EVALUATION OF INTROGRESSION LINES FOR RODUCTIVITY TRAITS AND RESISTANCE TO LATE LEAF SPOT AND RUST AND AN INITIATIVE TOWARDS MARKER ASSISTED BACKCROSSING IN TMV 2 IN GROUNDNUT (*Arachis hypogaea* L.)

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By

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CERTIFICATE

This is to certify that the thesis entitled "EVALUATION OF INTROGRESSION LINES FOR PRODUCTIVITY TRAITS AND RESISTANCE TO LATE LEAF SPOT AND RUST AND AN INITIATIVE TOWARDS MARKER ASSISTED BACKCROSSING IN TMV 2 IN GROUNDNUT (*Arachis hypogaea* L.) " submitted by Ms. SONALI PARATWAGH, for the degree of MASTER OF SCIENCE (AGRICULTURE) in MOLECULAR BIOLOGY AND BIOTECHNOLOGY, to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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ABBREVIATIONS

Particulars	Abbreviations
U	Unit
l	Liter/s
hr	Hour/s
m	Meter/s
g	Gram/s
sq	Square
mt	Metric Tonne/s
%	Per cent
min	Minute/s
sec	Second/s
mha	Million Hectare/s
bp	Base pair
µl	Microliter/s
mM	Millimolar
ml	Milliliter/s
^{0}C	Degree celsius
mg	Milligram/s
kg	Kilogram/s
$T_{10}E_1$	Tris (10 mM)-EDTA (1 mM)
pmol	Picomole/s
µmol	Micromole/s
ng	Nanogram/s
cm	Centimeter/s
cM	Centi Morgan
LLS	Late leaf spot
Tm	Melting temperature
QTL	Quantitative trait locus/loci
RPM	Revolutions Per Minute
IL	Introgression line
CTAB	Cetyl-trimethyl-ammonium Bromide
AhTE	*Arachis hypogaea* transposable element
SSR	Simple sequence repeat
LG	Linkage group
MABC	Marker assisted backcross breeding

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) also known as peanut, is one of the most important oilseed, food and feed legume crops belonging to Fabaceae. Groundnut originated in South and Central America, probably in the foothills of Andes Mountain where it was domesticated ~3,500 years ago and later spread to the tropical and sub-tropical countries. The crop is cultivated over 25.4 mha with a production of about 45.22 mt globally and Indian share accounts for 5.25 mha with a production of 9.47 mt (FAOSTAT 2013). The major states cultivating groundnut are Gujarat, Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra.

Several biotic and abiotic constraints limit the quantity and quality of the groundnut. Majority of the commercially grown varieties belong to Spanish bunch types (*Arachis hypogaea* ssp. *fastigiata*), and they are highly susceptible to foliar diseases namely, rust caused by *Puccinia arachidis* Speg. and late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx. (McDonald *et al*., 1985; Subrahmanyam *et al*., 1985). The yield loss due to the co-occurrence of rust and LLS can go up to 70% in India when fungicides are not applied (Subrahmanyam *et al*., 1984; Subrahmanyam *et al*., 1985; Waliyar, 1990). Chemical control method increases the cost of cultivation and also leads to environmental and health hazards. Hence, development of resistant cultivars is considered as the best strategy.

Significant progress has been made in developing resistant cultivars through conventional breeding approaches. But the success of breeding for disease resistance is influenced by the availability and identification of resistance sources and combining resistance with high productivity and desirable pod features. Not many cultivated varieties are resistant to LLS and rust. Valencia landraces and wild species of groundnut possess high level of resistance to foliar diseases, but the resistance is generally linked to low productivity, late maturity, poor adaptability and undesirable pod features (Wynne *et al*., 1991; Singh *et al*., 1997). Germplasm originating from secondary centers of diversity were resistant to foliar diseases with desirable agronomic backgrounds, but their productivity levels were low (Singh *et al*., 1997).

The genus *Arachis* contains 80 described species under nine sections according to their morphology, geographical distribution and cross-compatibility relationships (Krapovickas and Gregory, 1994; Valls and Simpson, 2005). Many of the wild *Arachis* species were found to be immune or highly resistant to rust and/or late leaf spot. Though intersectional hybridization is uncommon, intrasectional hybrids can be produced to harness the unexplored genetic variability (Gregory and Gregory, 1979). Attempts to introduce variability from wild diploid species into cultivated tetraploids have been made. Several interspecific derivatives have been developed. The first peanut cultivars released from interspecific hybridization were cv. Spancross (Hammons, 1970) and cv. Tamnut 74 (Simpson and Smith, 1975) from the cross *A. hypogaea* × *A. monticola* Krapov. and Rigoni. Subsequently, many interspecific derivatives like ICGV 87165, GPNCW 1, GPNCW2, GPNCW 3, GPNCW 4, ICGV 86699, ICGV 87167 were developed (Nigam *et al*., 1992; Stalker and Beute, 1993; Reddy *et al*., 1996; Moss *et al*., 1997). They possessed high levels of resistance to foliar diseases but the existence of strong association between undesirable traits like late maturity, inferior pod and grain characteristics with resistance prevented their commercial release.

Hence, combining higher level of resistance with high productivity and market-acceptable pod and seed feature has not been very successful though resistant sources are available among wild diploids, if not in cultivated types. This problem is mainly due to the factors such as interference among the foliar diseases, complex inheritance pattern (Bromfield and Bailey, 1972; Tiwari *et al*., 1984; Paramasivam *et al*., 1990) and undesirable linkages of resistance with other traits (Subrahamanyam *et al*., 1993) making the selection of superior genotypes difficult during the breeding programmes. But, integration of genomic tools like markers and marker assisted selection with conventional breeding approaches might enhance the precision and speedy development of improved groundnut cultivars for LLS and rust resistance (Varshney *et al*., 2014).

A considerable progress has been made in groundnut to develop and apply genomic resources in breeding for LLS and rust resistant genotypes. Groundnut genome has been sequenced (http://news.uga.edu/releases/article/first-peanut-genome sequenced). Genetic maps have been developed and analyzed for the

genomic regions governing LLS and rust resistance. A consensus map based on the recombinant inbred line (RIL) populations of TAG 24 × GPBD 4 and TG 26 × GPBD 4 has been constructed. QTL analysis in these populations identified a common genomic region on the linkage group XV governing both rust and LLS resistance (Khedikar *et al*., 2010; Sujay *et al*., 2012). Two QTL flanked by GM2009-GM1536 and IPAHM103-GM1954 were detected within this region. These two major QTL explained up to 82.27 and 82.96% phenotypic variance for rust and LLS respectively. In addition, a third QTL flanked by GM1536- GM2301/GM2079 was detected only in TAG 24 × GPBD 4, which alone could explain 62.35% of phenotypic variance for rust. GM2009-GM1536, IPAHM103-GM1954 and GM1536-GM2301/GM2079 were also linked to LLS resistance and contributed up to 67.98, 42.66 and 17.37% of phenotypic variance, respectively. Again, GM1536-GM2301/GM2079 was detected only in TAG 24 × GPBD 4. A second genomic region governing LLS was mapped on linkage group XII. This region carried a major QTL (GM1573/GM1009-pPGPseq8D09) contributing 10.27–62.34% phenotypic variance (Sujay *et al*., 2012). From a study involving mutants, a transposable element-specific marker was found to be associated with LLS resistance (Gowda *et al*., 2010). The markers linked to LLS and rust resistances were validated using diverse germplasm (Khedikar *et al*., 2010), other mapping populations (Sukruth *et al*., 2014) and near-isogenic lines (Yeri *et al*., 2014b). Therefore, these markers could be employed for marker assisted selection and marker assisted backcrossing (Varshney *et al*., 2014).

Breeding material with broad genetic base is highly desirable in order to develop LLS and rust resistant genotypes by employing the markers. Use of wild species of groundnut is known to broaden the genetic base in many crops (Gur and Zamir, 2004; Fernie *et al*., 2006; Fu *et al*., 2010; Nevo and Chen, 2010) including groundnut (Mallikarjuna *et al*., 2011). The genes from these wild species can be incorporated into cultivated groundnut through triploids, autotetraploids, amphiploids and directly from tetraploid wild species (Simpson, 2001; Bertioli *et al*., 2011). Several tetraploid groundnuts have been developed doubling the chromosomes in a diploid obtained by crossing different wild species representing A, B and K genomes. These tetraploids represented broader genetic base since they were not exposed to natural or artificial selection.

Of such tetraploids, two allotetraploids [ISATGR 278-18 (*A. duranensis* ICG 8138 × *A. batizocoi* ICG 13160, AK genomes) and ISATGR 5B (*A. magna* ICG 8966 × *A. batizocoi* ICG 8209, BK genomes)] (Robledo and Seijo, 2010) were found to be resistant to LLS and rust (Varshakumari, 2013). They were crossed to two high yielding but susceptible varieties, ICGS 76 and DH 86. F_1s of ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B were backcrossed to get BC_1 and subsequently BC_2. The selfed progenies (introgression lines, ILs) showed considerable variability for resistance to LLS and rust (Bhat *et al*., 2012). LLS and rust resistant linked markers were validated among these ILs (Sukruth *et al.*, 2014). Therefore, the advanced and stabilized generations of these ILs make up a promising genetic resource for selecting the superior lines.

