by total number of observations. Difference between the minimum and maximum values observed for each trait was calculated as range.

3.6.3 Analysis of variance

Analysis of variance (ANOVA) for different characters was carried out by using the mean phenotypic data on each genotype in order to partition the variation due to different sources following the method given by Panse and Sukhatme (1954).

Source of variation	Df	MSS	Expected value of MSS	F
Replication	(r-1)	M_1	-	
Genotypes	(g-1)	M_2	$\sigma^2_e + r\sigma^2_g$	M_2/M_3
Error	(r-1)(g-1)	M_3	σ^2_e	
Total	(rg-1)	$M_1 + M_2 + M_3$		

3.6.4 Frequency distribution

Mean values on each trait across the genotypes was subjected for frequency distribution using statistical package SPSS version 16.0 available at the Department of Genetics and Plant Breeding, UAS, Dharwad.

3.6.5 Estimation of genetic variability components

Phenotypic and genotypic variances were calculated as suggested by Singh and Chaudhary (1979).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{\text{MSS (genotypes)} - \text{MSS (error)}}{\text{Number of replications}} = \frac{M2 - M3}{r}$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \text{MSS (error)} = \frac{M2 - M3}{R} + M3$$

3.6.6 Coefficient of variation

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and De Vane (1953).

$$\text{GCV} = \frac{\sigma_g}{\bar{X}} \times 100$$

Where,

$$\text{PCV} = \frac{\sigma_p}{\bar{X}} \times 100$$

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

$\bar{X}$ = General mean of the character

GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Sivasubramanian and Menon (1973).

3.6.7 Heritability (h^2)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$\text{Heritability } (h^2) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

σ^2_g = genotypic variance

σ^2_p = phenotypic variance

The heritability in broad sense were categorized as low (0-30%), moderate (30-60%) and high (>60%) as indicated by Robinson *et al.* (1949).

3.6.8 Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson *et al.* (1955).

$$\text{GA} = h^2 k \sigma_p$$

Where,

h^2 : Heritability in broad sense

K: Selection differential which is equal to 2.06 at 5% intensity of selection (Lush, 1940)

σ_p : Phenotypic standard deviation.

3.6.9 Genetic advance as percent of mean (GAM)

$$\text{GAM} = \frac{\text{GA}}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean of the character.

Genetic advance as per cent of mean were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Johnson *et al.* (1955).

3.6.10 Correlation analysis

Phenotypic correlation was computed to determine the degree of association among the characters (Diseases, quality and productivity traits) by using the formula given by Weber and Moorthy (1952).

$$r_{xy}(p) = \frac{\text{Cov}_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

$\text{Cov}_{xy}(p)$ = Phenotypic covariance between characters x and y

$V_x(p)$ = Phenotypic variance of character x

$V_y(p)$ = Phenotypic variance of character y

$r_{xy}(p)$ = Phenotypic correlation coefficient between characters x and y

Phenotypic correlation coefficients were compared against table value at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Fisher and Yates, 1963).

3.7 Marker data analysis

3.7.1 Polymorphism information content (PIC) analysis

Polymorphism information content (PIC) was computed for each marker using software Power Marker V 3.25 (Liu and Muse, 2005).

3.7.2 Single marker analysis (SMA)

Single marker analysis (SMA) was performed to determine the significance and the contribution of the marker towards rust and LLS resistance using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). Analysis of variance (ANOVA) and linear regression (Haley and Knott, 1992) were used in SMA to check the significance and workout the coefficient of determination (R^2) of each marker towards the phenotypic variation.

3.7.3 Marker-trait association analysis

Marker-trait association was checked by carrying out Single Marker Analysis (SMA) using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007) for parametric traits (agronomic, productivity and nutritional traits). In order to gain more confidence, the marker with significant trait association was compared among mutants with independent origin, but showing common mutant phenotype for the alleles. The marker showing same allele type across such mutants was considered as a trait associated marker.

3.7.4 z test (standard normal deviate test for proportion)

Two proportions differing for marker or allele content were tested for significance by calculating z value (Rao, 2007), and comparing with the critical value of 1.96 at the 5% level of significance (irrespective of degrees of freedom).

EXPERIMENTAL RESULTS

Analysis of mutants that have independent origin but share common phenotypic shifts for variation in transpositions using transposon specific markers provides an opportunity to identify the genetic changes responsible for important mutations. An effort was made to investigate the variability for various traits within a large population of induced mutants, and to associate and validate specific genetic changes detected by transposon markers with the traits.

4.1 Analysis of variance (ANOVA)

Analysis of variance was carried out for a mutant population consisting of mutants and their parents. ANOVA showed significant genotypic differences for all the agronomic, productivity and nutritional traits studied. But the genotypes differed significantly for rust and LLS resistance only at 80 DAS (Table 2 and 3).

4.2 Components of variation

The nature and magnitude of variation was assessed by comparing phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). All agronomic traits showed high PCV and GCV. Among productivity traits, pod yield/plant, number of pods/plant and SMKW showed high PCV and GCV whereas, pod length, pod width, and test weight showed moderate PCV and GCV while low PCV and GCV observed for shelling percentage. However, moderate PCV was observed for oleic acid and protein content, while oil percentage showed low PCV. In general, a moderate variability was observed for resistance to rust and LLS at three stages (Table 4 and 5).

4.3 Genetic parameters

Agronomic, productivity, nutritional traits and resistance to LLS and rust were analysed for heritability (h^2) and genetic advance as per cent of mean (GAM). High heritability along with high genetic advance as per cent of mean was observed for all agronomic traits. Productivity traits like pod yield/plant, number of pods/plant and SMKW showed high heritability along with high GAM. But pod length, pod width and test weight showed moderate heritability with moderate GAM. Shelling percentage showed low heritability and GAM probably due to low variability. Oleic acid and protein content showed moderate heritability and GAM, while oil content exhibited low heritability and GAM. Though a moderately high heritability and GAM were found for rust at 80 DAS, both LLS and rust exhibited low heritability and GAM (Table 4 and 5).

4.4 Frequency distribution

The frequency distribution of the genotypes based on the mean data for various traits was tested for the kind of distribution. The value calculated as (mean-mode)/standard deviation close to zero indicated a possible normal distribution. Such a distribution was observed for most of the traits, except for number of primary branches, main stem length and rust (70 DAS), which showed skewed distribution towards positive direction.

4.5 Correlation coefficients

Correlation coefficients were computed to assess the magnitude and direction of association between the traits.

4.5.1 LLS and rust resistance

The association analysis between stages (70, 80 and 90 DAS) showed positive and significant correlation (both phenotypic and genotypic) for rust resistance. LLS scores at different stages were also positively correlated. In general, LLS and rust scores across stages were positively correlated (Table 6a).

4.5.2 Agronomic, productivity, nutritional traits and disease resistance

By and large, pod length and pod width showed significant association with test weight, pod yield/plant, and SMKW. Pod yield/plant showed positive and significant association both at phenotypic and genotypic level with mean stem length, length of primary branch, leaf length, leaf width, pod length and width, number of pods/plant and shelling percentage. Pod yield/plant was positively associated with test weight and SMKW. Rust and LLS at 90 DAS showed positive association with pod yield/plant, and negative association with SMKW and test weight (Table 6b).

Table 2: ANOVA for agronomic, productivity and nutritional trait in mutant population of groundnut

Trait	df	MSS			F	CD (5%)	SEm±	CV
		Repl.	Treat.	Error				
Agronomic traits								
NPB	44	0.10	8.35	0.46	18.0*	1.37	0.48	10.62
NSB	44	0.49	1.54	0.05	121.1*	0.12	0.04	6.98
MSL	44	0.71	92.85	0.56	167.1*	1.50	0.53	5.19
LPB	44	0.14	29.37	0.16	184.4*	0.80	0.28	4.94
LL	44	0.00	2.82	0.01	195.8*	0.24	0.08	2.89
LW	44	0.24	2.60	0.09	79.7*	0.36	0.13	8.18
Productivity traits								
PL	44	0.01	0.63	0.04	17.6*	0.38	0.13	6.07
PW	44	0.00	0.06	0.01	10.5*	0.16	0.06	6.22
NPP	44	21.51	54.46	1.67	32.6*	2.60	0.91	8.99
SP	44	4.44	51.15	11.13	4.6*	6.72	2.36	4.77
TW	44	15.63	93.57	14.23	6.6*	7.60	2.67	9.84
SMKW	44	289.80	245.00	45.61	5.4*	13.61	4.78	12.69
PYP	44	0.72	29.67	0.49	59.9*	1.42	0.50	7.45
Nutritional traits								
Oleic acid	44	0.10	68.03	6.30	10.8*	5.06	1.78	5.23
Protein	44	2.70	14.47	2.83	5.1*	3.39	1.19	6.19
OIL	44	12.13	12.75	4.36	2.9*	4.21	1.48	4.66

* Significant at 5% probability

NPB: Number of primary branches NSB: Number of secondary branches
MSL: Main stem length LPB: Length of primary branches
LL: Leaf length LW: Leaf width
PL: Pod length PW: Pod width
NPP: Number of pods/plant SP: Shelling percentage
TW: Test weight SMKW: Sound mature kernel weight
PYP: Pod yield/plant Protein: Protein percentage
OIL: Oil percentage

Table 3: ANOVA for rust and LLS resistance in mutant population of groundnut

Trait	df	MSS			F	CD (5%)	SEm±	CV
		Repl.	Treat.	Error				
R (70)	44	0.54	0.81	0.20	3.96*	0.91	0.32	11.91
R (80)	44	3.21	3.16	0.44	7.21*	1.33	0.47	10.47
R (90)	44	5.38	1.50	0.61	2.47*	1.57	0.55	11.18
LLS (70)	44	0.01	0.47	0.12	3.75*	0.71	0.25	9.90
LLS (80)	44	5.88	1.87	0.86	2.18*	1.86	0.65	14.63
LLS (90)	44	1.88	1.19	0.70	1.71*	1.68	0.59	10.41

* Significant at 5% probability

df: Degrees of freedom Repl.: Replication mean sum of squares
Treat.: Treatment mean sum of squares CD: Critical difference
SEm±: Standard error of mean CV: Coefficient of variation

Table 4: Mean, range and genetic variability components for agronomic, productivity and nutritional traits in mutant population of groundnut

Trait	Range		Mean	PCV	GCV	h ²	GA	GAM
	Min.	Max.						
	Agronomic traits							
NPB	4.00	9.50	6.41	32.74	30.97	89.48	3.87	60.35
NSB	0.71	2.12	0.88	54.58	54.13	98.36	0.98	110.59
MSL	4.40	26.60	14.36	47.58	47.29	98.81	13.91	96.84
LPB	2.60	13.05	8.08	47.54	47.28	98.92	7.83	96.87
LL	2.30	7.50	4.15	28.68	28.53	98.97	2.43	58.48
LW	1.10	7.50	2.21	51.93	51.28	97.52	2.30	104.33
	Productivity traits							
PL	1.30	4.10	3.13	18.50	17.48	89.25	1.06	34.02
PW	0.75	1.55	1.26	14.93	13.58	82.67	0.32	25.43
NPP	3.50	28.50	14.38	36.85	35.73	94.05	10.26	71.38
SP	62.50	88.00	69.87	7.99	6.40	64.27	7.39	10.57
SMKW	30.50	74.50	53.22	22.65	18.77	68.62	17.04	32.02
TW	22.50	54.50	38.34	19.15	16.43	73.60	11.13	29.04
PYP	2.10	20.85	9.44	41.16	40.48	96.72	7.74	82.00
	Nutritional traits							
Oleic acid	35.83	62.11	47.98	12.71	11.58	83.04	10.43	21.74
Protein	20.81	31.55	27.17	10.83	8.88	67.28	4.08	15.01
OIL	40.27	53.66	44.79	6.53	4.57	49.02	2.95	6.60

GCV: Genotypic coefficients of variation PCV: Phenotypic coefficients of variation
 h²: Heritability GA: Genetic advance
 GAM: Genetic advance as per cent of mean NPB: Number of primary branches
 NSB: Number of secondary branches MSL: Main stem length
 LPB: Length of primary branches LL: Leaf length
 LW: Leaf width PL: Pod length
 PW: Pod width NPP: Number of pods/plant
 SP: Shelling percentage TW: Test weight
 SMKW: Sound mature kernel weight PYP: Pod yield/plant
 Protein: Protein percentage OIL: Oil percentage

Table 5: Mean, range and genetic variability components for resistance to LLS and rust in mutant population of groundnut

Trait	Range		Mean	PCV	GCV	h ²	GA	GAM
	Min.	Max.						
R (70)	3.00	5.00	3.77	18.68	13.17	49.69	0.72	19.12
R (80)	4.50	8.00	6.32	21.22	18.46	75.65	2.09	33.08
R (90)	5.50	8.50	6.96	14.74	9.60	42.40	12.87	12.87
LLS (70)	3.00	4.50	3.57	15.27	11.62	57.92	0.65	18.22
LLS (80)	4.50	7.50	6.32	18.45	11.25	37.18	0.89	14.13
LLS (90)	6.00	9.00	8.01	12.13	6.22	26.31	0.53	6.57

GCV: Genotypic coefficients of variation PCV: Phenotypic coefficients of variation

h²: Heritability

GA: Genetic advance

GAM: Genetic advance as per cent of mean

Table 6a: Genotypic and phenotypic correlation coefficient between rust and LLS resistance

Trait	R (70)	R (80)	R (90)	LLS (70)	LLS (80)	LLS (90)
R (70)	1	0.6915**	0.3274**	0.3210**	0.1797	0.3693**
R (80)	0.9048**	1	0.5077**	0.2236*	0.2055	0.3592**
R (90)	0.8361**	0.8627**	1	0.0100	0.0087	-0.0089
LLS (70)	0.6070**	0.3702**	0.2456*	1	0.5243**	0.3713**
LLS (80)	0.3722**	0.1506	0.1037	0.9569**	1	0.4045**
LLS (90)	0.1951	0.6472**	0.2687*	0.1549	0.2065	1

*, ** Significant at 5% and 1% probability, respectively.

(Below diagonal are genotypic correlations and above diagonal are phenotypic correlations)

Table 6b: Genotypic and phenotypic correlation coefficient between agronomic, productivity, nutritional traits and rust and LLS resistance

Trait	NPB	NSB	MSL	LPB	LL	LW	PL	PW	NPP	SP	SMKW	TW	Oleic Acid	Protein	OIL	R (90)	LLS (90)	PYP
NPB	1	0.432**	0.247 *	0.456**	0.359**	0.035	0.029	0.3117**	0.098	-0.015	-0.3325**	-0.111	0.053	-0.050	-0.197	0.181	-0.018	0.075
NSB	0.432**	1	0.084	0.456**	0.354**	0.005	0.030	0.2374 *	-0.127	-0.207	-0.148	-0.181	0.190	0.178	-0.117	0.153	0.089	0.028
MSL	0.248	0.084	1	0.801**	0.754**	0.042	0.397**	0.483**	0.470**	0.015	-0.026	0.117	-0.219	-0.132	-0.31	0.375**	0.147	0.358**
LPB	0.457**	0.401**	0.802**	1	0.706**	0.009	0.409**	0.598**	0.371**	-0.037	-0.037	0.182	-0.056	-0.062	-0.345	0.403**	0.178	0.359**
LL	0.361**	0.356**	0.758*8	0.710**	1	0.098	0.205	0.479**	0.360**	-0.112	-0.070	-0.028	-0.074	-0.057	-0.212	0.424**	0.000	0.289**
LW	0.035	0.005	0.043	-0.009	0.099	1	-0.048	-0.0638	-0.156	-0.152	0.023	0.143	0.129	-0.197	0.047	0.074	-0.279	0.251**
PL	0.031	0.031	0.417**	0.431**	0.216	-0.051	1	0.532**	0.205	-0.067	0.222 *	0.437 **	0.006	-0.2424 *	-0.142	0.161	0.132	0.301**
PW	0.340**	0.2587	0.524**	0.651**	0.530**	-0.070	0.614**	1	0.301**	-0.074	0.200	0.347 **	0.082	-0.027	-0.236	0.232*	0.183	0.309**
NPP	0.101	-0.131	0.486**	0.384**	0.370**	-0.161	0.215	0.394	1	0.255 *	0.034	-0.031	-0.319	-0.200	-0.315	0.2538*	0.181	0.403**
SP	-0.016	-0.218	0.013	-0.037	-0.122	-0.160	-0.071	-0.064	0.270	1	0.130	-0.013	-0.253	0.061	-0.25	0.008	0.223	0.256*
SMKW	-	0.401**	-0.178	-0.031	-0.042	0.025	0.057	0.288**	0.220	0.128	1	0.275 * *	0.038	-0.049	-0.053	-0.157	-0.091	0.082
TW	-0.130	-0.211	0.137	0.209	-0.036	0.170	0.540**	0.357**	-0.023	-0.033	0.357**	1	0.032	-0.056	-0.030	-0.117	0.081	0.058
Oleic acid	0.054	0.194	-0.224	-0.056	-0.077	0.132	-0.342**	0.023	0.103	0.276**	0.04	0.064	1	-0.001	0.115	-0.110	-0.274	0.2674*
Protein	-0.051	0.183	-0.134	-0.062	-0.061	-0.204	-0.253	-0.010	-0.206	0.054	-0.078	-0.057	0.002	1	-0.113	-0.056	0.272	-0.064
OIL	-0.201	-0.120	0.317**	-0.353**	-0.216	0.049	-0.136	-0.243	-0.321**	-0.263	-0.033	-0.049	0.123	-0.108	1	-0.119	-0.299	0.2409*
R (90)	0.278	0.235	0.564**	0.617**	0.667**	0.127	0.129	0.416**	0.380**	0.007	-0.165	-0.151	-0.199	-0.070	-0.120	1	-0.009	0.051
LLS (90)	-0.035	0.174	0.284**	0.341*8	0.040	0.533**	0.218	0.149	0.387**	0.673**	-0.376**	-0.017	0.517*8	0.539**	0.533**	0.269	1	0.258*
PYP	0.086	0.032	0.407**	0.409**	0.333**	-0.286	0.385**	0.368**	0.478**	0.319**	0.055	0.108	-0.303	-0.073	-0.279	0.140	0.527**	1

*,** Significant at 5% and 1% probability, respectively.

(Below diagonal are genotypic correlations and above diagonal are phenotypic correlations)

NPB: Number of primary branches

LL: Leaf length

NPP: Number of pods/plant

Protein: Protein percentage

PYP: Pod yield/plant

NSB: Number of secondary branches

LW: Leaf width

SP: Shelling percentage

OIL: Oil percentage

MSL: Main stem length

PL: Pod length

SMKW: Sound mature kernel weight

R (90): Rust at 90 DAS

LPB: Length of primary branches

PW: Pod width

TW: Test weight

LLS (90): LLS at 90 DAS

4.6 Comparison of parents versus mutants

The genotypes used in this study included ten parents and their mutants. A parent with its mutant(s) was considered as a set. Thus, ten sets were evaluated in field during *kharif* 2012. The mutants were compared to their respective parent for selected traits like number of pods/plant, pod yield/plant, shelling percentage, SMKW, test weight, resistance LLS and rust and a few morphological traits like disease mimicking leaves, mosaic leaves, fused leaflet, crinkle leaf, and long and narrow leaf. The mutants with similar phenotypic shift or change (in positive or negative direction) across the sets were identified and grouped.

4.6.1 Number of pods/plant

In general, the mutants with significantly reduced number of pods/plant were more than those with significantly increased number of pods/plant. Five mutants with significantly increased number of pods/plant as compared to their respective parent were observed across four sets (I, V, IX and X). TG 2 showed highest of 28.5 pods/plant followed by TGM 50 (24.5 pods/plant). Both these mutants originated from Spanish Improved (18.5 pods/plant) (Table 7a). Except set V and VIII, all other sets contributed mutants (23) with significantly reduced number of pods/plant when compared to their parents. Mutants of both the classes (significantly higher as well as lower number of pods/plant) were observed in three sets (I, IX and X).