With the development of such a useful genomic resource, now it is important to develop LLS and rust resistant genotypes in other widely adapted elite varieties. TMV 2 is one such a variety which is under cultivation on a large area since 1950 for its quality. TMV 2 is highly susceptible to LLS and rust diseases. Initiation of MABC programme in TMV 2 involving GPBD 4 (Gowda *et al*., 2002) as the donor of resistance (rust and LLS) requires screening of markers for foreground and background selection. With this background, the objectives of this study were

1. To assess the genetic variability for resistance to LLS and rust among the introgression lines for selecting the superior lines in groundnut.
2. To assess the extent of polymorphism between TMV 2 × GPBD 4 using transposable element markers for use in backcross breeding in groundnut.

2. REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea* L.) is an important food crop worldwide with majority of the produce crushed for oil. Domesticated groundnut suffers from various diseases and insect pests. Late leaf spot and rust are widespread and cause intense economic losses. Therefore, groundnut breeding programs are focusing on improving disease resistance. It is well accepted that the conventional breeding methods need to be complemented with broadening of the genetic base of the crop and employing the genomic resources such as molecular markers, QTL and MAS. Hence, in the recent past, the breeding strategy has changed its paradigm from conventional breeding to marker assisted selection (MAS) and marker assisted backcrossing (MABC). Literature pertaining to foliar diseases, breeding strategies, molecular markers and MAS in groundnut is reviewed in this chapter.

2.1 Foliar diseases of groundnut

The major foliar diseases of groundnut caused by fungi are rust (*Puccinia arachidis* Speg), early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (*Phaeoisariopsis personata* [(Berk. and Curt) Deighton]). LLS and rust are the most widely distributed, destructive and economically important foliar diseases causing severe damage to the crop (McDonald *et al*., 1985; Kokalis-Burelle, 1997). LLS and rust can cause substantial yield loss but when they occur together losses are further increased. For instance, rust and LLS together can cause up to 70 per cent yield loss in India though the incidence and severity vary between locations and seasons (Subrahmanyam *et al*., 1984). These foliar diseases besides reducing the yield, have an adverse effect on seed quality, oil content and grade characteristics, deteriorate the quality of plant biomass and thus render the foliage unsuitable as animal feed.

2.2 Rust

Rust disease of groundnut was first noted by Spegazzini (1884). The disease occurs in most of the groundnut growing Indian states and more intensively in South Indian states as the conditions favour the development and spread of the disease (Subrahmanyam and McDonald, 1982). Rust is identified by the appearance of brown to dark reddish-brown pustules (uredinia) on the lower surface with the upper

surface developing yellow, chlorotic spots with necrotic brown areas in the center. At later stage, the primary pustules are surrounded by secondary sori. Symptoms are mainly confined to leaflets but pustules can be seen on all the aerial parts of a plant except flowers and pegs.

2.3 Leaf spots (early and late)

Among the leaf spot diseases, LLS is distributed throughout the world and more predominant compared to early leaf spot because of its fast spreading nature. The perfect stage *Mycosphaerella berkeleyii* was described by Jenkins (1938). But, recently, it was renamed as *Phaeoisariopsis personata* (Berk and Curt) V. Arx. The pathogen perpetuates from season to season only on volunteer groundnut plants and infected plant debris, building up an inoculum reservoir for the following season (Subrahmanyam *et al*., 1995).

Leaf spots cause small necrotic flecks that enlarge and become light to dark brown. The yellow halo is either absent or less conspicuous in late leaf spot. Sporulation is common on the abaxial (lower) surface of leaves. Leaf spot lesions are not only confined to the leaf lamina, but also occur on petioles, stems and pegs leading to direct deterioration of the developing pods. Lesions caused by *C. arachidicola* are sub circular and from 1-10 mm in diameter. They are dark brown on the adaxial (upper) leaflet surface where most sporulation occurs and a lighter shade of brown on the abaxial leaflet surface. Lesions caused by *P. personata* are usually smaller, more nearly circular, and darker in colour than those of *C. arachidicola*. On the lower surfaces where most sporulation occurs, the lesions are black with a slightly rough appearance.

Leaf spots cause reduction in photosynthetic area by way of defoliation (Boote *et al*., 1980) and premature leaflet abscission. Generally, 10 to 15 per cent yield losses were reported due to late leaf spot (McDonald *et al*., 1985) worldwide and reduced seed yield could be due to reduction in dry weight, chlorophyll, protein and sugar (Ghosh and Biswas, 1995). LLS fungus produces haustoria that penetrate individual plant cells and the leaves infected with the fungus showed a marked increase in respiration (Horne *et al*., 1976).

2.4 Source of resistance

Considerable effort has been made in identifying sources of resistance to rust and early and late leaf spot (Bromfield and Cevario, 1970; Chiteka *et al.*, 1988; Anderson *et al.*, 1993). Among cultivated types, not many are really immune or resistant to foliar diseases. However, most of the wild *Arachis* species in sections *Erectoides*, *Triseminale*, *Extranervosae*, and *Rhizomatosae* showed immunity to rust with no recognizable symptoms of the disease appearing even after an incubation period of 40 days (Subrahmanyam *et al.*, 1983). Many of the resistant lines possessed "rate-reducing" components, i.e. "slow rusting" epidemiological mechanisms.

A. batizocoi (PI 298639, PI 338312), *A. duranensis* (PI 219823), *A. cardenasii* (PI 262141), *A. chacoense* (PI 276235), *A. pusilla* (PI 338449), *A. villosa* (PI 210554) and *A. correntina* (PI 331194) had increased incubation period, decreased infection frequency and reduced pustule size, spore production and spore germ inability (Subrahmanyam *et al.*, 1983). Many interspecific derivatives with the cultivated types have also been screened for resistance to rust under both field and laboratory conditions (Subrahmanyam *et al.*, 1983). Most of the interspecific derivatives showed a high degree of resistance to rust (Nigam *et al.*, 1992; Stalker and Beute, 1993; Reddy *et al.*, 1996; Moss *et al.*, 1997), however, those belonging to Virginia group possessed agronomically undesirable traits like late maturity, inferior pod and seed characteristics (Tallury *et al.*, 2009). They had small and slightly depressed uredosori that did not rupture to release the comparatively few urediospores produced.

Valencia (*A. hypogaea* subsp. *fastigiata* var. *fastigiata*) landraces originating in Peru contains germplasm lines which are resistant to late leaf spot and/or rust but are limited for utilization in groundnut breeding because of many undesirable attributes they posses like thick shell, low productivity, poor adaptation, late maturity, highly reticulated and constricted pods which are commercially unacceptable (Anderson *et al.*, 1993; Hegde *et al.*, 1995; Singh *et al.*, 1997). However, later, screening of the germplasm originating from secondary centers of diversity resulted in identification of some resistant sources with good agronomic backgrounds, but even then low productivity is the major constraint (Singh *et al.*, 1997).

2.5 Genetic basis of disease resistance

Resistance to LLS has been reported as having a digenic recessive basis (Tiwari *et al*., 1984) or being conferred by a multiple genes (Nevill, 1980). It is controlled by a combination of both, nuclear and maternal gene effects. Among nuclear gene effects, additive effect controlled majority of the variation (Pasupuleti *et al*., 2013).

Resistance to rust has been consistently reported as genetically recessive, governed by only a few genes (Bromfield and Bailey, 1972; Tiwari *et al*., 1984; Paramasivam *et al*., 1990). Motagi (2000) reported that resistance to rust is conferred by duplicate complementary genes (9:7). Singh *et al.* (1984) concluded that rust resistance in diploid species is partially dominant as compared to the recessive nature in cultivated groundnut (Kalekar *et al*., 1984; Knauft, 1987; Paramasivam *et al*., 1990). It could be predominantly controlled by additive, dominance and additive × additive and additive × dominance genetic effects (Reddy *et al*., 1987; Varman *et al*., 1991). Bromfield and Bailey (1972) noticed digenic mode of inheritance with recessive genes during the F2 population of a spontaneous cross between rust-resistant female PI 298115 and an unknown male parent. In another experiment, Kishore (1981) reported digenic (15 susceptible: 1 resistant) and trigenic mode of inheritance (63 susceptible: 1 resistant) in a study involving three susceptible and three resistant parents. Knauft and Norden (1983) observed two recessive duplicate genes governing rust resistance. Also, Tiwari *et al*. (1984) registered recessive nature of resistance. The F_2 crosses between resistant and susceptible genotypes segregated as 9 susceptible: 7 resistance. Contrary to the earlier results, Kalekar *et al*. (1984) reported the role of single recessive gene conferring resistance. In yet another experiment, rust resistance was governed by one or two or three recessive genes as evidenced from the segregation ratios of 3:1, 15:1, and 63:1, respectively (Joel *et al*., 2006).