4.6.2 Pod yield/plant

All ten mutant-parent sets showed significant difference for pod yield/plant. Number of mutants with significantly lower pod yield/plant was more frequent than those with significantly higher values. Seven mutants with significantly higher pod yield/plant as compared to their respective parent were observed across five sets (III, V, VI, VII and VIII). TGM 117 had highest pod yield/plant (20.85 g/plant) as compared to its parent TAG 24 (12.85 g/plant) (Table 7b). Twenty mutants with significantly lower pod yield/plant were observed across six sets (I, II, III, IV, IX and X). Mutants with significantly higher or lower pods yield/plant when compared to their respective parent were observed in one set (III).

4.6.3 Shelling percentage

Significant difference for shelling percentage was observed within four mutant-parent sets. Four mutants across three sets (I, III and VII) exhibited significantly higher shelling percentage as compared to their parents. Six mutants with reduced shelling percentage as compared to respective parents were observed across three sets (I, III and V). Mutants of both the classes were observed in two sets (I and III). Mutants with reduced shelling percentage outnumbered those with increased shelling percentage. TGM 50 recorded the highest shelling percentage of 88.0%, while its parent Spanish Improved had 74.5%. Likewise, TGM 138 (81.0%) possessed higher shelling percentage than its parent TAG 24 (70.0%) (Table 7c).

4.6.4 Sound mature kernel weight (SMKW)

Sound mature kernel weight differed significantly among seven mutant-parent sets. Five mutants with significantly higher SMKW as compared to their respective parents were observed across four sets (S IV, VII, VIII and S IX). Four mutants with significantly lower SMKW were observed across three sets (II, III and X). However, none of the sets showed the mutants of both the classes (significantly higher or lower SMKW). In general, the mutants with increased SMKW were more in number. Mutant TGM 167 (74.5%) had significantly higher SMK than its parent TG 66 (57.0%), while mutant TGM 43 (68.0%) had higher SMKW than its parent TGM 8 (45.0%) (Table 7d).

4.6.5 Test weight

Four parent-mutant sets contained mutants significantly differing for test weight. Three mutants across two sets (I and VII) had significantly higher test weight when compared to their parents. Seven mutants with significantly lower test weight were observed across three sets (I, III and X). Set I had both the categories of the mutants. Again, the mutants with reduced test weight outnumbered the mutants with increased test weight. Mutant TGM 51 (54.5%) had significantly higher test weight than its parent Spanish Improved (38.75%), while the mutants (TGM 37 and TGM 54) originating from parents TG 18 A (37.5%) showed significantly higher test weight (46 g and 48 g, respectively) (Table 7e).

Table 7a: List of mutants significantly differing from their parents for number of pods/plant

Set	Parent		Mutants with change in			
	Name	Mean	Positive direction		Negative direction	
			Name	Mean	Name	Mean
I	Spanish Improved	18.5	TGM 50	24.5	TG 1	8.0
			TG 2	28.5	TGM 14	12.0
					TGM 8	15.5
					TGM 51	13.5
II	TG 2	28.5			TGM 19	3.5
III	TAG 24	23.5			TGM 46	16.0
					TGM 96	13.5
					TGM 101	8.5
					TGM 111	12.5
					TGM 112	9.0
					TGM 113	14.0
					TGM 117	20.5
					TGM 119	15.0
					TGM 156	3.5
					TGM 138	12.0
IV	TGM 8	15.5			TGM 43	9.0
V	TG 9	16.5	TGM 36	23.0		
VI	TG 16	16.0			TGM 44	13.5
VII	TG 18 A	17.0			TGM 20	13.0
					TGM 54	11.5
IX	TG 66	14.0	TGM 218	19.0	TGM 178	6.5
					TGM 189	9.0
X	TGM 51	13.5	TGM 223	19.0	TGM 196	8.0
					TGM 225	7.5

Table 7b: List of mutants significantly differing from their parents for pod yield/ plant

Set	Parent		Mutants with change in			
	Name	Mean	Positive direction		Negative direction	
			Name	Mean	Name	Mean
I	Spanish Improved	18.6			TG 1	14.5
					TG 2	12.4
					TGM 8	11.2
					TGM 14	9.1
					TGM 50	13.9
					TGM 51	9.3
					TGM 58	7.8
II	TG 2	12.4			TGM 119	2.1
III	TAG 24	12.8	TGM 117	20.8	TGM 46	8.6
					TGM 96	7.4
					TGM 101	10.3
					TGM 112	8.9
					TGM 113	7.4
					TGM 119	10.7
					TGM 159	2.2
					TGM 138	9.4
IV	TGM 8	11.2			TGM 43	9.1
V	TG 9	5.8	TGM 36	12.2		
VI	TG 16	7.2	TGM 44	16.3		
VII	TG 18 A	7.6	TGM 20	11.5		
			TGM 37	9.8		
			TGM 54	11.4		
VIII	Somnath	8.1	TGM 47	11.5		
IX	TG 66	7.6			TGM 196	5.6
X	TG 51	9.3			TGM 223	7.1
					TGM 225	4.8

Table 7c: List of mutants significantly differing from their parents for shelling percentage

Set	Parent		Mutants with change in			
	Name	Mean	Positive direction		Negative direction	
			Name	Mean	Name	Mean
I	Spanish Improved	74.5	TGM 50	88.0	TG 1	65.5
					TGM 8	64.0
					TGM 14	66.0
					TGM 51	66.0
III	TAG 24	70.0	TGM 138	81.0	TGM 156	62.5
V	TG 9	73.0			TGM 36	65.0
VII	TG 18 A	64.5	TGM 20	74.0		
			TGM 54	78.0		

Table 7d: List of mutants significantly differing from their parents for SMKW

Set	Parent		Mutants with change in			
	Name	Mean	Positive direction		Negative direction	
			Name	Mean	Name	Mean
II	TG 2	55.5			TGM 19	31.2
III	TAG 24	52.5			TGM 96	39.0
					TGM 101	30.5
IV	TGM 8	45.0	TGM 43	68.0		
VII	TG 18 A	43.5	TGM 37	59.5		
		41.0	TGM 54	59.0		
VIII	Somnath	57.0	TGM 47	56.0		
IX	TG 66	63.0	TGM 167	74.5		
X	TG 51	63.0			TGM 223	28.0

Table 7e: List of mutants significantly differing from their parents for test weight

Set	Parent		Mutants with change in			
	Name	Mean	Positive direction		Negative direction	
			Name	Mean	Name	Mean
I	Spanish Improved	38.7	TGM 51	54.5	TGM 8	30.0
III	TAG 24	40.5			TGM 58	22.5
					TGM 96	30.5
					TGM 101	29.0
					TGM 119	31.0
VII	TG 18 A	37.5	TGM 37	46.0		
			TGM 54	48.0		
X	TG 51	54.5			TGM 223	28.0
					TGM 225	28.0

Table 7f: List of mutants significantly differing from their parents for rust at 90 DAS

Set	Parent		Mutants with change in negative direction	
	Name	Mean	Name	Mean
III	TAG 24	8	TGM 101	6
VI	TG 16	8	TGM 44	6

Table 8: List of mutants with morphological changes as compared to their parents

Trait	Parent	Mutant
Disease mimicking leaves	TG 18 A	TGM 20
	TAG 24	TGM 113
	TG 66	TGM 189
		TGM 218
Mosaic leaves	Spanish Improved	TGM 51
	TAG 24	TGM 112
	TG 66	TGM 178
Crinkle leaves	Spanish Improved	TGM 8
Fused leaflet	Spanish Improved	TGM 14
	TG 66	TGM 193
		TGM 196
Long and narrow leaves	TG 51	TGM 225

4.6.6 Resistance to rust

Two mutants across two sets (III and VI) had significantly reduced disease score for rust (90 DAS). TGM 101 from set III and TGM 44 from set VI showed a reduced score of 6 when compared to their parent TAG 24 (8) and TG 16 (8), respectively. No mutants recorded increased rust score when compared to their parents (Table 7f). For LLS, none of the mutants showed either significant increase or decrease in disease score when compared to their parents.

4.6.7 Morphological traits

Mutants and their parents were evaluated for unusual morphological traits like disease mimicking leaves, mosaic leaves, crinkle leaves, fused leaflet and thin and narrow leaves. Four mutants (TGM 20, TGM 113, TGM 189 and TGM 218) belonging to set III, VIII and IX showed leaves mimicking rust lesions after 30-40 days of sowing. Three mutants (TGM 51, TGM 112 and TGM 178) belonging to three sets (I, III and IX, respectively) showed mosaic leaves. TGM 8 originating from Spanish Improved (SI) showed crinkle leaves. The crinkle leaves formed after 50% flowering. Three mutants (TGM 14, TGM 193 and TGM 196) from two sets (I and IX) showed fused top leaflets with trifoliate appearance. Fused leaflets were observed approximately 35 to 42 days after sowing. Mutant, TGM 225 showed long (5.70 cm) and narrow (1.35 cm) leaves when compared to its parent TG 51 (4.15 cm length and 1.75 cm breadth) (Table 8).

4.7 Genotypic data analysis

Forty five genotypes comprising of parents and mutants were genotyped with 60 transposon-specific markers in order to identify the genetic change(s) between parent and its mutant(s) at the marker loci, and also to measure the significance and strength of each marker in governing a phenotypic trait. Alleles containing *AhMITE1* (205 bp) were scored as “1” and those without *AhMITE1* were scored as “2”. Absence of the specific PCR product was scored as “0”. Of the 60 markers, only 47 could amplify the products which could be scored.

4.7.1 Polymorphism information content (PIC) analysis

The power of each marker to discriminate the genotypes was calculated by estimating polymorphism information content (PIC) value. Markers were classified as high (≥ 0.5), medium (0.26 to 0.49) and low (≤ 0.25) based on the PIC value. In total, 30 and 17 markers were identified as medium and low for their genotype-discriminating power, respectively (Table 9).

4.7.2 Polymorphism between parent and their mutant(s)

Based on the transposon-specific marker data, the extent of polymorphism was worked out between parent and its mutant(s) within each set (Table 10). The overall polymorphism across the sets was 27.7%. The polymorphism varied from 8.5% (Set VII) to 46.1% (Set IV). A considerable variation was also observed for per cent polymorphism within sets. Highest polymorphism of 72.3% was observed between Spanish Improved and TG 1. The same was 68.1% between TAG 24 and TGM 156, while it was 51.1% between TAG 24 and TGM 117. Spanish Improved and three of its mutants, TG 2, TGM 8 and TGM 58 failed to show any polymorphism for the markers tested.

4.8 Identification and validation of trait-specific marker

In an effort to identify trait-specific marker(s), single marker analysis (SMA) was carried out by calculating *F* statistic and simple linear regression coefficient R^2 (Haley and Knott, 1992) using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). Markers identified by SMA as significantly associated with the trait were validated using independent mutants displaying similar shift(s) in phenotype. For each trait, the mutants showing phenotypic shift in positive and negative direction as compared to their parents were grouped in two clusters. Within these clusters, the markers that were polymorphic between mutant and its parent were listed. Markers that were polymorphic across maximum number of mutant-parent pairs were identified as common polymorphic markers. Also the type of allele among the mutants sharing such common markers was determined. Statistical significance of these proportions was worked out by *z* test (standard normal deviate test for proportion).

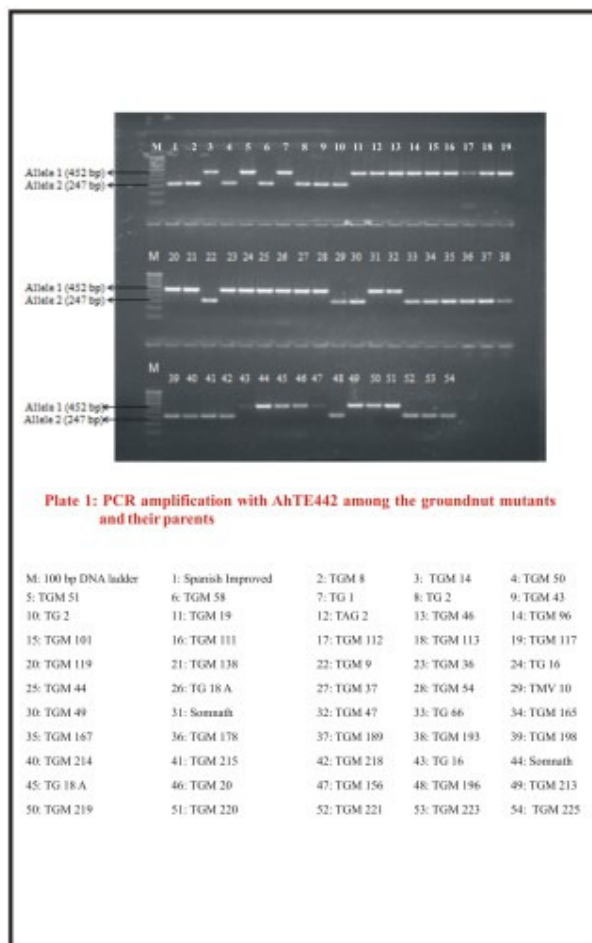


Plate 1: PCR amplification with AhTE422 among the groundnut mutants and their parents

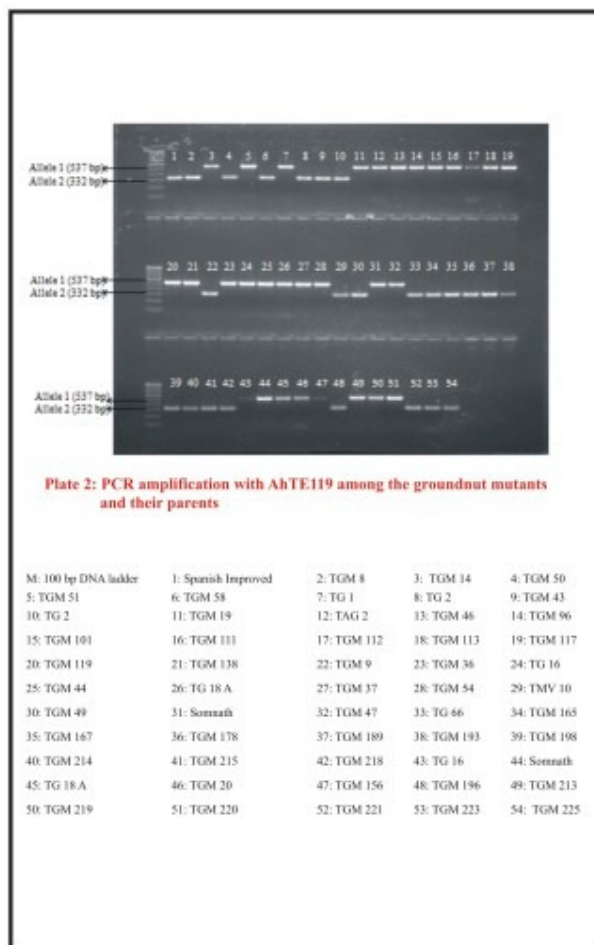


Plate 2: PCR amplification with AhTE119 among the groundnut mutants and their parents

Table 9: Polymorphism information content value for the markers

Sl. No.	Marker	PIC
1	AhTE107	0.16
2	AhTE113	0.30
3	AhTE119	0.49
4	AhTE121	0.46
5	AhTE130	0.21
6	AhTE148	0.12
7	AhTE159	0.04
8	AhTE164	0.48
9	AhTE180	0.12
10	AhTE181	0.08
11	AhTE190	0.23
12	AhTE205	0.46
13	AhTE213	0.23
14	AhTE218	0.42
15	AhTE222	0.40
16	AhTE248	0.04
17	AhTE254	0.49
18	AhTE268	0.04
19	AhTE278	0.43
20	AhTE282	0.34
21	AhTE296	0.49
22	AhTE303	0.44
23	AhTE305	0.47
24	AhTE324	0.36
25	AhTE333	0.04
26	AhTE335	0.24
27	AhTE343	0.08
28	AhTE359	0.35
29	AhTE360	0.31
30	AhTE361	0.08
31	AhTE372	0.47
32	AhTE373	0.08
33	AhTE398	0.04
34	AhTE416	0.24
35	AhTE422	0.27
36	AhTE426	0.48
37	AhTE437	0.45
38	AhTE442	0.23
39	AhTE445	0.12
40	AhTE446	0.49
41	AhTE459	0.35
42	AhTE467	0.35
43	AhTE477	0.38
44	AhTE489	0.43
45	AhTE491	0.48
46	AhTE501	0.49
47	AhTE504	0.20

4.8.1 Number of pods/plant

Five mutants with significantly increased number of pods/plant when compared to their parents across four sets were clubbed in cluster I, while 23 mutants with significantly reduced number of pods/plant across eight sets were placed in cluster II. Overall, 30 markers were found to be polymorphic between the parents and the five mutants across in cluster I (Table 11). The polymorphic markers ranged from two (TGM 50 versus Spanish Improved) to 19 (TGM 36 versus TG 9, and TGM 223 versus TGM 51) (Table 10). All forty seven markers were found to be polymorphic between the parents and twenty three mutants in cluster II (Table 11). The polymorphic markers ranged from two (TGM 96 and TGM 101 versus their parent TAG 24, and TGM 51 versus TG 18 A) to 34 markers (TG 1 versus Spanish Improved) (Table 10).

Single marker analysis showed significant association of nine markers with number of pods/plant (Table 12). R^2 values indicating the contribution of each marker towards phenotypic variance ranged from 9.71% (AhTE254) to 44.41% (AhTE324). AhTE324 was not a common polymorphic in cluster I. However, it was common to 14 (out of 23) mutants in (non-significant z of 1.47) in cluster II. Of them, 13 mutants showed the allele with *AhMITE1*.

AhTE164 with R^2 of 18.48% was common among two mutants in cluster I, but they carried contrasting alleles at this locus. In cluster II, AhTE164 was common among 12 mutant-parent pairs (non-significant z of 0.29). Nine mutants carried the allele without *AhMITE1*. AhTE254 (R^2 9.71%) was not a common polymorphic marker in cluster I. But it was polymorphic across 13 mutant-parent pairs in cluster II (non-significant z of 0.88). Of the 13 pairs, ten mutant-parent pairs had similar allele (with *AhMITE1* insertion) and other three had allele (without *AhMITE1* insertion).

4.8.2 Pod yield per plant

Thirty eight markers were found to be polymorphic between the parents and seven mutants with higher pod yield/plant across the five sets in cluster III (Table 11). Among these mutants, the number of polymorphic markers ranged from two (TGM 37 and TGM 54 versus their parent TG 18 A) to 24 (TGM 117 versus TAG 24) (Table 10). Twenty mutants with significantly reduced pod yield/plant across six sets in cluster IV showed a total of forty five polymorphic markers when compared to their parents (Table 11). Polymorphic markers ranged from 2 (TGM 50 versus Spanish Improved, and TGM 96 and TGM 101 versus their parent TAG 24) to 34 (TG 1 versus Spanish Improved) (Table 10).

Pod yield per plant was found to be significantly associated with six markers with R^2 ranging from 7.92% (AhTE121) to 22.80% (AhTE373) (Table 12). None of them were common polymorphic markers since their polymorphism was restricted to a maximum of one mutant-parent pair.

4.8.3 Shelling percentage

Four mutants with significantly higher shelling percentage over respective parents showed a total of eleven polymorphic markers in cluster V (Table 11). It ranged from two (TGM 50 versus Spanish Improved, and TGM 54 versus TG 18 A) to eight (TGM 20 versus TG 18 A) (Table 10).

Mutants (6) with reduced shelling percentage when compared to their parents showed 44 polymorphic markers (Table 11) against their parents with a range of 19 (TGM 14 and TGM 51 versus their parent Spanish Improved, and TGM 36 versus TG 9) to 34 (TG 1 versus Spanish Improved) in cluster VI (Table 10).

Single marker analysis showed significant association of only AhTE130 (R^2 of 11.85%) with shelling percentage (Table 12). Of the four mutants in cluster V, none were polymorphic for AhTE130 when compared to their parents. Among the six mutants with reduced shelling percentage in cluster VI, three (non-significant z value) had AhTE130 as common polymorphic marker. The allele (with *AhMITE1*) was common to all these three mutants at AhTE130 locus.

4.8.4 Sound mature kernel weight (SMKW)

A total of twenty six markers were found to be polymorphic between the parents and five mutants with increased sound mature kernel weight over their parents in cluster VII (Table 11). Polymorphic markers ranged from two (TGM 37 and TGM 54 versus their parent TG 18 A) to 22 (TGM 43 versus TGM 8) (Table 10). Four mutants with reduced SMKW showed a total of 29 polymorphic markers in cluster VIII (Table 11), which ranged from two (TGM 96 and TGM 101 versus their parent TAG 24) to 19 (TGM 19 versus TG 2, and TGM 223 versus TGM 51) (Table 10).

Table 10: Polymorphic markers between the mutant and parent

Set	Parent	Mutant	No. polymorphic markers	AhTE markers
I	Spanish Improved	TG 1	34 (72.3)	AhTE296, AhTE254, AhTE121, AhTE359, AhTE107, AhTE148, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE335, AhTE489, AhTE504, AhTE130, AhTE477, AhTE426, AhTE113, AhTE373, AhTE467, AhTE164, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE491
		TG 2	0	-
		TGM 8	0	-
		TGM 14	19 (40.4)	AhTE296, AhTE359, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE335, AhTE130, AhTE113, AhTE324, AhTE422, AhTE437, AhTE360, AhTE372, AhTE416, AhTE303, AhTE446, AhTE491
		TGM 50	2 (4.3)	AhTE282, AhTE372
		TGM 51	19 (40.4)	AhTE190, AhTE296, AhTE254, AhTE359, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE504, AhTE130, AhTE477, AhTE113, AhTE437, AhTE360, AhTE372, AhTE222, AhTE303, AhTE491
		TGM 58	0	-
		Average per cent polymorphism for set	39.3	
II	TG 2	TGM 19	19 (40.4)	AhTE296, AhTE254, AhTE121, AhTE359, AhTE119, AhTE205, AhTE489, AhTE130, AhTE426, AhTE113, AhTE467, AhTE164, AhTE324, AhTE360, AhTE459, AhTE222, AhTE446, AhTE501, AhTE491
III	TAG 24	TGM 46	3 (6.4)	AhTE254, AhTE324, AhTE360
		TGM 96	2 (4.3)	AhTE254, AhTE324
		TGM 101	2 (4.3)	AhTE254, AhTE324
		TGM 111	3 (6.4)	AhTE254, AhTE248, AhTE324
		TGM 112	8 (17.0)	AhTE296, AhTE254, AhTE130, AhTE148, AhTE164, AhTE324, AhTE360, AhTE282
		TGM 113	4 (8.5)	AhTE164, AhTE324, AhTE372, AhTE416

Contd...

		TGM 117	24 (51.1)	AhTE254, AhTE107, AhTE148, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE504, AhTE477, AhTE268, AhTE333, AhTE373, AhTE164, AhTE248, AhTE422, AhTE437, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE398
		TGM 119	5 (10.6)	AhTE324, AhTE446, AhTE181, AhTE213, AhTE442
		TGM 138	3 (6.4)	AhTE254, AhTE324, AhTE360
		TGM 156	32 (68.1)	AhTE296, AhTE254, AhTE121, AhTE359, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE489, AhTE504, AhTE477, AhTE268, AhTE426, AhTE113, AhTE373, AhTE467, AhTE164, AhTE248, AhTE324, AhTE437, AhTE459, AhTE372, AhTE446, AhTE501, AhTE398, AhTE361, AhTE213, AhTE442, AhTE445, AhTE190
		Average per cent polymorphism for set	18.3	
IV	TGM 8	TGM 43	22 (46.8)	AhTE251, AhTE121, AhTE205, AhTE218, AhTE305, AhTE489, AhTE504, AhTE130, AhTE426, AhTE113, AhTE467, AhTE164, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE303, AhTE446, AhTE501
V	TG 9	TGM 36	19 (40.4)	AhTE296, AhTE107, AhTE148, AhTE119, AhTE205, AhTE278, AhTE305, AhTE504, AhTE477, AhTE164, AhTE324, AhTE360, AhTE222, AhTE180, AhTE491, AhTE398, AhTE282, AhTE442, AhTE445
VI	TG 16	TGM 44	8 (17.0)	AhTE107, AhTE254, AhTE343, AhTE361, AhTE398, AhTE442, AhTE445, AhTE491
VII	TG 18 A	TGM 20	8 (17.0)	AhTE121, AhTE107, AhTE268, AhTE164, AhTE360, AhTE446, AhTE282, AhTE343
		TGM 37	2 (4.3)	AhTE335, AhTE164
		TGM 54	2 (4.3)	AhTE164, AhTE282
		Average per cent polymorphism for set	8.53	
VIII	Somnath	TGM 47	6 (12.8)	AhTE121, AhTE335, AhTE489, AhTE426, AhTE422, AhTE416
IX	TG 66	TGM 165	3 (6.4)	AhTE426, AhTE437, AhTE491
		TGM 167	6 (12.8)	AhTE205, AhTE426, AhTE164, AhTE437, AhTE446, AhTE491

Contd...

		TGM 178	6 (12.8)	AhTE205, AhTE426, AhTE164, AhTE437, AhTE446, AhTE491
		TGM 189	7 (14.9)	AhTE254, AhTE205, AhTE426, AhTE164, AhTE437, AhTE222, AhTE491
		TGM193	17 (36.2)	AhTE254, AhTE205, AhTE305, AhTE504, AhTE130, AhTE477, AhTE426, AhTE164, AhTE248, AhTE324, AhTE437, AhTE372, AhTE222, AhTE491, AhTE442, AhTE445, AhTE190
		TGM 196	9 (19.1)	AhTE254, AhTE121, AhTE205, AhTE164, AhTE437, AhTE372, AhTE222, AhTE501, AhTE491
		TGM 198	6 (12.8)	AhTE296, AhTE254, AhTE359, AhTE422, AhTE437, AhTE459
		TGM 213	11 (23.4)	AhTE296, AhTE121, AhTE119, AhTE205, AhTE164, AhTE437, AhTE372, AhTE501, AhTE491, AhTE282, AhTE190
		TGM 214	6 (12.8)	AhTE205, AhTE164, AhTE324, AhTE422, AhTE437, AhTE491
		TGM 215	21 (44.7)	AhTE121, AhTE359, AhTE205, AhTE218, AhTE305, AhTE335, AhTE504, AhTE130, AhTE113, AhTE164, AhTE248, AhTE437, AhTE372, AhTE222, AhTE416, AhTE491, AhTE181, AhTE213, AhTE442, AhTE343, AhTE190
		TGM 218	6 (12.8)	AhTE164, AhTE437, AhTE360, AhTE372, AhTE491, AhTE213
		Average per cent polymorphism for set	18.9	
X	TG 51	TGM 223	19 (40.4)	AhTE296, AhTE121, AhTE359, AhTE119, AhTE218, AhTE278, AhTE305, AhTE489, AhTE504, AhTE477, AhTE426, AhTE113, AhTE437, AhTE360, AhTE222, AhTE303, AhTE491, AhTE282, AhTE190
		TGM 225	18 (28.3)	AhTE296, AhTE121, AhTE119, AhTE205, AhTE218, AhTE305, AhTE489, AhTE504, AhTE426, AhTE164, AhTE324, AhTE437, AhTE360, AhTE222, AhTE303, AhTE501, AhTE491, AhTE213
		Average per cent polymorphism for set	34.0	
		Overall per Cent Polymorphism	27.7	

Values in parenthesis indicate per cent polymorphism

Table 11: Polymorphic markers for the mutant(s) with change in positive and negative direction

Trait	Mutants with change in			
	Positive direction		Negative direction	
	Name	Polymorphic markers	Name	Polymorphic markers
NPP	TGM 50, TG 2, TGM 36, TGM 218, TGM 223	AhTE296, AhTE121, AhTE359, AhTE119, AhTE107, AhTE148, AhTE205, AhTE218, AhTE278, AhTE305, AhTE489, AhTE504, AhTE477, AhTE426, AhTE113, AhTE164, AhTE324, AhTE437, AhTE360, AhTE372, AhTE222, AhTE180, AhTE303, AhTE491, AhTE398, AhTE282, AhTE213, AhTE442, AhTE445, AhTE190	TG 1, TGM 14, TGM 8, TGM 51, TGM 19, TGM 46, TGM 96, TGM 101, TGM 111, TGM 112, TGM 113, TGM 117, TGM 119, TGM 156, TGM 138, TGM 43, TGM 44, TGM 20, TGM 54, TGM 178, TGM 189, TGM 196, TGM 225	AhTE296, AhTE254, AhTE121, AhTE359, AhTE107, AhTE148, AhTE119, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE489, AhTE504, AhTE130, AhTE477, AhTE268, AhTE333, AhTE426, AhTE113, AhTE373, AhTE467, AhTE164, AhTE248, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE491, AhTE398, AhTE361, AhTE181, AhTE282, AhTE213, AhTE442, AhTE445, AhTE343, AhTE190
	Total	30	Total	47
PYP	TGM 117, TGM 36, TGM 44, TGM 20, TGM 37, TGM 54, TGM 47	AhTE296, AhTE254, AhTE121, AhTE107, AhTE148, AhTE119, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE489, AhTE504, AhTE477, AhTE268, AhTE333, AhTE426, AhTE373, AhTE164, AhTE248, AhTE324, AhTE422, AhTE437, AhTE360, AhTE222, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE491, AhTE398, AhTE361, AhTE282, AhTE442, AhTE445, AhTE343	TG 1, TG 2, TGM 8, TGM 14, TGM 50, TGM 51, TGM 58, TGM 119, TGM 46, TGM 96, TGM 101, TGM 112, TGM 113, TGM 119, TGM 159, TGM 138, TGM 43, TGM 196, TGM 223, TGM 225	AhTE296, AhTE254, AhTE121, AhTE359, AhTE107, AhTE148, AhTE119, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE489, AhTE504, AhTE130, AhTE477, AhTE268, AhTE426, AhTE113, AhTE373, AhTE467, AhTE164, AhTE248, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE491, AhTE398, AhTE361, AhTE181, AhTE282, AhTE213, AhTE442, AhTE445, AhTE190
	Total	38	Total	45

Contd...

SP	TGM 50, TGM 138, TGM 20, TGM 54	AhTE107, AhTE121, AhTE164, AhTE254, AhTE268, AhTE282 AhTE324, AhTE343, AhTE360, AhTE372, AhTE446	TG 1, TGM 8, TGM 14, TGM 51,TGM 156, TGM 36	AhTE296, AhTE254, AhTE121, AhTE359, AhTE107, AhTE148, AhTE119, AhTE159, AhTE205, AhTE218, AhTE278, AhTE305, AhTE335, AhTE489, AhTE504, AhTE130, AhTE477, AhTE268, AhTE426, AhTE113, AhTE373, AhTE467, AhTE164, AhTE248, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE180, AhTE303, AhTE416, AhTE446, AhTE501, AhTE491, AhTE398, AhTE161, AhTE282, AhTE213, AhTE442, AhTE445, AhTE190
	Total	11	Total	44
SMKW	TGM 43, TGM 37, TGM 54, TGM 47, TGM 167	AhTE254, AhTE121, AhTE205, AhTE218, AhTE305, AhTE489, AhTE504, AhTE130, AhTE426, AhTE113, AhTE467, AhTE164, AhTE324, AhTE422, AhTE437, AhTE459, AhTE360, AhTE372, AhTE222, AhTE303, AhTE446, AhTE501, AhTE335, AhTE282, AhTE416, AhTE491	TGM 19, TGM 96, TGM 101 TGM 223	AhTE296, AhTE121, AhTE254, AhTE359, AhTE119, AhTE205, AhTE218, AhTE278, AhTE305, AhTE489, AhTE504, AhTE130, AhTE477, AhTE426, AhTE113, AhTE467, AhTE164, AhTE324, AhTE442, AhTE437, AhTE459, AhTE360, AhTE222, AhTE303, AhTE446, AhTE501, AhTE491, AhTE282, AhTE190
	Total	26	Total	29
TW	TGM 51, TGM 37, TGM 54	AhTE296, AhTE254, AhTE359, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE504, AhTE130, AhTE477, AhTE113, AhTE437, AhTE360, AhTE372, AhTE222, AhTE303, AhTE491, AhTE335, AhTE164, AhTE190	TGM 8, TGM 58, TGM 96, TGM 101, TGM 119, TGM 223	AhTE296, AhTE254, AhTE121, AhTE359, AhTE119, AhTE205, AhTE218, AhTE278, AhTE305, AhTE489, AhTE504, AhTE477, AhTE426, AhTE113, AhTE164, AhTE324, AhTE437, AhTE360, AhTE222, AhTE303, AhTE446, AhTE501, AhTE491, AhTE181, AhTE282, AhTE213, AhTE442,
	Total	21	Total	27
R (90)			TGM 101, TGM 44	AhTE107, AhTE254, AhTE343, AhTE361, AhTE398, AhTE442, AhTE445, AhTE491, AhTE324
			Total	9

Four markers, AhTE372, AhTE296, AhTE119 and AhTE419 showed significant association with SMKW (Table 12). R^2 ranged from 10.34% (AhTE372) to 11.85% (AhTE491). All the four SMKW-associated markers were restricted to a maximum of one mutant-parent pair in cluster VII. But AhTE296 (R^2 of 11.30%), AhTE119 (R^2 of 11.41%) and AhTE491 (R^2 of 11.85%) were common among the two mutants (TGM 19 and TGM 223). Allele with *AhMITE1* insertion was found in TGM 19 at all the three marker loci, while the contrasting allele (without *AhMITE1*) was found in TGM 223, indicating a lack of strong co-segregation between the allele type and the phenotype.

4.8.5 Test weight

Twenty one markers were found to be polymorphic across three mutants with significantly higher test weight in cluster IX (Table 11). Polymorphic markers ranged from two (TGM 37 and TGM 54 versus their parent TG 18 A) to 19 (TGM 51 versus Spanish Improved) (Table 10). Twenty seven markers were polymorphic across seven mutants with reduced test weight in cluster X (Table 11). Polymorphic markers ranged from two (TGM 96 and TGM 101 versus their parent TAG 24) to nineteen (TGM 223 versus TG 51) (Table 10).

Single marker analysis showed significant association of 12 markers with test weight. The R^2 values of these markers ranged from 2.18% (AhTE268) to 23.55% (AhTE305) (Table 12). These markers were unique to each pair of mutant-parent in both the clusters (Table 10).

4.8.6 Rust and LLS resistance

SMA showed significant association of three markers AhTE359 (R^2 of 9.03%), AhTE422 (R^2 of 9.70%) and AhTE491 (R^2 of 10.52%) with rust resistance (Table 12). Cluster XI had two mutants with reduced rust score. TGM 101 differed from its parent TAG 24 for two markers, while TGM 44 differed from its parent TG 16 for eight markers (Table 10). AhTE254 was a common polymorphic marker between TGM 101 and TG 44 but they did not share the similar allele. In this study, mutants with increased rust score were not observed.

For resistance to LLS, five markers showed significant association with R^2 ranging from 18.00% (AhTE372) to 9.74% (AhTE501) (Table 12). However, the markers could not be checked for their validation as the mutants with significantly altered reaction to LLS were not observed in this study.

4.8.7 Morphological traits

The mutants showing unusual morphological phenotype were genotyped along with their respective parent using TE markers, and the polymorphism was worked out between the mutant and its parent (Table 13). TGM 8 with crinkle leaves did not show any polymorphic marker when compared to its parent Spanish Improved. TGM 225 with long and narrow leaves showed eighteen polymorphic markers as compared to its parent TG 51. Four mutants (TGM 20, TGM 113, TGM 189 and TGM 218) with disease mimicking leaves differed from their parents for a total of eighteen markers. Polymorphic markers ranged from four (TGM 113 versus TAG 24) to eight (TGM 20 versus TG 18 A). AhTE164 was a common polymorphic marker among three mutants (TGM 20, TGM 189 and TGM 218) (non-significant z value), where TGM 20 and TGM 189 had the allele without *AhMITE1* insertion.

Three mutants (TGM 51, TGM 112 and TGM 178) with mosaic leaves differed for twenty five markers from their parents. Polymorphic markers ranged from six (TGM 178 versus TG 66) to 19 (TGM 51 versus Spanish Improved). AhTE254 was found polymorphic across two mutants (TGM 51 and TGM 112), and both the mutants had the similar allele (with *AhMITE1* insertion). However, these proportions were statistically non-significant.

Three mutants (TGM 14, TGM 193 and TGM 196) with fused leaflet differed for a total 32 markers when compared to their parents. Polymorphic markers ranged from nine (TGM 196 versus TG 66) to 19 (TGM 14 versus Spanish Improved). AhTE372, AhTE437 and AhTE491 were common polymorphic markers among all the three mutants. But the alleles without *AhMITE1* insertion at these loci were found in a maximum of two mutants (non-significant z of 0.82).