Luo *et al*. (2005) identified genes for resistance to LLS using microarray and real time polymerase chain reaction (PCR). Fifty six genes in several functional categories were identified. Seventeen of the 20 most effective genes were validated and proposed as markers for molecular breeding. Molecular components of the initial stages of the resistance LLS was investigated by gene expression profiling

using suppression subtractive hybridization and differential screening of cDNA macroarray techniques (Nobile *et al.*, 2008). Gene expression analyses could identify more than 700 peanut unique expressed sequence tags (EST) involved in several aspects of the early stages of infection, such as components of defense signaling pathways, gene expression regulators, cell cycle controlling genes and components of the biosynthesis of transducer and antimicrobial compounds. The most significantly induced gene corresponded to a novel *O*'-methyltranferase, suggesting its involvement in the production of local lesions in *C. personatum*-resistant *A. hypogea* genotypes. These results contribute to elucidate the defense strategies of peanut and provide the framework for the generation of pathogen-resistant groundnut cultivars.

2.6 Genomic resources in groundnut

Genetic improvement of groundnut faces challenges like narrow genetic base of the cultivated gene pool and the tetraploid and complex nature of genome. Only limited genetic diversification has been achieved in the past through interspecific hybridization between cultivated groundnut and other species of section *Arachis* due to differences in ploidy levels and the linkage drag. Eliminating the linkage drag involves a lengthy process that also results in dilution of the level of resistance present in wild relatives of *Arachis*. Traits like resistance to rust and late leaf spot are difficult to manage through conventional phenotypic selection because they co-occur and lead to defoliation. Such traits can be handled with ease using markers.

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 that facilitate the identification of QTL and discovery of genes associated with tolerance/resistance to abiotic and biotic stresses and agronomic traits. These genomic resources would accelerate molecular breeding for several traits for development of superior genotypes.

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 (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) have been used in groundnut. 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 (Varshney *et al*., 2009a). SNPs have been detected from diploids (Alves *et al*., 2008; Nagy *et al*., 2012) as well as cultivated tetraploids (Chen *et al*., 2013).

The major application of molecular markers in crop improvement is in the construction of linkage maps. A linkage map (also called genetic map) shows the marker position and distance relative to each other in terms of recombination frequency worked out based on a mapping population. Genetic maps help researchers to locate gene(s) and QTL by testing for genetic linkage. This is the first step towards molecular breeding because these mapped gene/QTL controlling desired traits either can be introgressed individually or as several genes/QTL pyramided into an elite cultivar using tightly linked/perfect/functional markers.

In groundnut, molecular genetic studies initially progressed by using diploids rather than tetraploid cultivated types due to the greater simplicity of diploids as genetic models (Halward *et al*., 1993). Genetic maps for diploid groundnut have been reported by Moretzsohn *et al*. (2005) (1,230.89 cM, AA genome) and Moretzsohn *et al*. (2009) (1,294.0 cM, BB genome). The first SSR-based genetic linkage map for cultivated groundnut was developed on the recombinant inbred lines of TAG 24 × ICGV 86031 (Varshney *et al*., 2009b). It is now considered as a reference map for cultivated groundnut and has been saturated up to 191 SSR loci (Ravi *et al*., 2011). Two genetic maps based on RIL populations (ICGS 76 × CSMG 84-1 with 119 SSR loci and ICGS 44 × ICGS 76 with 82 SSR loci) segregating for drought tolerance related traits were also constructed.

Considering the importance of foliar diseases, several mapping populations segregating for LLS and rust resistance were developed using GPBD 4 as one of the parents at UAS, Dharwad (Bhat *et al*., 2012). Two hundred and sixty eight RILs of TAG 24 × GPBD 4 were used for map (462.24cM; 56 loci mapped on 14 LGs) construction using 59 SSR markers (out of total 67) (Khedikar *et al*., 2010). Similarly, a map (657.90 cM; 45 loci mapped on 8 LGs) was constructed using the RILs of TG

26 × GPBD 4 (Sarvamangala *et al*., 2011). Recently, improved genetic maps for the same populations, TAG 24 × GPBD 4 (1922.4 cM; 188 loci mapped on 20 LGs) and TG 26 × GPBD 4 (1963 cM; 181 loci mapped on 21 LGs) were constructed (Sujay *et al*., 2012). Further, using 143 markers common to the two maps, a consensus map with 225 SSR loci and total map distance of 1,152.9 cM was developed (Sujay *et al*., 2012).

Comprehensive QTL analysis using the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 could detect two genomic regions governing LLS and rust resistance (Sujay *et al*., 2012). The genomic region on LG XV carried three QTL, GM2009-GM1536, GM1536-GM2301/GM2009 and IPAHM103-GM1954, contributing for both rust and LLS resistance. The highest phenotypic variance explained (PVE) across the seasons ranged from 62.35% to 82.96% for rust resistance and 17.37% to 67.98% for LLS resistance among the three QTL. Another region on LG XII flanked by GM1573-GM1009-pPGPseq8D09 D exhibited 62.34% PVE for LLS resistance. Analysis of RILs from VG 9514 9 × TAG 24 showed strong linkage of SSR marker, GO340445 with rust resistance. The marker was closely linked (11.9 cM) to previously identified marker IPAHM103 (Mondal *et al*., 2012). Validation of these QTL and linked markers for rust and LLS resistance will be of great importance in molecular breeding for LLS and rust resistance in groundnut.

A considerable progress has been made to involve wild germplasm in the recent genomics approaches, which not only expedite QTL mapping, fine mapping and gene discovery, but also help variety development since they involve the simultaneous transfer of QTL into elite breeding lines. In general, when wild relatives are used, inbreeding after crossing results in sterility, thus making it difficult to generate a large, random array of segregants for mapping. Advanced backcross QTL (AB-QTL) populations help overcome this problem. Wild species (donor) are crossed to an elite variety (recurrent parent), and the F_1s are backcrossed. An array of BC_2 or BC_3 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).

In groundnut, such efforts began when 46 F_{10} lines (allotetraploid) derived from a cross between *Arachis hypogaea* L. (2n=4x=40) and *A. cardenasii* Krapov. & W.e. Gregory (2n=2x=20) were developed and analyzed with 73 RFLP probes and

70 RAPD primers to confirm the introgression of *A. cardenasii* chromosome segments, although markers detecting introgressed fragments could not be placed on the peanut linkage map (Garcia *et al.*, 1995). An F_2 population derived from the cross of *Arachis hypogaea* × *Arachis cardenasii* was used to identify two dominant genes conditioning resistance to the root-knot nematode *Meloidogyne arenaria.* Bulked segregant analysis could identify an *A. cardenasii* specific RAPD marker (Z3/265) linked to the resistance to the root-knot nematode (Garcia *et al.*, 1996). A diploid interspecific backcross population consisting of forty four BC_1F_1 [*Arachis stenosperma* × (*A. stenosperma* × *A. cardenasii*)] plants was developed and genotyped with RAPD and RFLP markers to construct the linkage map (Garcia *et al.*, 2005). One hundred sixty-seven RAPD loci and 39 RFLPs were mapped to 11 linkage groups, covering a total genetic map length of 800 cM.

A synthetic allotetraploid TxAG-6 (Simpson, 1991; Simpson *et al.*, 1993) was developed through the cross [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)], where *A. cardenasii* and *A. diogoi* were diploids with A genome and *A. batizocoi* has B genome diploid ancestor. A backcross population (78 BC_1F_1) was generated by crossing TxAG-6 (donor) with Florunner (recurrent parent) and used to construct a genetic map using RFLP markers (Burow *et al.*, 2001). This approach resulted in simultaneous introgression of chromatin from three wild diploid species into the two genomes of cultivated peanut.

In an effort to analyze the genome-wide segment introgressions into the background of a cultivated variety, a synthetic amphidiploid (*A. duranensis* and *A. ipaensis*) was crossed to a cultivated variety (Fleur 11) and 88 BC_1F_1 were developed (Fonceka *et al.*, 2009). The population was used to construct a SSR based genetic map. Subsequently, the BC_1F_1 population was used to develop an advanced backcross population of 142 plants (87 BC_3F_1 and 55 BC_2F_2). QTL analysis in this population identified total of 95 QTL for traits involved in peanut productivity and adaptation as well as domestication in the two water treatments (Fonceka *et al.*, 2012a) and produced 122 BC_4F_3 populations for QTL mapping for plant morphology using CSSL approach. The line × trait significant associations were assigned to 42 QTL for growth habit, plant height, plant spread and flower color (Fonceka *et al.*, 2012b).

Since RILs and ABLs are the progenies of a biparental cross involving two contrasting phenotypes, such populations may serve as the source of desirable genotypes with favourable combination of traits (Krishnappa *et al*., 2009). Hence, evaluation of RILs and ABLs developed from the parents differing for foliar disease resistance might identify superior genotypes for productivity and disease resistance traits. Also, the QTL/markers identified for LLS and rust resistance can be validated using such genotypes.

3. MATERIAL AND METHODS

The present study aims at evaluating a large number of introgression lines derived from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B for foliar disease resistance and productivity traits in order to identify superior lines. Genotyping of these introgression lines with rust and late leaf spot resistance-linked markers would not only indicate their association with disease resistance but also allow marker assisted selection. The material and methods used in evaluating and genotyping the introgression lines and screening of TMV 2 versus GPBD 4 for marker polymorphism are described in this chapter.

3.1 Experimental material

Introgression lines (ILs; BC_2F_5) (Table 1) developed from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B at the Department of Genetics and Plant Breeding, UAS, Dharwad were used along with the checks, ICGS 76, DH 86, TMV 2, JL 24, TG 26 and GPBD 4.

3.2 Experimental season and site

The field evaluation of the ILs and parents was carried out during the rainy season of 2013 at IABT Garden of Main Agricultural Research Station, UAS, Dharwad. This experimental site is located in the transitional tract of Karnataka at 15^0 13' north latitude and 75^0 07' east longitude with an altitude of 678 m above mean sea level. The soil type of the experimental block was vertisol with pH ranging from 7.0 to 7.5.

3.3 Raising the crop

The entries were sown on 3rd July 2013 in a Randomized Complete Block Design (RCBD) with a spacing of 30 cm between rows and 10 cm between plants in two replications. All the necessary agronomic practices were followed to raise a healthy crop. The crop was harvested on 25th October 2013.

Table 1: Details of groundnut introgression lines used in the study

Disease score* (DAS)				
Genotypes	**LLS (80)**	**LLS (90)**	**RUST (80)**	**RUST (90)**
ICGS 76 × ISATGR 278-18				
ICGS 76	5	7	6	8
ISATGR 278-18	3	3	3	3
11 (7-3)	6	6	6	6
14 (9-1)	5	4	5	6
20 (14-1)	8	4	8	4
39 (16-2)	8	4	8	4
24 (16-3)	5	4	5	6
25 (16-4)	4	4	4	4
27 (16-6)	4	4	4	4
28 (16-7)	4	4	4	4
29 (16-8)	4	4	4	4
30 (16-9)	4	4	4	4
31 (16-10)	4	4	4	4
32 (17-1)	4	4	4	4
38 (19-1)	4	4	4	4
39 (19-2)	4	4	4	4
40 (19-3)	4	4	4	4
41(19-4)	4	4	4	4
45 (20-2)	4	4	4	4
46 (20-3)	4	4	4	4
49 (22-3)	4	4	4	4
53 (26-1)	4	4	4	4
62 (27-3)	4	4	4	4
63 (27-4)	4	4	4	4
64 (30-2)	4	4	4	4
66 (32-3)	4	4	4	4
69 (39-2)	4	4	4	4
70 (39-3)	4	4	4	4
71(39-4)	4	4	4	4
72 (39-5)	4	4	4	4
73 (39-6)	4	4	4	4
74 (39-7)	4	4	4	4
75 (40-1)	4	4	4	4
78 (42-1)	4	4	4	4
84 (42-7)	4	4	4	4
88 (43-3)	4	4	4	4
90 (43-3)	4	4	4	4
94 (44-1)	4	4	4	4
103 (50-3)	4	4	4	4
109 (52-1)	4	4	4	4
110 (52-2)	4	4	4	4
112 (52-4)	4	4	4	4
113 (53-4)	4	4	4	4
118 (60-1)	4	4	4	4

Contd...

120 (92-2)	4	4	4	4
121 (92-3)	4	4	4	4
122 (92-4)	4	4	4	4
123 (92-5)	4	4	4	4
124 (98-1)	4	4	4	4
126 (99-5)	4	4	4	4
127 (99-6)	4	4	4	4
128 (100-1)	4	4	4	4
131 (101-3)	4	4	4	4
132 (102-3)	4	4	4	4
134 (105-3)	4	4	4	4
135 (105-4)	4	4	4	4
136 (105-5)	4	4	4	4
137 (105-6)	4	4	4	4
138 (105-7)	4	4	4	4
140 (106-1)	4	4	4	4
141 (107-1)	4	4	4	4
145 (107-10)	4	4	4	4
DH 86 × ISATGR 278-18				
DH 86	6	8	7	8
ISATGR 278-18	3	3	3	3
4 (8-2)	4	4	4	4
5 (8-3)	4	4	4	4
6 (8-4)	4	4	4	4
7 (8-5)	4	4	4	4
9 (8-7)	4	4	4	4
10 (8-8)	4	4	4	4
11 (8-9)	4	4	4	4
12 (8-10)	4	4	4	4
4 (4-3)	4	4	4	4
5 (5-7)	4	4	4	4
6 (6-11)	4	4	4	4
8 (8-11)	4	4	4	4
9 (11-1)	4	4	4	4
32 (47-11)	4	4	4	4
DH 86 × ISATGR 5B				
DH 86	5	7	6	8
ISATGR 5B	3	3	3	3
9 (17-1)	4	4	4	4
10 (17-2)	4	4	4	4
12 (17-4)	4	4	4	4
12 (2-4)	4	4	4	4

***:** Disease score as observed during the rainy season of 2012; DAS: Days after sowing;

LLS (80): Late leaf spot score at 80 days; LLS (90): Late leaf spot score at 90 days;

RUST (80): Rust score at 80 days and RUST (90): Rust score at 90 days

3.4 Observations recorded

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

3.4.1 Growth characters

1. Plant height (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 branch on the main stem was counted at the time of harvest.

3. Height of primary branches (cm)

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

4. Leaf length (cm)

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

5. Leaf width (cm)

   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.

6. Pod constriction (PC): It was recorded as absent, shallow, medium, deep or very deep based on the intensity of constriction of mature pods.

7. Pod beak (PB): Based on the intensity of pod beak, the mature pods were classified as absent, slight, medium, prominent or very prominent.

8. Pod reticulation (PR): It was recorded as absent, slight, medium, prominent or very prominent based on the extent of veination on mature pods.

Degrees of pod features	Pod constriction (PC)	Pod reticulation (PR)	Pod beak (PB)
1	Absent	Absent	Absent
3	Shallow	Slight	Slight
5	Medium	Medium	Medium
7	Deep	Prominent	Prominent
9	Very deep	Very prominent	Very prominent

3.4.2 Productivity parameters

1. Pod length (cm): The length of five dried and cleaned pods were measured and recorded using vernier callipers.
2. Pod width (cm): The width of five dried and cleaned pods were measured and recorded using vernier callipers.
3. Number of pods per plant: Number of pods per plant was counted at the time of harvest.
4. Pod yield per plant (g)

   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. Pod yield (kg/ha)

   Total weight of dried and cleaned pods obtained from net plot was used to calculate the plot yield expressed as kg/ha.

6. Test weight (g)

   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.

3.5 Screening for rust and late leaf spot resistance

LLS and rust inoculums were maintained on TMV 2 (susceptible to LLS) and mutant JL 24 (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 JL 24) were planted at every 10^{th} 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 uredinospores 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.

3.5.1 Disease scoring for rust and late leaf spot

Modified 9 point scale (1-9 score) (Subbarao *et al.*, 1990) was used for scoring rust (Table 2, Fig. 1) and LLS (Table 3, Fig. 2) disease at 90 DAS.

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 available at Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. The following statistical methods were employed for data analysis.

Table 2: Modified 9 point scale used for field screening of groundnut genotypes for resistance to rust disease

Disease score	Description	Disease severity (%)*
1	No disease	0
2	Pustules sparsely distributed, largely on lower leaves	1 - 5
3	Many pustules on lower leaves, necrosis evident, very few pustules on middle leaves	6 - 10
4	Number of pustules on lower and middle leaves, severe necrosis of lower leaves	11 – 20
5	Severe necrosis of lower and middle leaves, pustules may be present on top leaves but less severe	21 - 30
6	Extensive damage to lower leaves, middle leaves, necrotic with dense distribution of pustules on top leaves	31 - 40
7	Severe damage of lower and middle leaves, pustules densely distributed on top leaves	41 - 60
8	100 per cent damage to lower and middle leaves, pustules on top leaves	61 – 80
9	Almost all leaves withered, bare stems seen	81 - 100