Table 12: Marker-trait association as measured by Single marker analysis for important productivity traits and resistance to LLS and rust

Trait	Sl. No.	Marker	F	R ²
NPP	1	AhTE254	4.62*	9.71
	2	AhTE489	12.07**	11.36
	3	AhTE164	7.56**	18.48
	4	AhTE205	4.90*	20.54
	5	AhTE113	4.37*	20.67
	6	AhTE360	6.25*	22.69
	7	AhTE130	5.78*	23.34
	8	AhTE359	21.18**	23.79
PYP	9	AhTE324	10.83**	44.41
	1	AhTE121	5.40*	7.92
	2	AhTE107	8.81**	16.79
	3	AhTE180	9.78**	20.30
	4	AhTE148	11.03**	20.33
	5	AhTE333	7.93**	20.41
SP SMKW	6	AhTE373	7.00*	22.80
	1	AhTE130	4.77*	11.85
	1	AhTE372	5.08*	10.34
	2	AhTE296	5.54*	11.30
TW	3	AhTE119	4.28*	11.41
	4	AhTE491	5.83*	11.85
	1	AhTE268	4.67*	2.18
	2	AhTE335	8.03**	7.38
	3	AhTE296	9.57**	8.43
	4	AhTE218	5.81*	12.62
	5	AhTE491	12.96**	13.37
	6	AhTE477	12.82**	15.53
	7	AhTE359	5.32*	15.99
	8	AhTE303	8.16**	16.58
	9	AhTE119	4.08*	18.20
	10	AhTE437	11.79**	19.85
R (90)	11	AhTE278	8.54**	22.97
	12	AhTE305	5.04*	23.55
LLS (90)	1	AhTE359	4.107*	9.03
	2	AhTE422	4.315*	9.70
	3	AhTE491	5.431*	10.52
	1	AhTE121	5.341*	12.96
	2	AhTE119	7.899*	15.49
	3	AhTE489	4.828*	10.56
	4	AhTE372	7.292*	18.00
	5	AhTE501	3.488*	9.74

*, ** Significance at 5% and 1% probability, respectively

Table 13: Polymorphic markers between the mutant and parent for unusual phenotype

Unusual phenotype	Parent	Mutant	No. polymorphic markers	AhTE markers
Disease mimicking leaves	TG 18 A	TGM 20	8 (17.0)	AhTE121, AhTE107, AhTE268, AhTE164, AhTE360, AhTE446, AhTE282, AhTE343
	TAG 24	TGM 113	4 (8.5)	AhTE164, AhTE324, AhTE372, AhTE416
	TG 66	TGM 189	7 (14.9)	AhTE254, AhTE205, AhTE426, AhTE164, AhTE437, AhTE222, AhTE491
		TGM 218	6 (12.8)	AhTE164, AhTE437, AhTE360, AhTE372, AhTE491, AhTE213
	Average per cent polymorphism for set		22.8	
Mosaic leaves	Spanish Improved	TGM 51	19 (40.4)	AhTE190, AhTE296, AhTE254, AhTE359, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE504, AhTE130, AhTE477, AhTE113, AhTE437, AhTE360, AhTE372, AhTE222, AhTE303, AhTE491
	TAG 24	TGM 112	8 (17.0)	AhTE296, AhTE254, AhTE130, AhTE148, AhTE164, AhTE324, AhTE360, AhTE282
	TG 66	TGM 178	6 (12.8)	AhTE205, AhTE426, AhTE164, AhTE437, AhTE446, AhTE491
	Average per cent polymorphism for set		23.4	
Crinkle leaves	Spanish Improved	TGM 8	0	
Fused leaflet	Spanish Improved	TGM 14	19 (40.4)	AhTE296, AhTE359, AhTE119, AhTE218, AhTE278, AhTE282, AhTE305, AhTE335, AhTE130, AhTE113, AhTE324, AhTE422, AhTE437, AhTE360, AhTE372, AhTE416, AhTE303, AhTE446, AhTE491
		TGM 193	14 (29.8)	
		TGM 196	9 (19.1)	AhTE254, AhTE205, AhTE305, AhTE504, AhTE130, AhTE477, AhTE426, AhTE164, AhTE248, AhTE324, AhTE437, AhTE372, AhTE222, AhTE491, AhTE442, AhTE445, AhTE190
	Average per cent polymorphism for set		29.7	
Long and narrow leaves	TG 51	TGM 225	18 (38.3)	AhTE296, AhTE121, AhTE119, AhTE205, AhTE218, AhTE305, AhTE489, AhTE504, AhTE426, AhTE164, AhTE324, AhTE437, AhTE360, AhTE222, AhTE303, AhTE501, AhTE491, AhTE213

Values in parenthesis indicate per cent polymorphism

DISCUSSION

Groundnut is one of the most important cultivated grain legumes in the world. Recent years have witnessed accelerated development of genomic resources such as development of molecular markers, genetic and physical maps, generation of expressed sequenced tags (ESTs), development of mutant resources, and functional genomics platforms. These genomic resources facilitate the identification of gene(s)/QTL/marker(s) associated with tolerance/resistance to abiotic and biotic stresses and agronomic traits. The first step towards molecular breeding to develop superior genotypes is the identification of gene/QTL/marker(s) controlling desired traits.

Linkage mapping and association mapping are mainly used to identify gene/QTL/marker(s) governing traits of importance. These approaches employ phenotypic data and marker data from different populations. For the study of linkage, one needs to perform suitably designed crosses, sometimes leading to the development of mapping populations or near-isogenic lines (NILs). Alternatively, association analysis has the potential not only to identify and map QTLs, but also to identify causal polymorphism within a gene that is responsible for the difference in two alternative phenotypes.

In groundnut, initial efforts on mapping started with the diploids (Moretzsohn *et al.*, 2005; Moretzsohn *et al.*, 2009) as they represent simple genetic models (Halward *et al.*, 1993). Later, SSR-based genetic linkage maps were developed using recombinant inbred lines (RILs) for cultivated tetraploid groundnut (Varshney *et al.*, 2009; Ravi *et al.*, 2011). Several RIL populations differing for LLS and rust resistance were developed at UAS, Dharwad (Bhat *et al.*, 2012), and genetic maps were developed for TAG 24 × GPBD 4 (Khedikar *et al.*, 2010; Sujay *et al.*, 2012) and TG 26 × GPBD 4 (Sarvamangala *et al.*, 2011; Sujay *et al.*, 2012). A considerable progress has been made to involve wild germplasm in the recent genomics approaches. Wild species (donor) are crossed to an elite variety (recurrent parent), and the F₁s are backcrossed. An array of BC₂ or BC₃ lines, each containing a small number of random introgressions from the donor wild species in an elite varietal background is used as the AB-QTL population (Tanksley and Nelson, 1996). Linkage maps have been developed using backcross populations involving wild species (Burow *et al.*, 2001; Fonceka *et al.*, 2009; Fonceka *et al.*, 2012b; Fonceka *et al.*, 2012a). These maps were used to detect QTL for various traits like growth habit, plant height, plant spread, flower colour, LLS and rust resistance and drought related traits (Sujay *et al.*, 2012).

Markers play a key role in mapping and handling of QTL. In groundnut, a range of molecular markers such as restriction fragment length polymorphism, random amplified polymorphic DNA, amplified fragment length polymorphism, and simple sequence repeat (SSR) markers have been used (Hilu and Stalker, 1995; Kochert *et al.*, 1996; Subramanian *et al.*, 2000; Dwivedi *et al.*, 2001; He and Prakash, 2001; Herselman, 2003; Bravo *et al.*, 2006). Recently, Diversity Array Technology (DArT) marker platform has also been developed for groundnut (Kilian, 2008). Currently, the advent of next-generation sequencing and faster genotyping technologies have enabled the detection of single nucleotide polymorphisms (SNPs), which have emerged as the marker of choice in crop breeding and SNPs have been detected from diploids (Alves *et al.*, 2008; Nagy *et al.*, 2012) as well as cultivated tetraploids (Chen *et al.*, 2013).

Bhat *et al.* (2008) and Gowda *et al.* (2010) reported the development of a groundnut transposon-based marker and its strong association with late leaf spot resistance. Subsequently, a large number of such markers displaying very high level of polymorphism were developed and used for mapping in groundnut (Shirasawa *et al.*, 2012a; Shirasawa *et al.*, 2012b; Shirasawa *et al.*, 2013). With a wide distribution in the genome and frequent role in groundnut mutations and evolution (Gowda *et al.*, 1996; Gowda *et al.*, 2011), transposons make a useful template for the development of markers that can have immense value in mapping as well as identifying the key gene governing a trait. Use of a large number of transposon-based markers in combination with several independent mutants sharing common phenotypic changes for various traits of importance would not only enable understanding the basis of mutation but also provide a unique opportunity of identifying marker-trait association.

5.1 Genetic variability within mutant population

In this study, a mutant population consisting of thirty five independent mutants and 10 parents obtained from BARC, Mumbai was field evaluated during *kharif* 2012 for various agronomical, morphological, productivity and nutritional traits and for resistance to rust and late leaf spot. Mutants along with their parents were genotyped with a sizeable number of TE-specific markers to find out the

association of each marker with a given trait. The marker(s) showing strong and significant association were considered as trait-specific markers.

Frequency distribution of the genotypes for various traits followed normal distribution except for number of primary branches, main stem length and rust (70 DAS). Genotypic differences were significant for all the agronomic, productivity and nutritional traits studied. However, the genotypes differed significantly for rust and LLS resistance only at 80 DAS. The genotypes exhibited high PCV and GCV for all the agronomic traits. Pod yield/plant, number of pods/plant and SMKW showed high PCV and GCV whereas, test weight, oleic acid and protein content showed moderate variability and shelling percentage and oil percentage had low variability. In general, a moderate variability was observed for resistance to rust and LLS at three stages.

Genetic parameters like heritability and genetic advance as per cent of mean (GAM) were high for all agronomic traits. Productivity traits like pod yield/plant, number of pods/plant and SMKW showed high heritability along with high GAM. But test weight showed moderate heritability with moderate GAM. Shelling percentage showed low heritability and GAM probably due to low variability. Oleic acid and protein content showed moderate heritability and GAM, while oil content exhibited low heritability and GAM. Only rust at 80 DAS showed moderately high heritability and GAM among different stages of LLS and rust.

5.2 Correlation coefficients

Identification of a marker associated with a trait which in turn is correlated with other traits is of considerable value in molecular breeding. Hence a prior knowledge on character correlations helps to decide the worthiness of any trait-associated marker.

In general, pod yield/plant showed positive and significant association both at phenotypic and genotypic level with mean stem length, length of primary branch, leaf length, pod length and breadth, number of pods/plant and shelling percentage. Pod yield/plant was positively associated with test weight and SMKW. Rust and LLS at 90 DAS showed positive association with pod yield/plant, and negative association with SMKW and test weight.

The association analysis between stages (70, 80 and 90 DAS) showed positive and significant correlation (both phenotypic and genotypic) for rust resistance. LLS scores at different stages were also positively correlated. In general, LLS and rust scores across stages were positively correlated.

5.3 Marker-trait association

5.3.1 Productivity traits and disease resistance

The mutant population was classified into ten sets, each consisting of a parent and its mutant(s). All the 10 parents and thirty five mutants were genotyped with 60 transposon-specific markers (Shirasawa *et al.*, 2012a). Alleles containing *AhMITE1* (205 bp) were scored as "1" and those without *AhMITE1* were scored as "2". Absence of the specific PCR product was scored as "0". Of the 60 markers, only 47 could amplify the products which could be scored. The power of each marker to discriminate the genotypes was calculated by estimating polymorphism information content (PIC) value. In total, 30 and 17 markers were identified as medium (PIC 0.26 to 0.49) and low (PIC $\leq$ 0.25) for their genotype-discriminating power, respectively.

The extent of marker polymorphism was worked out between each mutant and its parent, and also within each set of a parent and its mutant(s) as average per cent polymorphism. Overall per cent polymorphism across all pairs of parent and mutant was 27.7%, indicating a very high average polymorphism between any parent and any mutant. The polymorphism between Spanish Improved and TG 1 was highest (72.3%). Three mutants, TG 2, TGM 8 and TGM 58 failed to show any polymorphism for the markers tested when compared to their parent (Spanish Improved). Average per cent polymorphism across the sets ranged from 8.5% (VII) to 46.1% (IV), indicating that the highest polymorphism was observed between Spanish Improved and its mutants. High rate polymorphism recorded in this study indicated the utility of transposon-specific markers in groundnut genomics as observed previously (Shirasawa *et al.*, 2012a; Shirasawa *et al.*, 2013).

Mutations are said to be random, recessive and rare. However, in this study, a considerably high polymorphism between many mutants and their parents indicated a possibility of several mutations contributing towards the origin of mutants. Zhao *et al.* (2013) reported high level of

polymorphism for SSR markers between salt tolerant mutants (M_3) and their parent (Huayu 22) in groundnut. Todorovska *et al.* (2013) found point mutations and small indels (2 bp) in (AT) n repeats located in the introns of *rubisco activase* and *waxy* genes among three gamma induced mutant lines of barley. Not all polymorphisms may lead to changes in gene structure and function. But considerable variation in the gene expression pattern was observed in microarray analysis between a mutant showing increased resistance to toxin produced by *Streptomyces scabies* and other *Streptomyces* species (the causative agents of common scab disease in potato and other taproot crops) and its parent in *Arabidopsis* (Scheible *et al.*, 2003). Thirty-one genes showed increased expression, and 20 genes showed decreased expression in the mutant. Transposon-based markers such as the ones used in this study detect the transposition of *AhMITE1* which has preference for genic regions, hence these markers have high chance of detecting changes in gene structure and function.

In an effort to identify trait-specific marker(s), single marker analysis was carried out by calculating F statistic and simple linear regression coefficient R^2 (Haley and Knott, 1992) using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). Recent developments indicate that single-marker tests can be used to construct powerful inference procedures for haplotype analysis. Chapman *et al.* (2003) found that SMA based on regression analysis is more powerful than the traditional haplotype analysis. Use of SMA in association mapping has been highlighted (Roeder *et al.*, 2005; Schaid *et al.*, 2005; Wang and Sheffield, 2005; Libioulle *et al.*, 2007).

Mutant population such as the one used in this study provides a unique opportunity of validating the marker identified by SMA. For marker validation, the mutants that showed phenotypic change or shift in positive or negative direction for each trait when compared to their parents were grouped into separate clusters. Markers that were polymorphic between a mutant and its parent were identified for each mutant in each cluster, and any such polymorphic marker shared by two or more independent mutants was identified and the type of allele among those mutants was recorded. A marker that is common and shows similar allele type across several mutants in a cluster indicates more evidence towards its validation. Thus any marker showing significant F value and high R^2 upon SMA, and validated across mutants might be suggested for trait selection.

Such an analysis requires identification of clusters consisting independent mutants significantly differing (in positive or negative direction) from their parents for various traits. In this study, attempt was made to construct such clusters for number of pods/plant, pod yield/plant, shelling percentage, SMKW, test weight and resistance LLS and rust. Mutants with significantly higher and lower means than their parent were observed for number of pods/plant, pod yield/plant, shelling percentage, SMKW and test weight. For rust reaction, mutants with only significantly lower score when compared to parent were observed. For none of the traits considered, mutants with only higher mean value were recorded. For reaction to LLS, no mutants with significant changes in the mean were observed. The mutants with significantly higher or lower mean than their parents were clubbed into clusters. In total, 11 clusters were constituted, and the cluster size varied from 2 (lower rust score) to 23 (higher number of pods/plant). A mutant population consisting of a large number of independent mutants with similar phenotypes is always useful in identifying or validating marker-trait association.

For number of pods/plant, single marker analysis showed significant association of nine markers with R^2 values, indicating the contribution of each marker towards phenotypic variance, ranging from 9.71% (AhTE254) to 44.41% (AhTE324). Validation of these markers was attempted by checking for the extent of co-segregation between a given transposition (detected by a marker) and the common phenotypic shift or change among the mutants. For this, five mutants with significantly increased number of pods/plant when compared to their parents across four sets were clubbed in cluster I, while 23 mutants with significantly reduced number of pods/plant across eight sets were placed in cluster II. Overall, 30 markers were found to be polymorphic between the parents and the five mutants across in cluster I. The polymorphic markers ranged from two (TGM 50 versus Spanish Improved) to 19 (TGM 36 versus TG 9, and TGM 223 versus TGM 51). Forty seven markers were found to be polymorphic between the parents and twenty three mutants in cluster II. The polymorphic markers ranged from two (TGM 96 and TGM 101 versus their parent TAG 24, and TGM 51 versus TG 18 A) to 34 markers (TG 1 versus Spanish Improved). Wide range of polymorphism in both the clusters indicated either change in the other related traits or the complex genetic nature of the trait (number of pods/plant). Hence, it was attempted to find from those markers that had significant F and high R^2 upon SMA, and were polymorphic across a maximum number of mutant-parent pairs. AhTE324 was not a common polymorphic marker in cluster I, But, it was common to 14 (out of 23) mutants in cluster II. Of them, 13 mutants showed the allele with *AhMITE1*. The proportions at both

the levels (being common polymorphic and carrying similar allele) were compared statistically using the z test (standard normal deviate test for proportion) (Rao, 2007). These proportions were non-significant, indicating little evidence for the marker validation. It was also concluded that the marker did not have significant role in the origin of mutants with significantly altered number of pods/plant.

AhTE164 with R^2 of 18.48% was common among two mutants in cluster I, but they carried contrasting alleles at this locus. In cluster II, AhTE164 was common among 12 mutant-parent pairs. Nine mutants carried the allele without *AhMITE1*. AhTE254 (R^2 9.71%) was not a common polymorphic marker in cluster I. But it was polymorphic across 13 mutant-parent pairs in cluster II. Of the 13 pairs, ten mutant-parent pairs had similar allele (with *AhMITE1* insertion) and other three had allele (without *AhMITE1* insertion). Again, AhTE164 and AhTE254 were not supported by significant z value.

Pod yield/plant was significantly associated with six markers with R^2 ranging from 7.92% (AhTE121) to 22.80% (AhTE373). For their validation, mutants within cluster III and IV were analysed. Thirty eight markers were found to be polymorphic between the parents and seven mutants with higher pod yield/plant across the five sets in cluster III. Among these mutants, the number of polymorphic markers ranged from two (TGM 37 and TGM 54 versus their parent TG 18 A) to 24 (TGM 117 versus TAG 24). Twenty mutants with significantly reduced pod yield/plant across six sets in cluster IV showed a total of forty five polymorphic markers when compared to their parents. Polymorphic markers ranged from 2 (TGM 50 versus Spanish Improved, and TGM 96 and TGM 101 versus their parent TAG 24) to 34 (TG 1 versus Spanish Improved). However, none of the six markers (with significant F) could be validated for their trait association.

Single marker analysis showed significant association of only AhTE130 (R^2 of 11.85%) with shelling percentage. Four mutants with significantly higher shelling percentage over respective parents showed a total of eleven polymorphic markers in cluster V. It ranged from two (TGM 50 versus Spanish Improved, and TGM 54 versus TG 18 A) to eight (TGM 20 versus TG 18 A). Mutants (6) with reduced shelling percentage when compared to their parents showed 44 polymorphic markers against their parents with a range of 19 (TGM 14 and TGM 51 versus their parent Spanish Improved, and TGM 36 versus TG 9) to 34 (TG 1 versus Spanish Improved) in cluster VI. Of the four mutants in cluster V, none were polymorphic for AhTE130 when compared to their parents. Though, AhTE130 as common polymorphic marker among three of the six mutants with reduced shelling percentage in cluster VI, the proportions were non-significant.

Four markers with R^2 ranging from 10.34% (AhTE372) to 11.85% (AhTE491) were significantly associated with SMKW. Among the two clusters of mutants employed for marker validation, a total of 26 and 29 markers were polymorphic between the mutants and their parents in cluster VII and VIII, respectively. Again, the number of polymorphic markers varied widely between these mutants and their parents. But the four SMKW-associated markers were restricted to a maximum of one mutant-parent pair in cluster VII. Of the four mutants with lower SMKW in cluster VIII, only two mutants (TGM 19 and TGM 223) shared AhTE296 (R^2 of 11.30%), AhTE119 (R^2 of 11.41%) and AhTE491 (R^2 of 11.85%). Allele with *AhMITE1* insertion was found in TGM 19 at all the three marker loci, while the contrasting allele (without *AhMITE1*) was found in TGM 223, indicating a lack of evidence for the marker validation.

Single marker analysis showed significant association of 12 markers with test weight with R^2 ranging from 2.18% (AhTE268) to 23.55% (AhTE305). A large number of (21 and 27 in cluster IX and X, respectively) were polymorphic across mutants and their parents. But none of the test weight-associated markers showed strong validation.

Most of the productivity traits are governed by quantitative genes as revealed by bulked segregant analysis for number of pods per plant, plant weight, and pod maturity (Gomez Selvaraj *et al.*, 2009). QTL with major effects have also been identified for the productivity traits using introgression line populations in groundnut (Varshakumari, 2013).

SMA showed significant association of three markers AhTE359 (R^2 of 9.03%), AhTE422 (R^2 of 9.70%) and AhTE491 (R^2 of 10.52%) with rust resistance. Cluster XI had two mutants with reduced rust score. In this study, mutants with increased rust score were not observed. TGM 101 differed from its parent TAG 24 for two markers, while TGM 44 differed from its parent TG 16 for eight markers. AhTE254 was a common polymorphic marker between TGM 101 and TG 44 but they did not share the similar allele. Till date, major QTL have been mapped (Khedikar *et al.*, 2010; Sujay *et al.*, 2012), and the linked markers have been identified and validated (Khedikar *et al.*, 2010; Yeri *et al.*, 2014) for

rust resistance in groundnut. Two TE markers AhTE360 and AhTE498 have been mapped and observed a strong association with rust resistance (Mondal *et al.*, 2013).

For resistance to LLS, five markers showed significant association with R^2 ranging from 18.00% (AhTE372) to 9.74% (AhTE501). However, the markers could not be checked for their validation as the mutants with significantly altered reaction to LLS were not observed in this study. Previous study with Recombinant Inbred Line populations has identified 28 QTL and linked SSR markers for LLS resistance (Sujay *et al.*, 2012). However, two QTL regions; one on linkage group XV and the other on XII had phenotypic variance explained (PVE) of 67.98 and 62.34, respectively.