***:** Per cent leaf area damaged by the disease (Subbarao *et al.,* 1990)

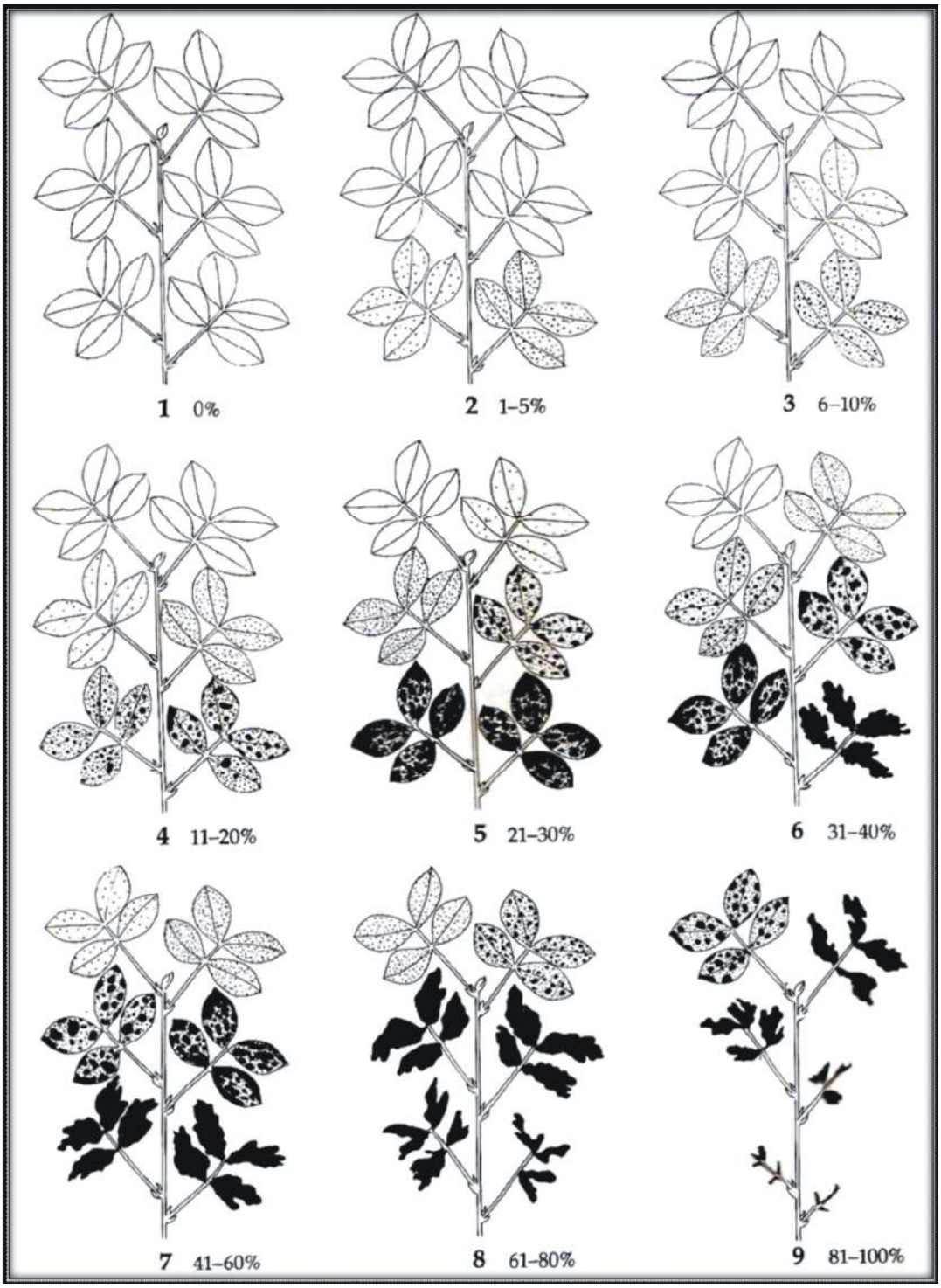


Fig. 1 Modified 9 point scale used for field evaluation of rust in groundnut

Table 3: Modified 9 point scale used for field screening of groundnut genotypes for resistance to late leaf spot disease

Disease score	Description	Disease severity (%)*
1	No disease	0
2	Lesions present largely on lower leaves; no defoliation	1 - 5
3	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaflets evident on lower leaves	6 - 10
4	Lesions present on lower and middle leaves but severe on lower leaves; defoliation of some leaflets evident on lower leaves	11 – 20
5	Lesions present on all lower and middle leaves; over 50% defoliation of lower leaves	21 - 30
6	Severe lesions on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; defoliation of some defoliation on middle leaves	31 - 40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves	41 - 60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves; some defoliation of top leaves evident.	61 – 80
9	Almost leaves defoliated, leaving bare stem; some leaflets may remain, but show severe leaf spots.	81 – 100

*: Per cent leaf area damaged by the disease (Subbarao *et al.,* 1990)

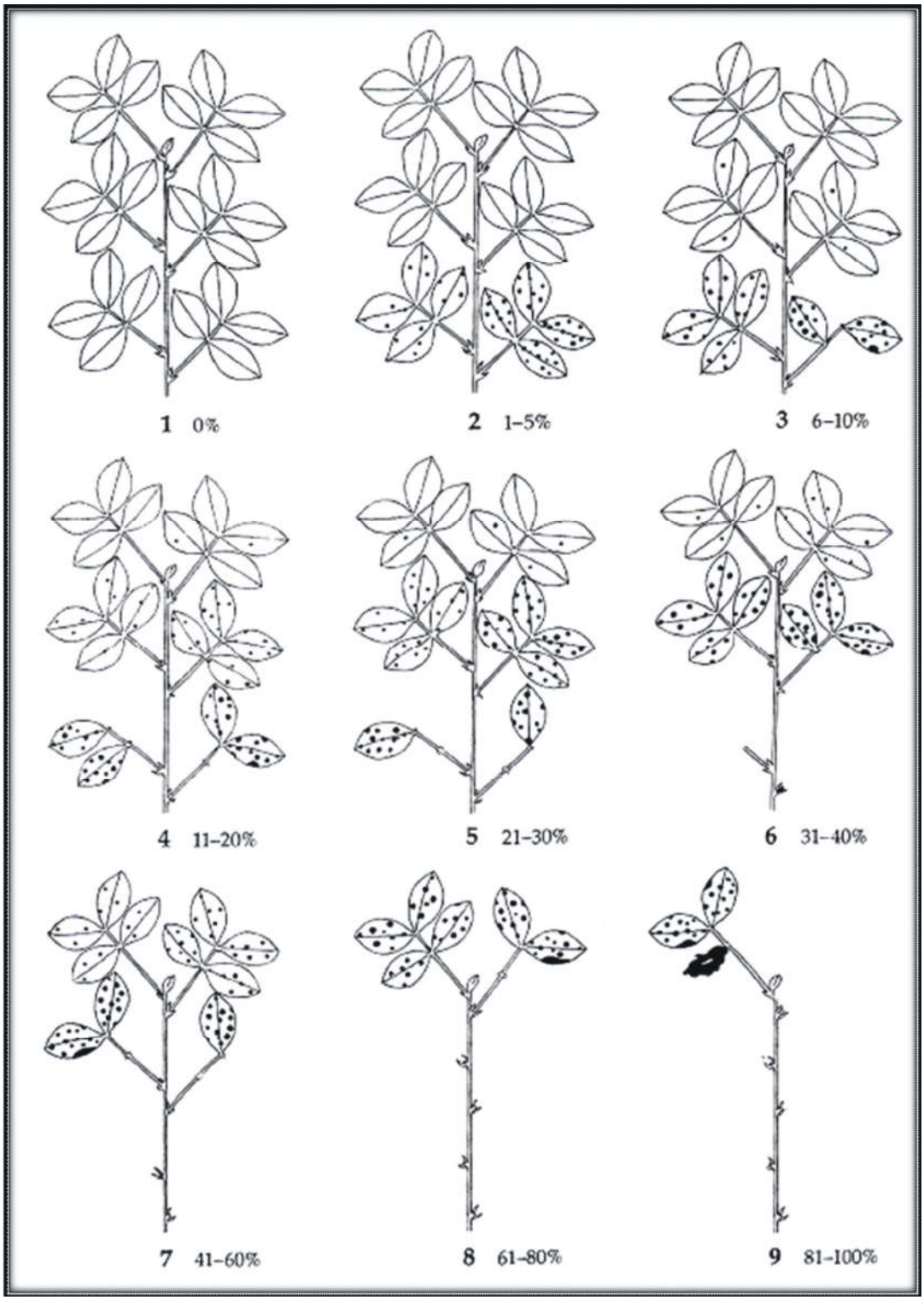


Fig. 2 Modified 9 point scale used for field evaluation of late leaf spot in Groundnut

3.6.1.1 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.

3.6.1.2 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	Cal F.
Replication	(r-1)	M_1	-	
Genotypes	(g-1)	M_2	$\sigma^2e + r\sigma^2g$	M_2/M_3
Error	(r-1) (g-1)	M_3	σ^2e	
Total	(rg-1)	$M_1+M_2+M_3$		

3.6.1.3 Estimation of genetic variability components

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

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

$$\text{Phenotypic variance } (\sigma^2p) = \sigma^2g + \text{MSS (error)} = \frac{M2 - M3}{r} + M3$$

3.6.1.4 Coefficient of variation

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

$$GCV = \frac{\sigma g}{\overline{X}} \times 100$$

$$PCV = \frac{\sigma p}{\overline{X}} \times 100$$

Where,

σg = Genotypic standard deviation

σp= Phenotypic standard deviation

$\overline{X}$= 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).

3.6.1.5 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).

3.6.1.6 Genetic advance (GA)

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

$$GA = h^2 k\sigma_p$$

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.

3.6.1.7 Genetic advance as percent of mean (GAM)

$$GAM = \frac{GA}{\overline{X}} \text{ X } 100$$

Where,

GA = Genetic advance

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

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

3.6.1.8 Correlation analysis

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

$$r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

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 Genotyping with markers

3.7.1 Genomic DNA isolation

DNA was isolated from the young leaves of introgression lines and parents grown in the field by following the modified cetyl trimethyl ammonium bromide (CTAB) method.