5.3.2 Morphological traits

Several mutants showed unusual morphological phenotype like mosaic leaves, disease mimicking leaves, crinkle leaves, fused leaflets etc. Mutants with independent origin but showing similar phenotype were grouped in clusters. They were genotyped along with their respective parent using TE markers, and the polymorphism was worked out between the mutant and its parent. TGM 8 with crinkle leaves did not show any polymorphic marker when compared to its parent Spanish Improved, indicating that its origin could not be explained with the markers used in this study. TGM 225 with long and narrow leaves showed eighteen polymorphic markers as compared to its parent TG 51, indicating the role of several transpositions in the origin of such mutants.

Mutations leading to disease mimicking leaves have been observed in several crops including groundnut. Leaves of disease lesion mimic mutants in groundnut mimic the symptoms of rust disease. Leaves contain drastically reduced chlorophyll content. The trait is normally inherited either as recessive or dominant trait (Johal *et al.*, 1995). However, the genetic analysis with two disease mimic mutants, TG18AM and TAG 24 M showed 13:3 segregation ratio. Four mutants, TGM 20, TGM 113, TGM 189 and TGM 218 with disease mimicking leaves were analysed for the polymorphic markers. They differed from their parents for a total of eighteen markers. Polymorphic markers ranged from four (TGM 113 versus TAG 24) to eight (TGM 20 versus TG 18 A). Of these polymorphic markers, AhTE164 was a common among three mutants (TGM 20, TGM 189 and TGM 218) (non-significant z value), where TGM 20 and TGM 189 had the allele without *AhMITE1* insertion at these marker loci.

Three independent mutants (TGM 51, TGM 112 and TGM 178) with mosaic leaves differed for twenty five markers from their parents. Polymorphic markers ranged from six (TGM 178 versus TG 66) to 19 (TGM 51 versus Spanish Improved). AhTE254 was found polymorphic across two mutants (TGM 51 and TGM 112), and both the mutants had the similar allele (with *AhMITE1* insertion). However, these proportions were statistically non-significant. Hence, the transposition detected by AhTE254 marker could not explain the origin of mutants with mosaic leaves.

Groundnut develops pinnately compound leaves with four to five leaflets. But the three fused top leaflet mutants (TGM 14, TGM 193 and TGM 196) produced three leaflets, where the top leaflet was formed by the fusion of the top two leaflets. The mutants were also characterized by the absence of the rachis between two pairs of leaflets. Thus, the whole leaf appeared as a trifoliate. The three mutants with fused leaflet differed for a total 32 markers when compared to their parents. Polymorphic markers ranged from nine (TGM 196 versus TG 66) to 19 (TGM 14 versus Spanish Improved). AhTE372, AhTE437 and AhTE491 were common polymorphic markers among all the three mutants with significant z (2.45). But the alleles without *AhMITE1* insertion at these loci were found in a maximum of two mutants (non-significant z of 0.82). Hence, these markers were not considered associated with fused leaflet mutation.

In groundnut, very few studies have reported marker-trait association. Mondal and Badigannavar (2009) found association of a few SSR markers with resistance late leaf spot and rust. Effort was also made to find association of SSR markers with biotic and abiotic stress responses and yield-quality traits using peanut minicore and Valencia germplasm (Kottapalli *et al.*, 2011). Wang *et al.* (Wang *et al.*, 2011) found strong association between a functional SNP marker from *FAD2A* with oleic acid, linoleic acid, and O/L ratio. The markers identified in this study as associated with traits need to be validated across other genotypes before employed for groundnut breeding.

SUMMARY AND CONCLUSIONS

Identification of molecular markers and genes governing yield, quality and resistance to biotic and abiotic stresses would assist in groundnut molecular breeding. Transposons being widely distributed in groundnut genome have played a key role in groundnut mutations and evolution. Markers detecting the transposition of a groundnut transposon (*AhMITE1*) were employed to detect genetic changes in a mutant population consisting of 35 mutants and their parents. An effort was made by evaluating the mutant population to detect and validate marker-trait association for important productivity traits and resistance to LLS and rust, and a few morphological mutant phenotypes. The significant findings of the study are listed hereunder.

- A mutant population consisting of thirty five independent mutants and 10 parents obtained from BARC, Mumbai was field evaluated during *kharif* 2012 for various agronomical, morphological, productivity and nutritional traits and for resistance to rust and late leaf spot.
- All the agronomic, productivity and nutritional traits exhibited significant genotypic difference. However, the genotypes differed significantly for rust and LLS resistance only at 80 DAS.
- The genotypes exhibited high PCV and GCV for all the agronomic traits. Yield related traits like pod yield/plant, number of pods/plant and SMKW showed high variability, test weight, oleic acid and protein content exhibited moderate variability and shelling percentage and oil percentage showed low variability. Reaction to LLS and rust showed moderate variability.
- Agronomic traits recorded high heritability and genetic advance as per cent of mean (GAM). Productivity traits like pod yield/plant, number of pods/plant and SMKW showed high heritability along with high GAM. But test weight showed moderate heritability with moderate GAM. Shelling percentage showed low heritability and GAM. Oleic acid and protein content showed moderate heritability and GAM, while oil content exhibited low heritability and GAM. Only rust at 80 DAS showed moderately high heritability and GAM among different stages of LLS and rust.
- In general, pod yield/plant showed positive and significant association both at phenotypic and genotypic level with mean stem length, length of primary branch, leaf length, pod length and breadth, number of pods/plant and shelling percentage. Pod yield/plant was also positively associated with test weight and SMKW.
- The association analysis between stages (70, 80 and 90 DAS) showed positive and significant correlation (both phenotypic and genotypic) for rust resistance. LLS scores at different stages were also positively correlated. By and large, LLS and rust scores across stages were positively correlated.
- Mutants with phenotypic change in positive and negative direction were clubbed into separate clusters for each trait studied. In total, 5, 7, 4, 5 and 3 mutants had positive phenotypic change for number of pods/plant, pod yield/plant, shelling percentage, SMKW and test weight, respectively. Similarly, 23, 20, 6, 4, 7 and 2 mutants had phenotypic change in negative direction for number of pods/plant, pod yield/plant, shelling percentage, SMKW, test weight and rust resistance, respectively.
- Mutants along with their parents were genotyped with 60 TE-specific markers, of which only 47 could amplify visible products. In total, 30 and 17 markers recorded medium (0.26 to 0.49) and low (≤ 0.25) PIC values, respectively.
- Overall per cent polymorphism across all pairs of mutant-parent was 27.7%. Highest polymorphism of 72.3% was observed between Spanish Improved and TG 1. Three mutants, TG 2, TGM 8 and TGM 58 failed to show any polymorphism for the markers tested when compared to their parent (Spanish Improved). Average per cent polymorphism across the sets ranged from 8.5% (set VII) to 46.1% (set III).
- In each cluster, the markers that were polymorphic between the mutants and their parents were identified, and the polymorphic markers were listed for each cluster. Cluster for significantly reduced number of pods/plant showed a maximum of 47 polymorphic markers followed by cluster with reduced pod yield/plant (45) and shelling percentage (44). Cluster with reduced rust score showed as low as nine polymorphic markers.

- Nine markers were significantly associated with number of pods/plant. AhTE324 had highest R^2 (44.41%) followed by AhTE164 (R^2 of 18.48%) and AhTE254 (R^2 of 9.71%). However, the independent mutants did not provide any evidence towards the validation of these markers.
- Six markers with R^2 ranging from 7.92% (AhTE121) to 22.80% (AhTE373) were significantly associated with pod yield/plant. But none of them was common across the independent mutants, indicating lack of marker validation.
- SMA showed significant association of only AhTE130 (R^2 of 11.85%) with shelling percentage. Three mutants with lower shelling percentage shared this marker as polymorphic against their parents. They also shared the common allele (with *AhMITE1*). But these proportions did not provide significant evidence towards validation of AhTE130.
- Four markers, AhTE372 (R^2 of 10.34%), AhTE296 (R^2 of 11.30%), AhTE119 (R^2 of 11.41%) and AhTE491 (R^2 of 11.85%) showed significant association with SMKW. Two mutants (TGM 19 and TGM 223) with lower SMKW shared AhTE296, AhTE119 and AhTE491. But the mutants did not show co-segregation between the allele and the phenotype.
- Twelve markers were significantly associated with test weight, and the R^2 ranged from 2.18% (AhTE268) to 23.55% (AhTE305). However, no mutants of both the classes shared any trait-associated marker as polymorphic when compared to their parents.
- Single marker analysis showed significant association of LLS resistance with five markers, and the R^2 ranged from 18.00% (AhTE372) to 9.74% (AhTE501).
- AhTE359 (R^2 of 9.03%), AhTE422 (R^2 of 9.70%) and AhTE491 (R^2 of 10.52%) were significantly associated with rust reaction. TGM 101 and TGM 44 with reduced rust incidence shared AhTE254 as common polymorphic marker. But the alleles did not show co-segregation with the phenotype.
- Four mutants (TGM 20, TGM 113, TGM 189 and TGM 218) with disease mimicking leaves showed a total of eighteen polymorphic markers. Though AhTE164 was found common among three mutants (TGM 20, TGM 189 and TGM 218), z test indicated non-significant association with the phenotype.
- Three mutants (TGM 51, TGM 112 and TGM 178) with mosaic leaves showed polymorphism for a total of twenty five markers, of which AhTE254 was found to be common among two mutants (TGM 51 and TGM 112), and both had the allele with *AhMITE1* insertion. However, these proportions were non-significant (z value 0.82).
- Three mutants (TGM 14, TGM 193 and TGM 196) with fused leaflet showed thirty two polymorphic markers when compared to their parents. AhTE372, AhTE437 and AhTE491 were common polymorphic markers among all the three mutants with significant z (2.45). But the alleles without *AhMITE1* insertion at these loci were found in a maximum of two mutants (non-significant z of 0.82). But the alleles at AhTE372, AhTE437 and AhTE491 did not show strong co-segregation with fused leaflet.
- TGM 8 with crinkle leaves did not show any polymorphic marker as compared to Spanish Improved, indicating the need for screening with more markers.
- TGM 225 with long and narrow leaves as compared to its parent TG 51 differed for eighteen markers, which indicated the role of multiple mutations.
- The markers identified in this study as associated with important yield related traits and resistance to LLS and rust need to be validated across other genotypes before employed for groundnut breeding.

REFERENCES

- Alves, D. M., Pereira, R. W., Leal-Bertioli, S. C., Moretzsohn, M. C., Guimarães, P. M. and Bertioli, D. J., 2008, Development and use of single nucleotide polymorphism markers for candidate resistance genes in wild peanuts (*Arachis* spp). *Genet. Mol. Res.*, 7 (3): 631-642.
- Ashri, A., 1972, Mutations and physiological reaction to several chemical mutagens in peanuts, *Arachis hypogaea* L. *Proc. Latin American study group on induced mutations and plant improvements*, 16-20 Nov. 1970, Vienna, Austria, pp. 253-264.
- Ashri, A., 1976, Plasmon divergence in peanuts (*Arachis hypogaea*): A third plasmon and locus affecting growth habit. *Theor. Appl. Genet.*, 48 (1): 17-21.
- Badigannavar, A., Kale, D. and Murty, G., 2002, Genetic base and diversity in groundnut genotypes. *Plant Breed.*, 121 (4): 348-353.
- Benjamin, B., 1981, Transposable elements and genetic instabilities in crop plants. *Proc. Stadler Symposium*, pp. 115-128.
- Bennetzen, J. L., 2000, Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol.*, 42 (1): 251-269.
- Bertioli, D. J., Seijo, G., Freitas, F. O., Valls, J. F. M., Leal-Bertioli, S. and Moretzsohn, M. C., 2011, An overview of peanut and its wild relatives. *Plant Genet. Resour.*, 9 (1): 134-149.
- Bertioli, D. J., Vidigal, B., Nielen, S., Ratnaparkhe, M. B., Lee, T. H., Leal-Bertioli, S. C., Kim, C., Guimaraes, P. M., Seijo, G., Schwarzacher, T., Paterson, A. H., Heslop-Harrison, P. and Araujo, A. C., 2013, The repetitive component of the A genome of peanut (*Arachis hypogaea*) and its role in remodelling intergenic sequence space since its evolutionary divergence from the B genome. *Ann. Bot.*, 112 (3): 545-559.
- Bhagwat, A., Krishna, T. G. and Bhatia, C. R., 1997, RAPD analysis of induced mutants of groundnut (*Arachis hypogaea* L.). *J. Genet.*, 76: 201-208.
- Bhat, R. S., Patil, V. U., Chandrashekar, T. M., Sujay, V., Gowda, M. V. C. and Kuruvinashetti, M. S., 2008, Recovering flanking sequence tags of miniature inverted-repeat transposable element by thermal asymmetric interlaced-PCR in peanut. *Curr. Sci.*, 95 (4): 452-453.
- Bhat, R. S., Sujay, V., Mukri, G., Khedikar, Y. P., Sarvamangala, C., Yeri, S. B., Jadhav, S. S., Pujer, S. B., Narasimhulu, R., Varshakumari, Azharudheen, T. P. M., Varshney, R. K., Nadaf, H. L. and Gowda, M. V. C., 2012, Utility of peanut mapping populations developed at UAS Dharwad, India. Paper presented In: *VI International Conference on Legume Genetics and Genomics (ICLGG)*, Hyderabad Marriott Hotel & Convention Centre Hyderabad, India, 2-7 October 2012.
- Bonavia, D., 1982, Precera'mico peruano, Los Gavilanes, oasis en la historia del hombre. In: *Corporacio'n Financiera de Desarrollo* Ed Cofide, S. A., e Instituto Arqueolo'gico Alema'n., Peru.
- Bonin, A., Paris, M., Després, L., Tetreau, G., David, J. P. and Kilian, A., 2008, A MITE-based genotyping method to reveal hundreds of DNA polymorphisms in an animal genome after a few generations of artificial selection. *BMC Genom.*, 9 (1): 459.
- Bravo, J. P., Hoshino, A. A., Angelici CMLCD, L. C. and Gimenes, M., 2006, Transferability and use of microsatellite markers for the genetic analysis of the germplasm of some *Arachis* section species of the genus *Arachis*. *Genet. Mol. Biol.*, 29 (3): 516-524.
- Britten, R. J. and Davidson, E. H., 1969, Gene regulation for higher cells: a theory. *Science*, 165 (891): 349-357.
- Burow, M., Simpson, C., Faries, M., Starr, J. and Paterson, A., 2009, Molecular biogeographic study of recently described B and A genome *Arachis* species, also providing new insights into the origins of cultivated peanut. *Genome*, 52 (2): 107-119.
- Burow, M. D., Simpson, C. E., Starr, J. L. and Paterson, A. H., 2001, Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): Broadening the gene pool of a monophyletic polyploid species. *Genetics*, 159 (2): 823-837.

- Burton, G. and De Vane, E., 1953, Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agron. J.*, 45 (10): 478-481.
- Chapman, J. M., Cooper, J. D., Todd, J. A. and Clayton, D. G., 2003, Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Hum. Hered.*, 56 (1-3): 18-31.
- Charlesworth, B., Sniegowski, P. and Stephan, W., 1994, The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature*, 371 (6494): 215-220.
- Chen, X., Zhu, W., Azam, S., Li, H., Zhu, F., Li, H., Hong, Y., Liu, H., Zhang, E. and Wu, H., 2013, Deep sequencing analysis of the transcriptomes of peanut aerial and subterranean young pods identifies candidate genes related to early embryo abortion. *Plant Biotechnol. J.*, 11 (1): 115-127.
- Chénais, B., Caruso, A., Hiard, S. and Casse, N., 2012, The impact of transposable elements on eukaryotic genomes: From genome size increase to genetic adaptation to stressful environments. *Gene*, 509 (1): 7-15.
- Cuc, L. M., E. S. Mace, J. H. Crouch, V. D. Quang, T. D. Long, and R. K. Varshney, 2008: Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). *BMC Plant Biol.*, 8: 55.
- Dwivedi, S. L., Gurtu, S., Chandra, S., Yuejin, W. and Nigam, S. N., 2001, Assessment of genetic diversity among selected groundnut germplasm. I: RAPD analysis. *Plant Breed.*, 120 (4): 345-349.
- FAO, 2010, Food and Agriculture Organization of The United Nation. FAOSTAT database, <http://www.fo.org/faostat/collections,subset>, Agri.
- Feng, Q., Zhang, Y., Hao, P., Wang, S., Fu, G., Huang, Y., Li, Y., Zhu, J., Liu, Y. and Hu, X., 2002, Sequence and analysis of rice chromosome 4. *Nature*, 420 (6913): 316-320.
- Fernandez, A. and Krapovickas, A., 1994, Cromosomas y evolucion en *Arachis* (*Leguminosae*). *Bonplandia*, 8 (1-4): 187-220.
- Fisher, R. A. and Yates, F., 1963, Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Edinburgh.
- Fonceka, D., Tossim, H. A., Rivallan, R., Faye, I., Sall, M. N., Ndoeye, O., Favero, A. P., Bertioli, D. J., Glaszmann, J. C. and Courtois, B., 2009, Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. *BMC Plant Biol.*, 9 (1): 103.
- Fonceka, D., Tossim, H. A., Rivallan, R., Vignes, H., Faye, I., Ndoeye, O., Moretzsohn, M. C., Bertioli, D. J., Glaszmann, J. C. and Courtois, B., 2012a, Fostered and left behind alleles in peanut: interspecific QTL mapping reveals footprints of domestication and useful natural variation for breeding. *BMC Plant Biol.*, 12 (1): 26.
- Fonceka, D., Tossim, H. A., Rivallan, R., Vignes, H., Lacut, E., de Bellis, F., Faye, I., Ndoeye, O., Leal-Bertioli, S. C. M. and Valls, J. F. M., 2012b, Construction of chromosome segment substitution lines in peanut (*Arachis hypogaea* L.) using a wild synthetic and QTL mapping for plant morphology. *PLoS One*, 7 (11): e48642.
- Gibbons, R. W., Bunting, A. H. and Smartt, J., 1972, The classification of varieties of groundnut (*Arachis hypogaea* L.). *Euphytica*, 21 (1): 78-85.
- Gomez, S. M., Narayana, M., Schubert, A. M., Ayers, J. L., Baring, M. R. and Burow, M. D., 2009, Identification of QTLs for pod and kernel traits in cultivated peanut by bulked segregant analysis. *Electron. J. Biotechnol.*, 12 (2): 3-4.
- Gowda, M. V. C., Bhat, R. S., Motagi, B. N., Sujay, V., Varshakumari and Bhat, S., 2010, Association of high-frequency origin of late leaf spot resistant mutants with *AhMITE1* transposition in peanut. *Plant Breed.*, 129 (5): 567-569.
- Gowda, M. V. C., Bhat, R. S., Sujay, V., Kusuma, P., Varshakumari, Bhat, S. and Varshney, R. K., 2011, Characterization of *AhMITE1* transposition and its association with the mutational