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 CTAB buffer (650 µl). Contents were mixed vigorously by vortexing and inverting. The tube was incubated at 65^0C 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 -20^0C.

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 either air dried by placing the tube in a hot dry bath at 37^0C for 3 hr.

9) The pellet was dissolved in 200 µl of $T_{10}E_1$.

3.7.2 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/µl, 100 ng/µl and 200 ng/µl. The DNA stocks of the samples were diluted to a working concentration of 5 ng/µl.

3.7.3 Polymerase chain reaction (PCR)

Introgression lines and their parents were genotyped with rust and LLS resistance-linked markers (Table 4) (Shirsawa *et al.*, 2012b). In addition, a marker (GO340445) linked to rust resistance (Mondal *et al.*, 2012) was also used to genotype the introgression lines. DNA amplification was performed in a 20 µl reaction mixture (Table 6) using a PCR profile provided by eppendorf Mastercycler® pro and BIO-RAD T100^{TM} Thermal cyclers (Table 7 and Table 8). GO340445 was mapped onto LG XV. Both LLS and rust resistance-linked markers (AhTE360, AhTE498, AhTE621, AhTE618 and AhTE231) were mapped onto LG XV and only LLS resistance-linked marker (AhTE487 and AhTE555) was mapped onto LG XII.

3.8 Marker data analysis

Z test (standard normal deviate test for proportion) (Rao, 2007) was performed to know the strength of co-segregation between a marker and disease resistance. Each introgression line was tested for co-segregation by looking at the type of allele and the phenotype. Genotype showing resistance allele at linked marker loci and disease resistance (score less than 5.0) was considered positive for co-segregation. Z values were calculated across and within the three crosses. In each cross, the proportion of the genotypes showing

Table 4: SSR and AhTE markers used for genotyping the introgression lines

Sl. No.	Primer	Primer sequence (5′ to 3′)	Tm (°C)
Rust and LLS resistance-linked markers			
1	GO340445_F	GGCGGCGGCTGAGGAAGAAG	58.6
	GO340445_R	ACGCGACGCAGAGTGAAAGAA	
2	AhTE360_F	GGATATGATGCCCATAGCTGA	51.5
	AhTE360_R	TGCTGACTACTTGCAATGCC	
3	AhTE498_F	ATGACTTACATGTAGCAATTG	45.7
	AhTE498_R	TGAAAGGAGTCAAAGGTCATG	
4	AhTE621_F	CACTTTGGAGTTTGGACAGAAA	51.3
	AhTE621_R	CGAATCTTGATCGCATCTCTC	
5	AhTE618_F	TTCCGATTTGAAAGAAAATCAAA	60.0
	AhTE618_R	CTTACCCATCCTCGTGCTTG	
6	AhTE231_F	TTTGGAGGCCAATTATGTGTTT	52.7
	AhTE231_R	TAGGTGGGTACTCCCCTGAAG	
LLS resistance-linked markers			
7	AhTE487_F	GATTCTACAATGAGTGGAATGA	48.0
	AhTE487_R	TCCTGCCTCAACCAATTTTC	
8	AhTE555_F	ACAAGTCAAATTCCTTCGCA	53.5
	AhTE555_R	TTTGCCACTTAGGCGTCTTT	

Table 5: SSR markers used for genotyping TMV 2 and GPBD 4

Sl. No.	Primer	Primer sequence (5′ to 3′)	Tm (°C)
LLS resistance-linked markers			
1	GM1009_F	TTTCCTTCTTTCCCTTCTTCTTC	59.6
	GM1009_R	CGTTGTTGCCGTTAAACTGA	
2	pPGPseq8D09_F	TGAGTTTCCCCAAAAGGAGA	51.5
	pPGPseq8D09_R	CAACAACAATACGGCCAACA	
LLS and rust resistance-linked markers			
1	GM2009_F	CAAACGCATACACCCCATAAC	58.7
	GM2009_R	TTTGGTTCTCGTTTGTGTTTT	
2	GM2301_F	GTAACCACAGCTGGCATGAAC	60.3
	GM2301_R	TCTTCAAGAACCCACCAACAC	
3	GM2079_F	GGCCAAGGAGAAGAAGAAAGA	60.0
	GM2079_R	GAAGGAGTAGTGGTGCTGCTG	
4	GM1536_F	AAAGCCCTGAAAAGAAAGCAG	60.3
	GM1536_R	TATGCATTTGCAGGTTCTGGT	
5	GM1954_F	GAGGAGTGTGAGGTTCTGACG	59.7
	GM1954_R	TGGTTCATTGCATTTGCATAC	
6	IPAHM103_F	GCATTCACCACCATAGTCCA	59.0
	IPAHM103_R	TCCTCTGACTTTCCTCCATCA	

Table 6: PCR Components

Components	Concentration (per µl)	Volume (µl)
Nuclease free H_2O	-	13.2
Taq buffer with Mg^{2+}	10 X	2.0
dNTP	2 mM	2.0
Primers (F + R)	10 pmol	(0.5 + 0.5)
Taq DNA polymerase	5 U	0.3
DNA template	5 ng	1.5
		20.0

Table 7: PCR profile used for SSR markers

Steps	Temperature (°c)	Time	Cycles
Initial denaturation	95.0	3 min	
Denaturation	94.0	20 sec	5
Annealing	65.0*	20 sec	
Primer extension	72.0	30 sec	
Denaturation	94.0	20 sec	30
Annealing	60.0	20 sec	
Primer extension	72.0	30 sec	
Final extension	72.0	20 min	
Hold	4.0	-	

Table 8: PCR profile used for AhTE markers

Steps	**Temperature (°c)**	**Time**	**Cycles**
Initial denaturation	95.0	3 min	38
Denaturation	94.0	1 min	
Annealing	65.0*	45 sec	
Primer extension	72.0	1 min	
Final extension	72.0	10 min	
Hold	4.0	-	

*Decreased at the rate of 1°C per cycle for 5 cycles

co-segregation was compared with that of genotypes not showing co-segregation using z test (standard normal deviate test for proportion) where the z value was compared with the critical value of 1.96 at 5% level of significance (irrespective degrees of freedom). High proportion of individuals showing co-segregation and a significant z value was considered as a good case of marker validation in a population.

3.9 Genotyping in TMV 2 and GPBD 4 for MABC

Seeds of TMV 2 and GPBD 4 were obtained from the Department of Genetics and Plant Breeding, UAS, Dharwad. The seeds were sown in pots. DNA was extracted from fresh leaves TMV 2 and GPBD 4 using the modified cetyl trimethyl ammonium bromide (CTAB) extraction method (section 3.7.1). DNA was quantified as described in the section 3.7.2. In total, 294 TE markers were screened among TMV 2 and GPBD 4 using the PCR (Table 8). The two parents were also screened for polymorphism at GM2009, GM1954, GM2079, GM1536, GM2301 and IPAHM103 for foreground selection for both LLS and rust resistance, and at GM1009 and pPGPseq8D09 (Table 5) for foreground selection for only LLS resistance (Sujay *et al.*, 2012). The PCR products were scored to work out the polymorphism.

4. EXPERIMENTAL RESULTS

Introgression lines derived from cultivated varieties and synthetic tetraploids were evaluated for productivity traits and resistance to late leaf spot and rust to select the superior lines. The selected lines were also confirmed for the presence of QTL governing LLS and rust resistance by genotyping with linked markers. Also, with an objective of developing backcross populations in TMV 2 to transfer LLS and rust resistance QTL from GPBD 4, a parental marker polymorphism was checked between TMV 2 and GPBD 4. The results of these experiments are presented here.

4.1 Analysis of variance

Sixty introgression lines from ICGS 76 × ISATGR 278-18 (BC_2F_6), 14 from DH 86 × ISATGR 278-18 (BC_1F_6, BC_2F_6) and 4 from DH 86 × ISATGR 5B (BC_2F_6) were evaluated in a randomized block design during the rainy season of 2013 with two replications for estimating the variability and trait association. Analysis of variance for the phenotypic traits was performed among the introgression lines of three crosses. Plant height, number of primary branches, height of primary branches, leaf length, leaf width, LLS score, rust score, pod width, pod length, pod yield per plant, number of pods per plant, pod yield (kg/ha), test weight and shelling percentage differed significantly among the introgression lines (Table 9).

4.2 Components of variation

The nature and magnitude of variation was assessed by phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). High PCV was recorded for plant height, height of primary branches, LLS score, rust score and pod yield (kg/ha). Moderate PCV was recorded for number of primary branches, leaf length, leaf width, pod length, pod width, pod yield per plant, number of pods per plant, test weight and shelling percentage. Similarly, high GCV was observed for plant height, height of primary branches, late leaf spot and pod yield (kg/ha). Moderate GCV was observed for leaf length, leaf width, rust, pod width, pod yield per plant, number of pods per plant, test weight and shelling percentage. Number of primary branches and pod length recorded low GCV.