- and evolutionary origin of botanical types in peanut (*Arachis* spp.). *Plant Syst. Evol.*, 291 (3-4): 153-158.
- Gowda, M. V. C., Nadaf, H. L. and Sheshagiri, R., 1996, The role of mutations in intraspecific differentiation of groundnut (*Arachis hypogaea* L.). *Euphytica*, 90 (1): 105-113.
- Grabiele, M., Chalup, L., Robledo, G. and Seijo, G., 2012, Genetic and geographic origin of domesticated peanut as evidenced by 5S rDNA and chloroplast DNA sequences. *Plant Syst. Evol.*, 298 (6): 1151-1165.
- Green, M. M., 1988, Mobile DNA elements and spontaneous gene mutation. *Banbury Rep.*, 30: 41-50.
- Gregory, W., 1968, A radiation breeding experiment with peanuts. *Radiat. Bot.*, 8 (2): 81-147.
- Grzebelus, D., Gładysz, M., Macko-Podgórn, A., Gambin, T., Golis, B., Rakoczy, R. and Gambin, A., 2009, Population dynamics of miniature inverted-repeat transposable elements (MITs) in *Medicago truncatula*. *Gene*, 448 (2): 214-220.
- Haley, C. S. and Knott, S. A., 1992, A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, 69 (4): 315-324.
- Halward, T., Stalker, H. T. and Kochert, G., 1993, Development of an RFLP linkage map in diploid peanut species. *Theor. Appl. Genet.*, 87 (3): 379-384.
- Hanson, C., Robinson, H. and Comstock, R., 1956, Biometrical studies of yield in segregating populations of Korean Lespedeza. *Agron. J.*, 48 (6): 268-272.
- He, G. and Prakash, C., 2001, Evaluation of genetic relationships among botanical varieties of cultivated peanut (*Arachis hypogaea* L.) using AFLP markers. *Genet. Resour. Crop Evol.*, 48 (4): 347-352.
- Herselman, L., 2003, Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. *Euphytica*, 133 (3): 319-327.
- Hilu, K. W. and Stalker, H. T., 1995, Genetic relationships between peanut and wild species of *Arachis* sect. *Arachis* (*Fabaceae*): Evidence from RAPDs. *Plant Syst. Evol.*, 198 (3): 167-178.
- Husted, L., 1933, Cytological studies on the peanut, *Arachis*. I. *Cytologia*, 5 (1): 109-117.
- Husted, L., 1936, Cytological studies on the peanut, *Arachis*. II. *Cytologia*, 7 (3): 396-423.
- IBPGR/ICRISAT, 1992, Descriptors for groundnut. International Board of Plant Genetic Resources and International Crops Research Institute For the Semi-Arid Tropics, Rome, Italy and Patancheru, Andhra Pradesh, India.
- IndiaStat, 2013, Groundnut production statistical department of agriculture and cooperation, Govt. of India, <http://www.indiastat.com/default.aspx>.
- Jiang, H., Huang, L., Ren, X., Chen, Y., Zhou, X., Xia, Y., Huang, J., Lei, Y., Yan, L. and Wan, L., 2013, Diversity characterization and association analysis of agronomic traits in a Chinese peanut (*Arachis hypogaea* L.) mini-core collection. *J. Integr. Plant Biol.*, 56 (2): 159-69.
- Jiang, N., Bao, Z., Zhang, X., Eddy, S. R. and Wessler, S. R., 2004, Pack-MULE transposable elements mediate gene evolution in plants. *Nature*, 431 (7008): 569-573.
- Jiang, X. and Zhou, Y., 1987, The value of the radiation induced mutation "Fushi" in peanut crop breeding. *Mutat. Breed. Newslett.*, No. 30: 2-3.
- Johal, G. S., Hulbert, S. H. and Briggs, S. P., 1995, Disease lesion mimics of maize: a model for cell death in plants. *Bio-Essays*, 17 (8): 685-692.
- Johnson, H. W., Robinson, H. and Comstock, R., 1955, Genotypic and phenotypic correlations in soybeans and their implications in selection. *Agron. J.*, 47 (10): 477-483.
- Khedikar, Y., Gowda, M. V. C., Sarvamangala, C., Patgar, K., Upadhyaya, H. and Varshney, R., 2010, A QTL study on late leaf spot and rust revealed one major QTL for molecular

breeding for rust resistance in groundnut (*Arachis hypogaea* L.). *Theor. Appl. Genet.*, 121 (5): 971-984.

- Kilian, A., 2008, DARt-based whole genome profiling and novel information technologies in support system of modern breeding of groundnut. *Proc. 3rd International Conference for Peanut Genomics and Biotechnology on "Advances in Arachis through Genomics and Biotechnology"* (AAGB), 4-8 November 2008, ICRISAT, Patancheru, India, p. 2.
- Kochert, G., Stalker, H. T., Gimenes, M., Galgaro, L., Lopes, C. R. and Moore, K., 1996, RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *Am. J. Bot.*, 83 (10): 1282-1291.
- Koilkonda, P., Sato, S., Tabata, S., Shirasawa, K., Hirakawa, H., Sakai, H., Sasamoto, S., Watanabe, A., Wada, T., Kishida, Y., Tsuruoka, H., Fujishiro, T., Yamada, M., Kohara, M., Suzuki, S., Hasegawa, M., Kiyoshima, H. and Isobe, S., 2012, Large-scale development of expressed sequence tag-derived simple sequence repeat markers and diversity analysis in *Arachis* spp. *Mol. Breed.*, 30 (1): 125-138.
- Kottapalli, P., Upadhyaya, H. D., Kottapalli, K. R., Payton, P., Dwivedi, S., Burow, M., David, K. O., Sanogo, S. and Puppala, N., 2011, Population structure and diversity in Valencia peanut germplasm collection. *Crop Sci.*, 51 (3): 1089-1100.
- Krapovickas, A., 1969, The origin, variability and spread of the groundnut (*Arachis hypogaea*). In: *The domestication and exploitation of plant and animals*. Eds. Ucko, R. J. and Dimbleby, W. C., Greald Duckworth Co. Ltd, London, pp. 427-441.
- Krapovickas, A., 1973, Evolution of the genus *Arachis*. In: *Agricultural Genetics-Selected Topics*. Ed Moav, R., John Wiley & Sons, New York, USA, pp. 135-151.
- Krapovickas, A. and Gregory, W. C., 1994, Taxonomía del género *Arachis* (Leguminosae). *Bonplandia*, 8: 1-186.
- Kuang, H., Padmanabhan, C., Li, F., Kamei, A., Bhaskar, P. B., Ouyang, S., Jiang, J., Buell, C. R. and Baker, B., 2009, Identification of miniature inverted-repeat transposable elements (MITEs) and biogenesis of their siRNAs in the *Solanaceae*: New functional implications for MITEs. *Genome Res.*, 19 (1): 42.
- Kumar, A. and Bennetzen, J. L., 1999, Plant retrotransposons. *Annu. Rev. Genet.*, 33: 479-532.
- Larkin, P. J., Ryan, S., Brettell, R. and Scowcroft, W., 1984, Heritable somaclonal variation in wheat. *Theor. Appl. Genet.*, 67 (5): 443-455.
- Lavia, G. I., Ortiz, A. M. and Fernández, A., 2009, Karyotypic studies in wild germplasm of *Arachis* (Leguminosae). *Genet. Resour. Crop Evol.*, 56 (6): 755-764.
- Levy, A., 1976, The mutagenic activity of the acridine ethidium bromide in peanuts, *Arachis hypogaea* L. Thesis, Hebrew University, Rehovot, ISRAEL (Irsel).
- Libioulle, C., Louis, E., Hansoul, S., Sandor, C., Farnir, F., Franchimont, D., Vermeire, S., Dewit, O., De Vos, M. and Dixon, A., 2007, Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13. 1 and modulates expression of PTGER4. *PLoS Genetics*, 3 (4): e58.
- Lim, J., 1979, Site-specific instability in *Drosophila melanogaster*: the origin of the mutation and cytogenetic evidence for site specificity. *Genetics*, 93 (3): 681-701.
- Liu, K. and Muse, S. V., 2005, PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21 (9): 2128-2129.
- Lodha, M., Johari, R., Sharma, K. and Mehta, S., 1985, Photosynthesis and translocation rate in *Arachis hypogaea* L. mutants. *Biochemie und Physiologie der Pflanzen*, 180 (5): 337-343.
- Lu, C., Chen, J., Zhang, Y., Hu, Q., Su, W. and Kuang, H., 2012, Miniature inverted-repeat transposable elements (MITEs) have been accumulated through amplification bursts and play important roles in gene expression and species diversity in *Oryza sativa*. *Mol. Biol. Evol.*, 29 (3): 1005-1017.

- Lush, J. L., 1940, Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. *J. Anim. Sci.*, 1940 (1): 293-301.
- Ma, J. and Bennetzen, J. L., 2004, Rapid recent growth and divergence of rice nuclear genomes. *Proc. Natl. Acad. Sci.*, 101 (34): 12404-12410.
- Mao, L., Wood, T. C., Yu, Y., Budiman, M. A., Tomkins, J., Woo, S., Sasinowski, M., Presting, G., Frisch, D. and Goff, S., 2000, Rice transposable elements: a survey of 73,000 sequence-tagged-connectors. *Genome Res.*, 10 (7): 982-990.
- Mathur, R. K., Manivel, P. and Samdur, M. Y., 1999, Girnar 1 nlm—A new narrow leaf mutant of groundnut. *Indian J. Genet. Plant Breed.*, 59 (4): 527-530.
- Mathur, R. K., Manivel, P., Samdur, M. Y. and Paria, P., 2001, Girnar 1 CLM—a new chemically induced curly leaf groundnut mutant. *Indian J. Genet. Plant Breed.*, 61 (2): 196.
- McClintock, B., 1978, Mechanisms that rapidly reorganize the genome. *Proc. Stadler Symp.*, pp. 25-47.
- Mondal, S. and Badigannavar, A. M., 2009, Molecular diversity and association of SSR markers to rust and late leaf spot resistance in cultivated groundnut (*Arachis hypogaea* L.). *Plant Breed.*, 129 (1): 68-71.
- Mondal, S., Hande, P. and Badigannavar, A. M., 2013, Identification of transposable element markers for a rust (*Puccinia arachidis* Speg.) resistance gene in cultivated peanut. *J. Phytopatho.*, 10.1111/Jph.12220.
- Monden, Y., Naito, K., Okumoto, Y., Saito, H., Oki, N., Tsukiyama, T., Ideta, O., Nakazaki, T., Wessler, S. R. and Tanisaka, T., 2009, High potential of a transposon mPing as a marker system in *japonica* × *japonica* cross in rice. *DNA Res.*, 16 (2): 131-140.
- Moretzsohn, M. C., Barbosa, A. V. G., Alves-Freitas, D. M. T., Teixeira, C., Leal-Bertioli, S., Guimarães, P. M., Pereira, R. W., Lopes, C. R., Cavallari, M. M. and Valls, J. F. M., 2009, A linkage map for the B-genome of *Arachis* (*Fabaceae*) and its synteny to the A-genome. *BMC Plant Biol.*, 9 (1): 40.
- Moretzsohn, M. C., Gouvea, E. G., Inglis, P. W., Leal-Bertioli, S. C., Valls, J. F. and Bertioli, D. J., 2013, A study of the relationships of cultivated peanut (*Arachis hypogaea*) and its most closely related wild species using intron sequences and microsatellite markers. *Ann. Bot.*, 111 (1): 113-126.
- Moretzsohn, M. C., Leoi, L., Proite, K., Guimarães, P. M., Leal-Bertioli, S. C. M., Gimenes, M. A., Martins, W. S., Valls, J. F. M., Grattapaglia, D. and Bertioli, D. J., 2005, A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (*Fabaceae*). *Theor. Appl. Genet.*, 111 (6): 1060-1071.
- Mouli, C. and Kale, D. M., 1982, Gamma ray induced Spanish bunch mutant with large pod groundnut. *Oleagineux*, 37: 583-588.
- Mouli, C., Kale, D. M. and Patil, S. H., 1979, Sequential flowering large pod Trombay groundnuts (Abst.). *Proc. Symposium on the Role of Induced Mutations in Crop Improvement*, 9-11 September, Hyderabad, India, pp. 248-253.
- Mouli, C., Kale, D. M. and Patil, S. H., 1986, Inheritance of sequential flowering pattern in the mutants of groundnut cultivar, Robut 33-1. *Curr. Sci.*, 55: 1185-1187.
- Mouli, C., Kale, D. M. and Patil, S. H., 1989, Mutation research on groundnut in India. In: *Recent Advances in Genetics and Cytogenetics*. Eds. Farook, S. A. and Khan, I. A., Premier Publication House, Hyderabad, India, pp. 141-153.
- Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E. and Buckler, E. S., 2009, Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell*, 21 (8): 2194-2202.
- Nagaraj, G., 1988, Chemistry and utilization in groundnut. Indian Council of Agricultural Research, New Delhi, India.

- Nagy, E. D., Guo, Y., Tang, S., Bowers, J. E., Okashah, R. A., Taylor, C. A., Zhang, D., Khanal, S., Heesacker, A. F. and Khalilian, N., 2012, A high-density genetic map of *Arachis duranensis*, a diploid ancestor of cultivated peanut. *BMC Genom.*, 13 (1): 469.
- Nevers, P., Shepherd, N. S. and Saedler, H., 1986, Plant transposable elements. *Adv Bot Res.*, 12: 103-203.
- Nielen, S., Campos-Fonseca, F., Leal-Bertioli, S., Guimarães, P., Seijo, G., Town, C., Arrial, R. and Bertioli, D., 2010, FIDEL-a retrovirus-like retrotransposon and its distinct evolutionary histories in the A and B genome components of cultivated peanut. *Chromosome Res.*, 18 (2): 227-246.
- Nielen, S., Vidigal, B. S., Leal-Bertioli, S. C., Ratnaparkhe, M., Paterson, A. H., Garsmeur, O., D'Hont, A., Guimaraes, P. M. and Bertioli, D. J., 2012, Matita, a new retroelement from peanut: characterization and evolutionary context in the light of the *Arachis* A-B genome divergence. *Mol. Genet. Genom.*, 287 (1): 21-38.
- Norden, A. J., 1973, Breeding of the cultivated peanut (*Arachis hypogaea* L.). *Proc. Peanuts Culture and Uses*, Oklahoma, pp. 175-208.
- Oki, N., Yano, K., Okumoto, Y., Tsukiyama, T., Teraishi, M. and Tanisaka, T., 2008, A genome-wide view of miniature inverted-repeat transposable elements (MITEs) in rice, *Oryza sativa* ssp. *japonica*. *Genes Genet. Syst.*, 83 (4): 321-329.
- Pandey, M. K., Gautami, B., Jayakumar, T., Sriswathi, M., Upadhyaya, H. D., Gowda, M. V. C., Radhakrishnan, T., Bertioli, D. J., Knapp, S. J., Cook, D. R. and Varshney, R. K., 2012, Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea*). *Plant Breed.*, 131 (1): 139-147.
- Panse, V. G. and Sukhatme, P. V., 1954, Statistical methods for agricultural workers. ICAR, New Delhi.
- Patel, M., Jung, S., Moore, K., Powell, G., Ainsworth, C. and Abbott, A., 2004, High-oleate peanut mutants result from a MITE insertion into the *FAD2* gene. *Theor. Appl. Genet.*, 108 (8): 1492-1502.
- Patil, S. H., 1966, Mutations induced in groundnut by X rays. *Indian J. Genet. Plant Breed.*, 26A: 334-348.
- Perry, A., 1968, A radiation breeding experiment with peanuts, iv. Effects of the vegetative and reproductive phases of the branching system (NC4-18.5 KR). *Radiat. Bot.*, 8: 109-119.
- Prasad, M. V. R., 1989, Genetic differentiation in *Arachis hypogaea* L. In: *Recent Advances in Genetics and Cytogenetics*. Eds. Farook, S. A. and Khan, I. A., Premier Publishing House, Hyderabad, India, pp. 53-55.
- Prasad, M. V. R., Kaul, S. and Jain, H. K., 1984, Induced mutants of peanut for canopy and pod bearing characters. *Indian J. Genet. Plant Breed.*, 44: 25-34.
- Qingshu, Q., Wenguang, H. and Fuyu, S., 1994, Mutation breeding in China: achievements and prospects in groundnut. *Int. Arachis Newslet.*, 14: 11-12.
- Rao, N. G., 2007, Statistics for Agricultural Sciences. Oxford and IBH Publishing company, New Delhi, India, pp. 98-99.
- Ratnaparkhe, M. B., 2012, Comparative and evolutionary studies of major peanut allergen genes. Paper presented In: *Plant and Animal Genome XX Conference*, San Diego, CA, USA, 14-18 January 2012, pp. W345.
- Ratnaparkhe, M. B., Wang, X., Li, J., Compton, R. O., Rainville, L. K., Lemke, C., Kim, C., Tang, H. and Paterson, A. H., 2011, Comparative analysis of peanut NBS-LRR gene clusters suggests evolutionary innovation among duplicated domains and erosion of gene microsystemy. *New Phytol.*, 192 (1): 164-178.
- Ravi, K., Vadez, V., Isobe, S., Mir, R. R., Guo, Y., Nigam, S. N., Gowda, M. V. C., Radhakrishnan, T., Bertioli, D. J. and Knapp, S. J., 2011, Identification of several small main-effect QTLs and

a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). *Theor. Appl. Genet.*, 122 (6): 1119-1132.

- Reddy, P., Reddi, M., Raju, B. and Ali, S., 1977, Creation of genetic variability by recourse to irradiation in groundnut (*Arachis hypogaea* L.). *Oleagineux*, 32 (2): 59-62.
- Ribaut, J., William, H., Khairallah, M., Worland, A. and Hoisington, D., 2001, Genetic basis of physiological traits. *Application of Physiology in Wheat Breeding*. Eds. MP Reynolds, JI Ortiz-Monasterio and A McNab: 29-47.
- Robinson, H., Comstock, R. E. and Harvey, P., 1949, Estimates of heritability and the degree of dominance in corn. *Agron. J.*, 41(8): 353-359.
- Roeder, K., Bacanu, S. A., Sonpar, V., Zhang, X. and Devlin, B., 2005, Analysis of single-locus tests to detect gene/disease associations. *Genet. Epidemiol.*, 28 (3): 207-219.
- Sambrook, J. and Russell, D. W., 2001, Rapid isolation of yeast DNA. In: Sambrook, J. & Russell, D.W., eds. *Molecular Cloning, a Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, pp. 631–632.
- Santiago, N., Herráiz, C., Goñi, J. R., Messeguer, X. and Casacuberta, J. M., 2002, Genome-wide analysis of the emigrant family of MITEs of *Arabidopsis thaliana*. *Mol. Biol. Evol.*, 19 (12): 2285-2293.
- Sarvamangala, C., Gowda, M. V. C. and Varshney, R. K., 2011, Identification of quantitative trait loci for protein content, oil content and oil quality for groundnut (*Arachis hypogaea* L.). *Field Crops Res.*, 122 (1): 49-59.
- Schaid, D. J., McDonnell, S. K., Hebring, S. J., Cunningham, J. M. and Thibodeau, S. N., 2005, Nonparametric tests of association of multiple genes with human disease. *Am. J. Hum. Genet.*, 76 (5): 780-793.
- Scheible, W.-R., Fry, B., Kochevenko, A., Schindelasch, D., Zimmerli, L., Somerville, S., Loria, R. and Somerville, C. R., 2003, An *Arabidopsis* mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from *Streptomyces* species. *Plant Cell*, 15 (8): 1781-1794.
- Seijo, G., Lavia, G. I., Fernandez, A., Krapovickas, A., Ducasse, D. A., Bertioli, D. J. and Moscone, E. A., 2007, Genomic relationships between the cultivated peanut (*Arachis hypogaea*, *Leguminosae*) and its close relatives revealed by double GISH. *Am. J. Bot.*, 94 (12): 1963-1971.
- Seijo, J. G., Lavia, G. I., Fernandez, A., Krapovickas, A., Ducasse, D. and Moscone, E. A., 2004, Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaensis* are the wild diploid progenitors of *A. hypogaea* (*Leguminosae*). *Am. J. Bot.*, 91 (9): 1294-1303.
- Shirasawa, K., Bertioli, D. J., Varshney, R. K., Moretzsohn, M. C., Leal-Bertioli, S. C., Thudi, M., Pandey, M. K., Rami, J. F., Fonceka, D., Gowda, M. V., Qin, H., Guo, B., Hong, Y., Liang, X., Hirakawa, H., Tabata, S. and Isobe, S., 2013, Integrated consensus map of cultivated peanut and wild relatives reveals structures of the A and B genomes of *Arachis* and divergence of the legume genomes. *DNA Res*, 10.1093/dnares/dss1042.
- Shirasawa, K., Hirakawa, H., Tabata, S., Hasegawa, M., Kiyoshima, H., Suzuki, S., Sasamoto, S., Watanabe, A., Fujishiro, T. and Isobe, S., 2012a, Characterization of active miniature inverted-repeat transposable elements in the peanut genome. *Theor. Appl. Genet.*, 124 (8): 1429-1438.
- Shirasawa, K., Koilkonda, P., Aoki, K., Hirakawa, H., Tabata, S., Watanabe, M., Hasegawa, M., Kiyoshima, H., Suzuki, S., Kuwata, C., Naito, Y., Kuboyama, T., Nakaya, A., Sasamoto, S., Watanabe, A., Kato, M., Kawashima, K., Kishida, Y., Kohara, M., Kurabayashi, A., Takahashi, C., Tsuruoka, H., Wada, T. and Isobe, S., 2012b, In silico polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. *BMC Plant Biol.*, 12 (1): 80.
- Shivaraj, A. and Rao, G., 1962, Effects of fast neutrons on seeds of groundnut. *Ind. Oilseeds J.*, 11: 24-30.