Table 9: ANOVA for agronomic, productivity and disease resistance traits among the introgression lines of groundnut

Source of variation	df	Traits													
		PH	**NPB**	**HPB**	**LL**	**LW**	**LLS**	**RUST**	**PW**	**PL**	**PYP**	**NPP**	**PY**	**TW**	**SP**
Treatment MSS	**85**	234.60	1.06	76.73	0.85	0.38	1.94	0.90	0.07	0.13	10.84	41.84	975587	49.39	108.60
Replication MSS	**1**	6.69	1.32	0.00	1.81	2.18	0.12	0.00	0.00	0.11	68.48	17.38	17197	58.72	326.56
Error MSS	**85**	9.15	0.60	9.69	0.25	0.14	0.18	0.08	0.03	0.03	3.22	5.87	136062	6.95	16.53
F value		25.63**	1.75**	7.91**	3.37**	2.61**	10.70**	11.10**	2.18**	4.31**	3.35**	7.12**	7.17**	7.09**	6.57**
CV (%)		12.89	13.20	12.97	11.65	14.90	12.96	8.87	15.03	6.91	11.88	8.74	15.40	6.94	6.08
CD (5%)		6.01	1.54	6.19	0.99	0.76	0.84	0.56	0.35	0.35	3.57	4.81	733.40	5.24	8.08
CD (1%)		7.90	2.01	8.20	1.32	1.01	1.12	0.75	0.47	0.46	4.73	6.38	971.90	6.95	10.71
SEm$\pm$		2.12	0.54	2.18	0.35	0.27	0.29	0.20	0.12	0.12	1.26	1.70	259.30	1.85	2.85

*, **: Significant at 5% and 1% level of probability, respectively
df: Degrees of freedom; CD: Critical difference; CV: Coefficient of variation (%); SEm$\pm$: Standard error of mean; MSS: Mean sum of square; LL: Leaf length (cm)
NPB: Number of primary branches; PH: Plant height (cm); RUST: Rust score; LLS: Late leaf spot score; LW: Leaf width (cm); PL: Pod length (cm); HPB: Height of primary branches (cm); PYP: Pod yield per plant; PY: Pod yield (kg/ha); NPP: Number of pods per plant; SP: Shelling percentage; TW: Test weight (g) and PW: Pod width (cm)

LLS score, rust score and pod yield (kg/ha) recorded high PCV and GCV, indicating the scope for selecting the superior genotypes for these traits. For selection of the traits, broad sense heritability, genetic advance (GA) and genetic advance over mean (GAM) were also considered. Plant height, height of primary branches, leaf length, leaf width, late leaf spot, rust, pod length, pod yield per plant, number of pods per plant, pod yield (kg/ha), test weight and shelling percentage recorded high heritability, while number of primary branches and pod width exhibited moderate heritability (Table 10). Plant height, height of primary branches, leaf length, leaf width, late leaf spot, rust, pod yield per plant, number of pods per plant, pod yield (kg/ha) and test weight also recorded high genetic advance over mean. Number of primary branches, pod length, pod width and shelling percentage recorded moderate GAM. Scores for late leaf spot and rust, and pod yield (kg/ha) recorded high heritability, GAM along with high PCV and GCV, indicating a great scope for selecting the superior lines based on these traits.

4.3 Association studies

An effort was made to study the correlation between various productivity traits, morphological traits and disease resistance. Number of primary branches had significantly negative correlation with plant height and height of primary branches (Table 11). In general, pod yield per plant and number of pods per plant were positively correlated with plant height and height of the primary branches. Pod yield per plant was positively correlated with test weight and shelling percentage. However, number of pods per plant showed significantly negative correlation with test weight. Pod yield (kg/ha) showed significant and positive correlation with pod yield per plant and number of pods per plant. Incidence of LLS and rust were significantly and positively correlated. Occurrence of LLS and rust led to significantly reduced pod yield per plant and number of pods per plant.

4.4 Pod features

ILs were scored for pod constriction, pod reticulation and pod beak using a standard score (IBPGR/ICRISAT, 1992). Of the total 78 ILs, 74 were

Table 10: Mean, range and genetic variability components for agronomic, productivity and disease traits among the introgression lines of groundnut

Traits	Mean	Range		Coefficient of variation (%)		h^2_{bs}	GA	GAM
		Min	Max	PCV	GCV			
PH	23.46	16.05	92.40	46.16	45.25	96.0	21.40	91.30
NPB	5.88	4.00	7.61	12.37	8.11	43.0	0.64	10.97
HPB	24.00	12.85	72.00	25.80	24.12	87.0	11.14	46.44
LL	4.31	2.45	7.08	15.12	12.68	70.0	0.94	21.91
LW	2.58	2.15	3.65	17.03	13.38	61.7	0.56	21.65
LLS	3.28	2.99	8.50	30.00	28.57	90.6	1.84	56.04
Rust	3.21	3.00	7.00	20.90	19.94	90.9	1.25	39.18
PW	1.20	0.90	2.35	15.72	11.59	54.0	0.21	17.60
PL	2.55	1.70	3.50	10.15	8.90	76.8	0.41	16.06
PYP	15.12	9.35	21.58	15.40	12.90	70.0	3.36	22.28
NPP	27.69	9.10	31.60	16.51	15.31	86.0	8.10	29.24
PY	2393.00	549.80	3881.60	29.17	27.06	86.0	1238	51.72
TW	38.00	27.50	48.50	13.07	12.12	85.9	8.79	23.14
SP	66.84	30.25	76.50	11.02	10.15	84.0	12.87	19.25

GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; h^2_{bs}: Heritability in broad sense; GA: Genetic advance

GAM: Genetic advance as percent of mean; PH: Plant height (cm); LL: Leaf length (cm); LLS: Late leaf spot; NPB: Number of primary branches

LW: Leaf width (cm); HPB: Height of primary branches (cm); PW: Pod width (cm); PYP: Pod yield per plant; PL: Pod length (cm); PY: Pod yield (kg/ha)

NPP: Number of pods per plant; SP: Shelling percentage and TW: Test weight (gm)

Table 11: Phenotypic correlation coefficients for agronomic traits, disease traits with productivity traits among the introgression lines

	PH	NPB	HPB	LL	LW	LLS	RUST	PW	PL	PYP	NPP	TW	SP	PY
PH	1													
NPB	-0.3918**	1												
HPB	0.6224**	-0.1461	1											
LL	-0.2669**	0.3776**	-0.1827*	1										
LW	-0.0753	0.1013	-0.0685	0.4152**	1									
LLS	-0.0778	0.0562	-0.2158**	0.3812**	0.1542*	1								
RUST	-0.0179	0.1373	-0.2568**	0.4595**	0.1880*	0.8096**	1							
PW	-0.1668	0.2618**	0.0199	0.0900	0.1408	0.1084	0.2644**	1						
PL	-0.2352**	0.2319**	0.1309	-0.0822	-0.0888	-0.1731*	-0.2262**	0.2876**	1					
PYP	0.0714	-0.0335	0.2555**	-0.1810*	0.0455	-0.2797**	-0.4133**	-0.2225**	0.0513	1				
NPP	0.0338	-0.2128**	0.2689**	-0.4395**	-0.0889	-0.4913**	-0.5840**	0.0661	0.2482**	0.2104**	1			
TW	-0.2414**	0.4158**	-0.1128	0.3917**	0.1359	0.2765**	0.1918*	0.0812	0.0024	0.0032	-0.3342**	1		
SP	-0.5210**	0.2332**	-0.3892**	0.0517	-0.1124	-0.0614	-0.1317	0.0050	-0.0134	0.0672	0.0919	0.2237**	1	
PY	-0.3160**	0.2735**	0.0498	0.1156	0.0487	-0.2547**	-0.3526**	-0.0872	0.1003	0.3735**	0.1755*	0.4094**	0.3405**	1

*, **: Significant at 5 % and 1% level of probability, respectively with n-2 degrees of freedom. If correlation ・ 0.1492 (5%) and 0.1959 (1%).

PH: Plant height (cm); LL: Leaf length (cm); LLS: Late leaf spot; PYP: Pod yield per plant; NPP: Number of pods per plant; NPB: Number of primary branches

LW: Leaf width (cm); PL: Pod length (cm); PW: Pod width (cm);PY: Pod yield (kg/ha); TW: Test weight (g); HPB: Height of primary branches (cm) and SP: Shelling percentage

classified as medium for pod constriction and 73 as medium for pod reticulation. Sixty nine introgression lines showed a slight beak.

4.5 Selection of superior lines

An attempt was made to identify introgression lines that are superior for resistance to LLS and rust over the susceptible parents (ICGS 76 and DH 86). Of the 60 introgression lines from ICGS 76 × ISATGR 278-18, 59 were superior to LLS and rust resistance over ICGS 76. All 14 ILs from DH 86 ×ISATGR 278-18 were superior to LLS and rust resistance over DH 86. Of the four ILs of DH 86 × ISATGR 5B, 3 were superior for LLS and rust resistance over DH 86. Of the 59 introgression lines selected for LLS and rust resistance from ICGS 76 × ISATGR 278-18, two lines 41 (19-4) and 49 (22-3) recorded significantly superior pod yield (kg/ha) over ICGS 76. Of the seventeen introgression lines superior to DH 86 for LLS and rust resistance, five (all from DH 86 × ISATGR 278-18) recorded significantly superior pod yield (kg/ha) over DH 86.