- Singh, A. and Simpson, C., 1994, Biosystematics and genetic resources. In: *The Groundnut Crop. A Scientific Basis for Improvement* (Ed. J. Smartt), Chapman and Hall, London, United Kingdom, pp. 96-137.
- Singh, A. K., 1988, Putative genome donors of *Arachis hypogaea* (Fabaceae), evidence from crosses with synthetic amphidiploids. *Plant Syst. Evol.*, 160 (3): 143-151.
- Singh, R. K. and Chaudhary, B., 1979, Biometrical methods in quantitative genetic analysis. Kalyani Publishers, Ludhiana, pp. 55-90.
- Sivasubramanian, S. and Menon, M., 1973, Heterosis and inbreeding depression in rice. *Madras Agric. J.*, 60: 1139.
- Smartt, J., 1960, Genetic instability and outcrossing in the groundnut variety Mani Pintar. *Nature*, 180: 1070-1071.
- Smartt, J., 1990, The groundnut, *Arachis hypogaea* L. . In: *Grain legumes: evolution and genetic resources* (2nd Ed.) Cambridge University Press, Cambridge, UK, pp. 30-84.
- Smartt, J. and Gregory, W., 1967, Interspecific cross-compatibility between the cultivated peanut *Arachis hypogaea* L. and other members of the genus *Arachis*. *Oleagineux*, 22: 455-459.
- Subbarao, P. V., Subramanyam, P. and Reddy, P. M., 1990, A modified nine points diseases scale for assessment of rust and late leaf spot of groundnut. *Proc. Second International Congress of French Phytopathological Society*, 28-30 November 1990, Montpellier, France, p. 25.
- Subrahmanyam, P., McDonald, D., Waliar, F., Reddy, L. J., Nigam, S. N., Gibbons, R. W., Rao, V. R., Singh, A. K., Pande, S., Reddy, P. M. and Rao, P. V. S., 1995, Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information Bulletin, 47, ICRISAT, Patancheru, Andhra Pradesh.
- Subramanian, V., Gurtu, S., Rao, R. C. N. and Nigam, S. N., 2000, Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome*, 43 (4): 656-660.
- Sujay, V., Gowda, M. V. C., Pandey, M. K., Bhat, R. S., Khedikar, Y. P., Nadaf, H. L., Gautami, B., Sarvamangala, C., Lingaraju, S., Radhakrishnan, T., Knapp, S. J. and Varshney, R. K., 2012, QTL analysis and construction of consensus genetic map for foliar disease resistance based on two RIL populations in cultivated groundnut (*Arachis hypogaea* L.). *Mol. Breed.*, 30 (2): 773-788.
- Tanksley, S. D. and Nelson, J. C., 1996, Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.*, 92 (2): 191-203.
- Todorovska, E., Dimitrova, A., Stoilov, L., Christov, N., Vassilev, D. and Gecheff, K., 2013, Molecular variability in barley structural mutants produced by gamma irradiation. *Plant Mutat. Rep.*, 3 (1): 4-8.
- Valls, J. F. and Simpson, C. E., 2005, New species of *Arachis* (Leguminosae) from Brazil, Paraguay and Bolivia. *Bonplandia*, 14: 35-63.
- Van, E. A., Van Der Werf, J. and Goddard, M., 2011, The value of using DNA markers for beef bull selection in the seedstock sector. *J. Anim. Sci.*, 89 (2): 307-320.
- Varshakumari. 2013, Introgression of foliar disease resistance using synthetic amphidiploids and identification of associated QTLs in groundnut (*Arachis hypogaea* L.). *Ph. D. Thesis*, Univ. Agril. Sci., Dharwad.
- Varshakumari, Gowda, M. V. C. and Bhat, R. S., 2009, Molecular characterization of induced mutants in groundnut using RAPD markers. *Karnataka J. Agric. Sci.*, 22 (2): 276-279.
- Varshney, R. K., Bertoli, D. J., Moretzsohn, M. C., Vadez, V., Krishnamurthy, L., Aruna, R., Nigam, S. N., Moss, B. J., Seetha, K. and Ravi, K., 2009, The first SSR-based genetic linkage map for cultivated groundnut (*Arachis hypogaea* L.). *Theor. Appl. Genet.*, 118 (4): 729-739.
- Venkatachalam, P. and Jayabalan, N., 1997, Frequency and spectrum of viable mutations in groundnut induced by physical and chemical mutagens. *Crop Res.*, 14: 61-76.

- Wang, K. and Sheffield, V. C., 2005, A Constrained-Likelihood Approach to Marker-Trait Association Studies. *Am. J. Hum. Genet.*, 77 (5): 768.
- Wang, M. L., Sukumaran, S., Barkley, N. A., Chen, Z., Chen, C. Y., Guo, B., Pittman, R. N., Stalker, H. T., Holbrook, C. C. and Pederson, G. A., 2011, Population structure and marker-trait association analysis of the US peanut (*Arachis hypogaea* L.) mini-core collection. *Theor. Appl. Genet.*, 123 (8): 1307-1317.
- Wang, S., Basten, C. and Zeng, Z., 2007, Windows QTL cartographer 2.5. *Thesis*, North Carolina State University, Raleigh, NC, USA.
- Weber, C. R. and Moorthy, B., 1952, Heritable and nonheritable relationships and variability of oil content and agronomic characters in the F₂ generation of soybean crosses. *Agron. J.*, 44 (4): 202-209.
- Wessler, S. R., 1988, Phenotypic diversity mediated by the maize transposable elements Ac and Spm. *Science*, 242 (4877): 399-405.
- Wessler, S. R., 1998, Transposable elements associated with normal plant genes. *Physiol. Plant.*, 103 (4): 581-586.
- Wessler, S. R., Bureau, T. E. and White, S. E., 1995, LTR-retrotransposons and MITEs: Important players in the evolution of plant genomes. *Curr. Opin. Genet. Dev.*, 5 (6): 814-821.
- Wright, S. I., Agrawal, N. and Bureau, T. E., 2003, Effects of recombination rate and gene density on transposable element distributions in *Arabidopsis thaliana*. *Genome Res.*, 13 (8): 1897-1903.
- Wynne, J. C. and Coffelt, T. A., 1982, Genetics of *Arachis hypogaea* L. In: *Peanut Science and Technology*. Eds. Pattee, H. E. and Young, C. T., APRES, Yoakum/Ty, pp. 50-94.
- Yeri, S. B., Shirasawa, K., Pandey, M. K., Gowda, M. V. C., Sujay, V., Shriswathi, M., Nadaf, H. L., Motagi, B. N., Lingaraju, S., Bhat, A. R. S., Varshney, R. K., Krishnaraj, P. U. and Bhat, R. S., 2014, Development of NILs from heterogeneous inbred families for validating the rust resistance QTLs in peanut (*Arachis hypogaea* L.). *Plant Breed.*, 133: 80-85.
- Yu, A., Lepère, G., Jay, F., Wang, J., Bapaume, L., Wang, Y., Abraham, A. L., Penterman, J., Fischer, R. L. and Voinnet, O., 2013, Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense. *Proc. Natl. Acad. Sci.*, 110 (6): 2389-2394.
- Zhao, M. X., Sun, H. Y., Ji, R. R., Hu, X. H., Sui, J. M., Qiao, L. X., Chen, J. and Wang, J. S., 2013, In vitro mutagenesis and directed screening for salt-tolerant mutants in peanut. *Euphytica*, 193 (1): 89-99.

Appendix I: Mean performance of groundnut mutants and their parents used in study

Sl. No.	NPB	NSB	LPB	MSL	LL	LW	PL	PW	NPP	PYP	SP	SM KW	TW	Oleic acid	Protein	OIL	R (90)	LLS (90)
1	6.5	6.5	9.6	18.5	6.4	3.0	2.8	1.2	18.5	18.6	74.5	55.5	38.8	35.8	27.9	47.3	8.0	8.0
2	8.5	8.5	13.1	16.9	5.3	2.1	3.3	1.4	8.0	14.5	65.5	44.0	33.5	52.9	26.2	44.9	6.5	7.5
3	6.5	6.5	9.5	26.6	5.2	2.3	3.0	1.4	28.5	12.4	72.0	48.0	33.5	40.1	29.4	43.5	7.0	8.5
4	4.0	4.0	8.0	19.2	3.4	1.6	3.7	0.9	15.5	11.3	64.0	45.0	30.0	46.0	24.7	40.3	7.0	8.5
5	4.0	4.0	7.3	16.3	5.4	2.3	3.4	1.4	12.0	9.1	66.0	49.0	39.0	56.3	23.7	47.6	7.0	7.5
6	5.0	5.0	4.0	8.6	3.8	2.0	1.3	0.8	3.5	2.1	66.5	31.3	42.0	44.7	26.8	46.8	6.0	7.5
7	9.5	9.5	6.0	14.9	4.0	2.0	3.0	1.2	13.0	11.5	74.0	35.5	37.0	46.7	24.2	47.0	7.0	7.5
8	6.0	6.0	12.2	17.7	5.5	2.4	3.3	1.6	23.0	12.3	65.0	57.5	44.0	43.3	26.1	47.1	7.5	7.0
9	8.0	8.0	10.8	17.9	4.7	2.1	4.0	1.4	17.0	9.8	67.5	59.5	46.0	47.3	26.5	41.8	8.0	7.5
10	4.0	4.0	10.5	22.8	5.1	2.0	3.0	1.4	9.0	9.05	65.5	68.0	26.0	50.1	27.0	44.1	7.5	7.5
11	8.0	8.0	10.5	17.1	4.4	1.7	3.3	1.6	13.5	16.4	72.5	53.0	48.0	47.2	29.7	41.8	6.0	9.0
12	5.5	5.5	5.6	14.5	3.1	2.1	3.2	1.2	16.0	8.6	69.0	50.0	37.5	48.3	26.3	45.5	7.0	8.5
13	6.5	6.5	12.0	19.1	4.2	2.2	3.7	1.6	17.0	11.6	70.0	56.0	51.5	62.1	29.8	42.8	6.5	8.5
14	6.0	6.0	11.6	22.7	5.4	2.9	3.1	1.2	24.5	13.9	88.0	67.5	36.0	36.3	24.6	43.0	7.0	8.5
15	5.0	5.0	9.6	17.2	4.2	1.8	4.1	1.5	13.5	9.3	66.0	63.0	54.5	36.5	24.5	48.1	6.5	9.0
16	9.0	9.0	12.4	20.7	4.5	1.4	3.6	1.5	11.5	11.4	78.0	59.0	48.0	45.8	26.6	40.5	6.5	9.0
17	4.0	4.0	4.7	10.6	3.0	1.7	2.2	1.0	19.5	7.8	74.5	37.5	22.5	45.5	27.2	43.3	7.0	8.5
18	6.0	6.0	3.3	7.1	3.3	1.3	2.4	1.1	13.5	7.4	76.5	39.0	30.5	48.2	31.0	45.5	6.5	9.0
19	8.5	8.5	4.2	5.3	2.4	1.3	2.9	1.2	8.5	10.4	70.5	30.5	29.0	49.2	28.6	53.7	6.0	8.5
20	5.5	5.5	3.6	7.3	3.1	1.4	2.8	1.2	12.5	13.2	71.5	57.5	34.5	49.0	26.7	43.9	6.5	9.0
21	4.0	4.0	4.8	8.4	2.6	1.6	3.2	1.2	9.0	8.9	75.5	63.5	44.5	43.8	30.3	42.8	7.5	8.5
22	8.5	8.5	5.5	10.5	3.7	1.7	2.8	1.2	14.0	7.4	70.0	47.5	33.0	40.7	31.5	42.5	7.5	9.0
23	6.0	6.0	11.3	15.9	4.8	2.4	3.4	1.4	20.5	20.9	73.0	50.0	44.0	55.2	24.0	42.4	8.0	9.0
24	8.0	8.0	9.0	18.8	7.5	2.6	3.3	1.5	15.0	10.8	67.0	40.0	31.0	50.9	26.9	44.6	8.5	7.5
25	4.0	4.0	2.6	5.8	2.8	7.7	3.2	1.1	3.5	2.2	62.5	44.0	46.0	51.6	26.1	44.5	7.0	7.0
26	4.5	4.5	5.6	7.1	2.9	1.1	4.0	1.3	12.0	9.4	81.0	56.0	42.0	45.9	25.7	48.5	8.0	8.0
27	4.5	4.5	3.9	5.5	2.5	1.4	3.0	1.2	14.0	7.0	68.5	55.5	33.0	51.3	20.8	46.2	6.5	7.0

Contd...

28	5.5	5.5	3.4	4.6	3.4	4.1	2.2	1.1	14.5	6.7	73.0	74.5	36.0	49.3	29.1	44.7	6.5	8.0
29	6.5	6.5	3.4	4.4	2.3	5.3	2.5	1.1	6.5	6.4	68.5	68.0	39.0	56.6	22.8	46.1	5.5	6.0
30	8.5	8.5	4.0	6.3	3.3	1.9	3.3	1.2	9.0	7.2	68.5	54.5	34.5	47.8	25.7	47.6	6.5	8.0
31	7.0	7.0	4.3	7.4	3.2	1.1	2.6	1.2	14.5	6.5	70.5	59.5	39.0	46.9	27.1	45.4	5.0	8.0
32	4.5	4.5	5.4	6.0	2.9	1.4	2.7	1.1	8.0	5.6	75.0	61.5	42.0	49.1	30.4	44.3	6.0	8.0
33	9.0	9.0	10.4	17.4	4.2	2.4	2.7	1.3	16.5	8.7	64.5	61.5	37.5	48.1	28.1	45.6	8.0	6.0
34	4.0	4.0	9.3	18.1	4.1	1.8	4.2	1.3	15.0	15.6	70.5	67.0	40.0	44.1	32.4	44.5	5.5	8.5
35	4.5	4.5	4.5	7.3	2.9	1.5	3.6	1.4	14.0	6.3	63.5	69.5	40.5	48.5	26.9	45.1	7.0	7.5
36	5.0	5.0	3.4	6.2	4.1	2.1	3.5	1.2	15.0	7.4	66.5	57.5	43.5	63.3	29.2	45.4	5.5	7.0
37	6.5	6.5	6.6	13.3	4.2	1.7	3.7	1.3	19.0	6.9	63.5	59.5	40.0	47.1	25.7	42.0	5.5	7.5
38	11.0	11.0	11.3	17.8	5.4	2.3	2.7	1.4	19.0	7.1	66.0	28.0	28.0	40.4	28.4	40.7	8.0	8.0
39	7.0	7.0	12.4	13.9	5.7	1.4	2.7	1.2	7.5	4.8	66.0	60.0	28.0	55.0	33.4	48.0	8.0	8.0
40	4.5	4.5	10.7	13.6	3.2	1.2	3.0	1.4	23.5	12.9	70.0	52.5	40.5	46.7	28.0	44.5	8.0	8.5
41	4.0	4.0	10.8	29.1	5.5	3.3	3.7	1.4	16.0	7.2	67.5	58.0	43.5	42.3	31.7	44.1	8.0	8.5
42	9.5	9.5	19.2	28.9	5.2	3.2	4.0	1.4	15.5	8.1	70.0	41.0	46.0	52.6	24.5	42.6	8.0	8.5
43	11.5	11.5	15.5	15.7	5.3	3.3	3.4	1.5	17.0	7.6	64.5	43.5	37.5	49.6	27.0	42.8	7.5	9.0
44	6.0	6.0	9.7	22.6	5.4	2.9	2.9	1.6	16.5	5.8	73.0	59.0	35.0	50.0	23.5	45.0	7.5	8.0
45	8.5	8.5	9.1	22.0	4.8	2.1	3.2	1.2	14.0	7.6	68.5	57.0	39.5	50.7	25.7	47.4	7.0	7.0
CD	1.4	0.1	0.8	1.5	0.2	0.4	0.4	0.2	2.6	1.4	6.7	13.6	7.6	5.1	3.4	4.2	1.6	1.7
CV	10.6	7.0	4.9	5.2	2.9	8.2	6.1	6.2	9.0	7.5	4.8	12.7	9.8	5.2	6.2	4.7	11.2	10.4
SEm±	0.5	0.4	0.3	0.5	0.1	0.1	0.1	0.1	0.9	0.5	2.4	4.8	2.7	1.8	1.2	1.5	0.6	0.6

NPB: Number of primary branches
LL: Leaf length
NPP: Number of pods/plant
TW: Test weight
LLS (90): LLS at 90 DAS

NSB: Number of secondary branches
LW: Leaf width
PYP: Pod yield/plant
Protein: Protein percentage

LPB: Length of primary branches
PL: Pod length
SP: Shelling percentage
OIL: Oil percentage

MSL: Main stem length
PW: Pod width
SMKW: Sound mature kernel weight
R (90): Rust at 90 DAS

Appendix II: Sequence information of AhTE markers used in study

Sl. No.	Marker Name	Sequence	
		Forward primer (5'-3')	Reverse primer (5'-3')
1	AhTE107	CACTCGGAAAACCAAACCTCC	CAAGAAGCCTACAAAAATGGTG
2	AhTE113	TTATGGCATCCAAGGGTCAT	TCCCTAGTACCCGGATGATT
3	AhTE119	AAGCTAGCGATGGCGATAAC	TTTATGCCCGCAAACCTTTTT
4	AhTE121	CTGGCAATACGCCCCAAG	AAAACCTGCATTGTTGAAATTGG
5	AhTE130	TGGCTTGTCTCACTTATCCTCA	TGAAAAATTCATTAACCTTAACACCTT
6	AhTE148	GAATCCAGAGCTGCCATTGT	ATTTTTGCCTCTCAGCCTCA
7	AhTE159	CGCCATGACCACCTCTAAGT	GCAAAGAAATGCCTGCAAAC
8	AhTE164	AACCACCACCATCTTTTAAGG	TCGTCTCCTCTAAGGTTTGCTT
9	AhTE180	GAAAGAATCCGTGGGATATTCA	TTCACAATGAAAGAGGAGAATCAA
10	AhTE181	GAAAGTACGCCATGTTGTGGTA	TTTAAGTCGAGTTTTCCGCTAA
11	AhTE190	AAATTTATCTGTTTCGATCGAGTG	TCGAACCGACTCTGGTTTTAAT
12	AhTE202	GAGAGCGGTAATTTTTATGATTG	TACTTTTGCGACTTTGTCTCCA
13	AhTE205	TTCATTTCCATCTGTCTCATGC	TCGTCTCCTCTAAGGTTTGCTT
14	AhTE213	CCTTAAATATCCACCACCCAAA	CATCAGTCATCCATGCAGATTT
15	AhTE218	ATGGAGTTTCAGCAAATGAGGT	GAAAATCAAACTGGCTGCAT
16	AhTE222	ATTCACACCCAAAACAAAACAT	ACGAGGATCGATCGTTATACAT
17	AhTE231	TTTGGAGGCCAATTATGTGTTT	TAGGTGGGTACTCCCTGAAG
18	AhTE248	CAGCCTTAACCCTAGCACTTTTT	TTAGAATTGTAGGTGGTGGGTTG
19	AhTE254	ACTTCAAATCTGACGCACCTACT	CAATTCATGTTCTAGCAAGGGTTA
20	AhTE268	GAAGGCCTATCTCATTTTGTGTTG	CGCCGTTACCTAGGTTTTAGAAT
21	AhTE278	CTTTTCTTTTGTAATGAATCTGTTTTT	GCAATGCTAATATGCTAAATCGTT
22	AhTE282	CCCAAAAACCTACTTGGTTATGGTT	GAATGCGAAGAAGAAGGAGAAA
23	AhTE287	CACTGAGTTTAAATGGTATTTCCAA	AATTCAATTATACATGTTCCGTGT
24	AhTE288	CACCTGTCATGTCACCTAAAACA	TGAATGGTAAATTGTGTAAGAGGAA
25	AhTE296	ATTTTAAGAAACCCGAAACCAAA	CTTCACACGAGTGAGTAGTGACC
26	AhTE300	GGTTGTGTAATCCAATTTCCACT	TCGTCCCATTATAGTTCTTTTG
27	AhTE303	AAGATGCATACCTTTGGTTTTCT	CTGCACTTAAGCCAACCTTCTCAT
28	AhTE305	CTCATTGACCATGACTATCACGA	CGAAACGTTCTCCTATTTGAATG
29	AhTE324	TTTCAACAATGCAAACCCAA	GTGGATGTGGGAGTTTGTA
30	AhTE333	TTGTGCTTTTGGGATGTTCA	ATGTCGGTAATTGATTCGGC
31	AhTE335	GACAAAGGTAGAGGTTAATCCCA	CCTAACATTGGGTTGGAGGA
32	AhTE343	CGATCGCTACTTGCTACCAC	GGACATCAATCAAGAGGCGT
33	AhTE347	ACACTGAGTCACGCAGCAAT	TGAAGTTTGGAGAGGATGCC
34	AhTE359	ATCGTTGCTTGTTAGCACGG	AAAAGCGCAAAGAAATGGGT
35	AhTE360	GGATATGATGCCCATAGCTGA	TGCTGACTACTTGCAATGCC
36	AhTE361	TTGGAGTTTATCTCAAACCTCTGAAG	CCGGTAGTCCGGTAAATTA
37	AhTE363	GAAGCTTCCGTTGCCTTAA	TAATGGATAGATGCTCCGCC

Contd...

38	AhTE372	GCAATTTGGCATAGCCTCTC	CGTATTGACAAGGGTTCGGT
39	AhTE373	TGGTGTTGGAGATGGAGACA	CACGTGAACAGCTTCCAAAA
40	AhTE376	TGCAATGCAATGTGGATGAT	ATGCCAGCCTCACGTTTTAT
41	AhTE388	CCAGGGGATCAGAACCAAAT	TTTTTATTAAAGCGGTCTCCTTATC
42	AhTE398	TCAGCCCAACAGAATACACAA	GGACTTTGTTGGGGTGGTAG
43	AhTE416	GAGTTACATCGGTATTGAATATGTGA	CTCCGTGATTGAGGCGTTA
44	AhTE422	TGGCGTAATCTTTTAAGAACCAA	AGAATTAATGTCATCAAACGAATG
45	AhTE426	CAACCCATGATTTGTGAATTAAG	TGACTACAATGTTTGGTCATTTTG
46	AhTE437	TGGCTTTTGGGTGTGTATGA	GCCACGAGAGAATCCAAAAA
47	AhTE442	TTGTCTTTTCCCCTCCCTT	AAAGATGGTCAATGATTCTTTGTT
48	AhTE445	ACACTGCTCGCAGTTTGAGA	AAAGCAGGTGATTAGTGTACCTT
49	AhTE446	GTGCCACGAGGTACACGATA	AGACACACACCACACGCATT
50	AhTE459	CTCGACGAGCAGATTCTTC	CACGTCGACACTCAAATAAGTAAAA
51	AhTE467	AGGTTCCCGTTGAATGAAAT	TTCGCGAGCGTAAGGTTAGT
52	AhTE471	CGCCATTGTGAACTCGATAG	TTGCCTTGTGGTCCAAAAAT
53	AhTE477	GGTGGTGGCCATTTCTCTT	TTTGCCCATTTTTGGTTCTG
54	AhTE489	TGACTACAATGTTTGGTCATTTTG	GAACCACCCATGATTTGTGA
55	AhTE491	ATTATAACAGTATACAATTTCTAT	GAAGTTTTATTTGGTTCAGAATT
56	AhTE496	TTGTGTGTTTGAGACATGGATTT	TCCCGTAATTTCTGTAACATTTTA
57	AhTE500	ACTGCCTGATCCGAGCTTAG	CAGTTAATTATTTATGACCACACA
58	AhTE501	GAAGCAGGGAACACCCACTA	TCTCATGCCCTTAATTAACACAAA
59	AhTE502	TTCAGCAGAGCCCTTTCAAT	TTGCCAATTACCATTTATTTT
60	AhTE504	CTTCACAAGTCCAACGCAGA	TTCCACGAAGGTTCAATTTCA

Appendix III: Allele type among the groundnut mutants and their parents at AhTE marker loci

Sl. No.	Mutant	AhTE 296	AhTE 254	AhTE 121	AhTE 359	AhTE 107	AhTE 148	AhTE 119	AhTE 159	AhTE 205	AhTE 218	AhTE 278	AhTE 305	AhTE 335	AhTE 489	AhTE 504	AhTE 130
1	Spanish Improved	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
2	TG 1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1
3	TG 2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
4	TGM 8	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
5	TGM 14	1	2	2	1	2	2	1	2	1	1	1	1	1	2	2	1
6	TGM 19	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
7	TGM 20	1	2	0	1	0	2	1	2	2	1	1	1	2	2	2	1
8	TGM 36	1	1	1	2	2	1	1	2	2	1	1	1	2	1	1	1
9	TGM 37	1	2	2	1	2	2	1	2	2	1	1	1	0	2	2	1
10	TGM 43	2	1	1	2	2	2	2	2	2	1	2	1	2	1	1	1
11	TGM 44	2	2	2	1	1	2	1	2	1	2	1	1	1	2	2	1
12	TGM 46	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
13	TGM 47	1	2	2	1	2	2	1	2	2	1	1	1	2	2	2	1
14	TGM 50	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
15	TGM 51	1	1	2	1	2	2	1	2	1	1	1	1	2	2	1	1
16	TGM 54	1	2	2	1	2	2	1	2	2	1	1	1	2	2	2	1
17	TGM 58	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
18	TGM 96	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
19	TGM 101	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
20	TGM 111	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
21	TGM 112	0	1	1	1	2	0	1	2	2	2	2	2	2	1	2	0
22	TGM 113	1	2	1	1	2	2	1	2	2	2	2	2	2	1	2	1
23	TGM 117	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	TGM 119	1	2	1	1	2	2	1	2	2	2	2	2	2	1	2	1

Contd...

25	TGM 156	0	1	0	0	2	2	1	0	0	0	0	0	0	0	0	0
26	TGM 138	1	1	1	1	2	2	1	2	2	2	2	2	2	1	2	1
27	TGM 165	2	1	1	1	2	2	2	2	1	2	2	2	2	1	2	1
28	TGM 167	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	1
29	TGM 178	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	1
30	TGM 189	2	2	1	1	2	2	2	2	2	2	2	2	2	1	2	1
31	TGM 193	2	2	1	1	2	2	2	2	2	2	2	0	2	1	0	0
32	TGM 196	2	2	2	1	2	2	2	2	2	2	2	2	2	1	2	1
33	TGM 198	1	2	1	2	2	2	2	2	1	2	2	2	2	1	2	1
34	TGM 213	1	1	2	1	2	2	1	2	2	2	2	2	2	1	2	1
35	TGM 214	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	1
36	TGM 215	2	1	0	0	2	2	2	2	0	0	2	0	0	1	0	0
37	TGM 218	2	1	1	1	2	2	2	2	1	2	2	2	2	1	2	1
38	TGM 223	2	1	0	2	2	2	2	2	1	2	2	2	2	0	2	1
39	TGM 225	2	1	0	1	2	2	2	2	2	2	1	2	2	1	2	1
40	TAG 24	1	2	1	1	2	2	1	2	2	2	2	2	2	1	2	1
41	TG 16	2	1	2	1	2	2	1	2	1	2	1	1	1	2	2	1
42	Somnath	1	2	1	1	2	2	1	2	2	1	1	1	1	1	2	1
43	TG 18 A	1	2	2	1	2	2	1	2	2	1	1	1	2	2	2	1
44	TG 9	2	1	1	2	1	2	2	2	1	1	2	0	2	1	2	1
45	TG 66	2	1	1	1	2	2	2	2	1	2	2	2	2	1	2	1

Contd...

Sl. No.	Mutant	AhTE 477	AhTE2 68	AhTE 333	AhTE 426	AhTE 113	AhTE 373	AhTE 467	AhTE 164	AhTE 248	AhTE 324	AhTE 422	AhTE 437	AhTE 459	AhTE 360	AhTE 372	AhTE 222
1	Spanish Improved	2	2	2	2	2	2	1	1	2	2	1	2	1	2	1	2
2	TG 1	1	2	2	1	1	1	0	2	2	1	2	1	0	1	2	1
3	TG 2	2	2	2	2	2	2	1	1	2	2	1	2	1	2	1	2
4	TGM 8	2	2	2	2	2	2	1	1	2	2	1	2	1	2	1	2
5	TGM 14	2	2	2	2	1	2	1	1	2	1	0	1	1	0	2	2
6	TGM 19	2	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1
7	TGM 20	1	2	2	2	1	2	2	2	2	1	1	1	2	0	1	1
8	TGM 36	1	2	2	1	1	2	2	2	2	2	2	1	2	0	2	1
9	TGM 37	1	0	2	2	1	2	2	2	2	1	1	1	2	1	1	1
10	TGM 43	2	2	2	1	1	2	2	2	2	1	2	1	2	1	1	1
11	TGM 44	2	2	2	2	2	2	2	1	2	1	1	1	2	2	1	1
12	TGM 46	2	2	2	1	1	2	2	2	2	1	1	2	2	0	1	1
13	TGM 47	1	2	2	2	1	2	2	2	2	1	1	1	2	1	1	1
14	TGM 50	2	2	2	2	2	2	1	1	2	2	1	2	1	2	2	2
15	TGM 51	1	2	2	2	1	2	1	1	2	2	1	1	1	1	2	0
16	TGM 54	1	0	2	2	1	2	2	2	2	1	1	1	2	1	1	1
17	TGM 58	2	2	2	2	2	2	1	1	2	2	1	2	1	2	1	2
18	TGM 96	2	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1
19	TGM 101	2	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1
20	TGM 111	2	2	2	1	1	2	2	2	0	1	1	2	2	1	1	1
21	TGM 112	2	2	2	1	1	2	2	0	2	1	1	2	2	0	1	1
22	TGM 113	2	2	2	1	1	2	2	0	2	1	1	2	2	1	2	1
23	TGM 117	1	1	1	1	1	1	2	1	1	2	2	1	2	1	1	1
24	TGM 119	2	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1

Contd...

25	TGM 156	0	0	2	0	0	0	0	0	0	0	1	0	0	1	0	1
26	TGM 138	2	2	2	1	1	2	2	2	2	1	1	2	2	0	1	1
27	TGM 165	2	2	2	2	1	2	2	1	2	1	1	2	2	1	2	1
28	TGM 167	2	2	2	2	1	2	2	2	2	1	1	2	2	1	2	1
29	TGM 178	2	2	2	2	1	2	2	2	2	1	1	2	2	1	2	1
30	TGM 189	2	2	2	2	1	2	2	2	2	1	1	2	2	1	2	2
31	TGM 193	0	2	2	0	1	2	2	0	0	0	1	2	2	1	2	2
32	TGM 196	2	2	2	1	1	2	2	2	2	1	1	2	2	1	1	2
33	TGM 198	2	2	2	1	1	2	1	1	2	1	2	2	1	1	2	1
34	TGM 213	2	2	2	1	1	2	2	2	2	1	1	2	2	1	0	1
35	TGM 214	2	2	2	1	1	2	2	0	2	0	0	2	2	1	2	1
36	TGM 215	2	2	2	1	0	2	2	0	0	1	1	2	2	1	1	2
37	TGM 218	2	2	2	1	1	2	2	0	2	1	1	2	2	0	0	1
38	TGM 223	2	2	2	1	2	2	1	1	2	2	1	2	1	0	2	2
39	TGM 225	1	2	2	1	1	2	1	0	2	1	1	2	1	0	2	0
40	TAG 24	2	2	2	1	1	2	2	2	2	2	1	2	2	1	1	1
41	TG 16	2	2	2	2	2	2	2	1	2	1	1	1	2	2	1	1
42	Somnath	1	2	2	1	1	2	2	2	2	1	2	1	2	1	1	1
43	TG 18 A	1	0	2	2	1	2	2	0	2	1	1	1	2	1	1	1
44	TG 9	2	2	2	1	1	2	2	0	2	0	2	1	2	1	2	2
45	TG 66	2	2	2	1	1	2	2	1	2	1	1	0	2	1	2	1

Contd...

Sl. No.	Mutant	AhTE 180	AhTE 303	AhTE 416	AhTE 446	AhTE 501	AhTE 491	AhTE 398	AhTE 181	AhTE 282	AhTE 213	AhTE 442	AhTE 445	AhTE 343	AhTE 190	AhTE 361
1	Spanish Improved	2	2	1	2	2	2	2	2	0	2	0	2	2	2	2
2	TG 1	1	1	2	1	1	1	2	2	1	2	2	2	2	2	2
3	TG 2	2	2	1	2	2	2	2	2	0	0	2	2	2	2	2
4	TGM 8	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2
5	TGM 14	2	1	2	1	2	1	2	2	1	2	2	2	2	2	2
6	TGM 19	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2
7	TGM 20	2	1	1	2	1	1	2	2	0	2	2	2	0	2	2
8	TGM 36	1	1	1	1	1	1	2	2	0	2	2	2	2	2	2
9	TGM 37	2	1	1	1	1	1	2	2	0	2	2	2	2	2	2
10	TGM 43	2	1	1	1	1	2	2	2	1	2	2	2	2	2	2
11	TGM 44	2	1	2	2	1	2	2	2	1	2	2	2	2	2	2
12	TGM 46	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2
13	TGM 47	2	1	1	1	1	1	2	2	1	2	2	2	2	2	2
14	TGM 50	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2
15	TGM 51	2	1	1	2	2	1	2	2	1	2	2	2	2	0	2
16	TGM 54	2	1	1	1	1	1	2	2	1	2	2	2	2	2	2
17	TGM 58	2	2	1	2	2	2	2	2	0	2	2	2	2	2	2
18	TGM 96	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2
19	TGM 101	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2
20	TGM 111	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2
21	TGM 112	2	2	1	1	1	1	2	2	0	2	2	2	2	2	2
22	TGM 113	2	2	0	1	1	1	2	2	1	2	2	2	2	2	2
23	TGM 117	1	1	2	2	2	1	1	2	1	2	2	2	2	2	2
24	TGM 119	2	2	1	2	1	1	2	0	1	0	0	2	2	2	2

Contd...

25	TGM 156	2	2	1	2	0	1	0	2	1	0	0	0	2	0	0	2
26	TGM 138	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2	2
27	TGM 165	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2	2
28	TGM 167	2	2	1	1	2	2	2	2	1	2	2	2	2	2	2	2
29	TGM 178	2	2	1	1	2	2	2	2	1	2	2	2	2	2	2	2
30	TGM 189	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2	2
31	TGM 193	2	2	1	2	2	2	2	2	1	2	0	0	2	0	2	2
32	TGM 196	2	2	1	2	1	2	2	2	1	2	2	2	2	2	2	2
33	TGM 198	2	2	1	2	2	1	2	2	1	2	2	2	2	2	2	2
34	TGM 213	2	2	1	2	1	2	2	2	0	2	2	2	2	0	2	2
35	TGM 214	2	2	1	1	2	2	2	2	1	2	2	2	2	2	2	2
36	TGM 215	2	2	0	2	2	0	2	0	1	0	0	2	0	0	2	2
37	TGM 218	2	2	1	2	2	2	2	2	1	0	2	2	2	2	2	2
38	TGM 223	2	2	1	2	2	2	2	2	0	2	2	2	2	2	2	2
39	TGM 225	2	2	1	2	1	2	2	2	1	0	2	2	2	0	2	2
40	TAG 24	2	2	1	1	1	1	2	2	1	2	2	2	2	2	2	2
41	TG 16	2	1	2	2	1	0	2	2	1	2	2	2	2	2	2	2
42	Somnath	2	1	2	1	1	1	2	2	1	2	2	2	2	2	2	2
43	TG 18 A	2	1	1	1	1	1	2	2	0	2	2	2	2	2	2	2
44	TG 9	0	1	1	1	1	0	0	2	1	2	0	0	2	2	2	0
45	TG 66	2	2	1	2	2	1	2	2	1	2	2	2	2	2	2	2

1: With *AhMITE1* insertion

2: Without *AhMITE1* insertion

0: Absence of the specific PCR product

ASSOCIATION ANALYSIS FOR YIELD RELATED TRAITS AND FOLIAR DISEASE RESISTANCE USING TRANSPOSON SPECIFIC MARKERS IN A MUTANT POPULATION OF GROUNDNUT

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2014

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Major advisor

ABSTRACT

An effort was made to identify association of transposable element (TE)-specific markers with productivity and nutritional traits in a mutant population consisting of thirty five mutants and their parents in groundnut. Field evaluation of the genotypes during *kharif* 2012 showed significant genotypic differences and considerable variability for majority of the traits.

Genotyping of mutant population with 47 TE markers showed very high genotype-discriminating power in terms of polymorphism information content (PIC) for a large proportion of the markers. The overall polymorphism across mutant-parent pairs was 27.7%. Highest polymorphism of 72.3% was observed between Spanish Improved and TG 1. Three mutants, TG 2, TGM 8 and TGM 58 failed to show any polymorphism for the markers tested when compared to their parent (Spanish Improved).

Marker-trait association was analysed by single marker analysis. Markers exhibiting significant association with number of pods/plant, pod yield/plant, shelling percentage, sound mature kernel weight (SMKW), test weight and disease resistance were identified. AhTE324 (44.41%), AhTE305 (23.55%), AhTE372 (18.00%), AhTE491 (10.52%), AhTE373 (22.80%), AhTE130 (11.85%) and AhTE491 (11.85%) showed the highest R^2 (phenotypic variance explained) towards number of pods/plant, test weight, resistance to LLS resistance and rust, pod yield/plant, shelling percentage and SMKW, respectively. The markers identified as significantly associated with the traits were validated by employing the independent mutants with phenotypic change in positive and negative direction, but no evidence in favour of marker-trait association could be observed.

Mutants with unusual phenotypes like disease mimicking leaves, mosaic leaves, fused leaflets and crinkle leaves, and long and narrow leaves were observed. However, these phenotypes did not show any significant association with any of the markers tested. Hence, the markers identified in this study as associated with important yield related traits and resistance to LLS and rust need to be validated across other genotypes before employed for groundnut breeding.