Apart from resistance to LLS and rust, and pod yield (kg/ha), significant superiority for other productivity traits like number of pods per plant, test weight and shelling percentage were also checked among the selected lines. IL 41 (19-4) from ICGS 76 × ISATGR 278-18 exhibited significant superiority for shelling percentage over ICGS 76. IL 49 (22-3) was marginally superior for number of pods per plant, test weight and shelling percentage over ICGS 76 (Table 12).

Of the five ILs that were superior to DH 86 for LLS and rust resistance and pod yield (kg/ha), IL 4 (8-2) exhibited significant superiority for test weight over DH 86. Similarly, IL 5 (5-7) was significantly superior for number of pods per plant, and IL 8 (8-11) was significantly superior for shelling percentage over DH 86. Line 12 (8-10) was significantly superior over DH 86 for test weight. Line 9 (11-1) recorded marginally superiority for number of pods per plant, test weight and shelling percentage. Two ILs superior to ICGS 76 and five superior to DH 86 had medium pod constriction and pod reticulation with slight beak.

Introgression lines were also compared with GPBD 4 (a national check for late leaf spot and rust with high productivity) for disease resistance and productivity

Table 12: Performance of the superior introgression lines

Cross	Genotypes	PY	NPP	TW	SP	LLS	RUST
ICGS 76 × ISATGR 278-18	41 (19-4)	3306.7	25.2	31.8	72.0	3.0	3.0
	49 (22-3)	3568.3	31.4	43.5	65.0	3.0	3.0
DH 86 × ISATGR 278-18	4 (8-2)	3881.6	23.6	48.5	56.5	3.0	3.0
	12 (8-10)	3720.0	25.6	47.5	63.5	3.0	3.0
	5 (5-7)	3653.3	30.1	42.0	68.0	3.0	3.0
	8 (8-11)	3456.7	26.0	41.0	76.5	3.0	3.5
	9 (11-1)	3376.7	27.7	45.0	70.5	3.0	3.5
Checks	ICGS 76	2410.0	28.1	40.5	62.5	5.0	5.5
	DH 86	2603.4	26.0	42.0	66.5	7.0	4.0
	GPBD 4	2872.9	17.40	43.50	74.25	3.0	3.0
	CD (5%)	733.4	4.8	5.2	8.0	0.8	0.5
	CV (%)	15.4	8.7	6.9	6.0	12.9	8.8

PY: Pod yield (kg/ha); LLS: Late leaf spot score; NPP: Number of pods per plant RUST: Rust score; SP: Shelling percentage and TW: Test weight (gm)

traits. Three lines, IL 4 (8-2), 12 (8-10) and 5 (5-7) were significantly superior for LLS and rust resistance, number of pods per plant, pod yield (kg/ha). They were marginally superior for test weight and shelling percentage over GPBD 4 (Table 12).

4.6 Genotypic analysis of introgression lines

Seventy eight introgression lines and their parents were genotyped with a LLS and rust resistance-linked SSR marker (GO340445). In addition, AhTE (AhTE360, AhTE498, AhTE621, AhTE618 and AhTE231) markers mapped on the genomic region on LG XV governing both LLS and rust resistance and those (AhTE487 and AhTE555) mapped on the genomic region on LG XII governing only LLS resistance were also used. The alleles at GO340445 were scored as 1 (susceptible) and 2 (resistant). Alleles at AhTE markers were scored as 1 (susceptible allele with *AhMITE1* insertion) and 2 (resistant allele without *AhMITE1* insertion).

One SSR (GO340445) and seven AhTE markers (AhTE231, AhTE360, AhTE487, AhTE498, AhTE555, AhTE618 and AhTE621) were screened among parents, ICGS 76 versus ISATGR 278-18, DH86 versus ISATGR 278-18 and DH 86 versus ISATGR 5B. GO340445, AhTE360, AhTE498, AhTE621 and AhTE618 were polymorphic between all the three pairs of parents. However, AhTE487 was polymorphic between ICGS 76 and ISATGR 278-18 only. AhTE231 and AhTE555 were monomorphic between all the three pairs of parents (Plate 1).

4.7 Co-segregation study

All introgression lines were found to be homozygous at the marker loci tested in this study. The extent of co-segregation between the type of the allele and the phenotype was assessed using z-test. Among the markers linked to both LLS and rust resistance, GO340445, AhTE360, AhTE621 and AhTE498 showed co-segregation with LLS resistance among the ILs of both these crosses, however, only the ILs of ICGS 76 × ISATGR 278-18 showed co-segregation with rust resistance (Table 13a). GO340445, AhTE360, AhTE498, AhTE621 and AhTE618 also showed significant co-segregation with LLS resistance among the ILs of ICGS 76 × ISATGR 278-18 and DH 86 × ISATGR 278-18. But the marker (AhTE487) linked to only LLS resistance revealed significant co-segregation with LLS resistance in ICGS 76 × ISATGR 278-18, where it as polymorphic (Table 13b). Thus

M: 100 bp DNA ladder; P_1: ICGS 76; P_2: ISATGR 278-18; P_3: DH 86; 1: 11 (7-3); 2: 14 (9-1); 3: 20 (14-1); 4: 39 (16-2); 5: 24 (16-3); 6: 25 (16-4); 7: 27 (16-6); 8: 28 (16-7); 9: 29 (16-8); 10: 30 (16-9); 11: 4 (4-3); 12: 5 (5-7); 13: 6 (6-11); 14: 8 (8-11); 15: 9 (11-1) and 16: 32 (47-11)

Plate 1 Marker profile for AhTE618 among the introgression lines

Table 13a: Z values showing the strength of co-segregation between markers and rust resistance among the introgression lines

Cross	GO340445	AhTE360	AhTE498	AhTE621	AhTE618
ICGS 76 × ISATGR 278-18	4.38*	8.76*	9.49*	8.40*	9.86*
DH 86 × ISATGR 278-18	3.02*	3.78*	1.51	5.29*	5.29*
DH 86 × ISATGR 5B	0.00	1.41	1.41	1.41	0.00
Pooled	5.12*	9.61*	9.29*	9.93*	11.53*

Table 13b: Z values showing the strength of co-segregation between markers and late leaf spot resistance among the introgression lines

Cross	GO340445	AhTE360	AhTE498	AhTE621	AhTE618	AhTE487
ICGS 76 × ISATGR 278-18	4.75*	9.49*	9.86*	8.76*	10.22*	9.86*
DH 86 × ISATGR 278-18	2.27*	3.02*	2.27*	4.54*	4.54*	NA
DH 86 × ISATGR 5B	0.00	1.41	1.41	1.41	0.00	NA
Pooled	5.12*	9.93*	9.93*	9.93*	10.89*	NA

*: Significant at 5% (≥1.96)

NA: Not applicable since the marker was monomorphic between the parents

majority of the LLS and rust resistance markers revealed significant co-segregation with the trait among the ILs of ICGS 76 × ISATGR 278-18 and DH 86 × ISATGR 278-18 (Table 13a and Table 13b).

4.8 Allelic pattern of marker among the superior lines

Selected superior lines were checked for the type of allele at LLS and rust resistance-linked marker loci. Line 41 (19-4) superior to ICGS 76 possessed resistant allele at all six marker loci. However, IL 49 (22-3) carried resistant allele at four markers (out of total 6). Similarly, from the lines superior to DH 86, IL 8 (8-11) and 9 (11-1) showed resistant allele at all the five marker loci (Plate 2). IL 12 (8-10) and 5 (5-7) carried resistant allele at four marker loci. IL 4 (8-2) exhibited resistant allele at three marker loci (Table 14a).

4.9 Initiative towards marker assisted backcrossing

Considering the utility of the LLS and rust resistance-linked markers, an initiative for marker assisted backcross breeding in TMV 2, a disease susceptible but highly adapted groundnut variety was launched. GPBD 4, an improved and disease resistant variety was selected as the donor. As a pre-requisite, TMV 2 and GPBD 4 were screened with LLS and rust resistance-linked markers for foreground selection and with a large number of AhTE markers for background selection.

LLS and rust resistance-linked markers such as GM2009, GM1954, GM2079, GM1536, GM2301, IPAHM103 belonging to LG XV, and only LLS resistance-linked marker GM1009 and pPGPseq8D09 belonging to LG XII were employed for parental screening between TMV 2 and GPBD 4. All these markers, except GM1009, showed polymorphism between TMV 2 and GPBD 4 (Plate 3).

Background selection helps in reducing the number of backcrosses in a marker assisted backcrossing programme. An effort was made to identify a large number of polymorphic markers to be used for background selection. Of the 294 AhTE markers screened for polymorphism between TMV 2 and GPBD 4, 42 showed polymorphism (Table15). The number of polymorphic markers corresponded to 14.28% of the total markers used for screening. Out of 42 AhTE polymorphic markers 26 markers were already mapped (Shirasawa *et al.*, 2013).

Table 14a: Allele pattern at LLS and rust resistance-linked marker loci among the superior introgression lines

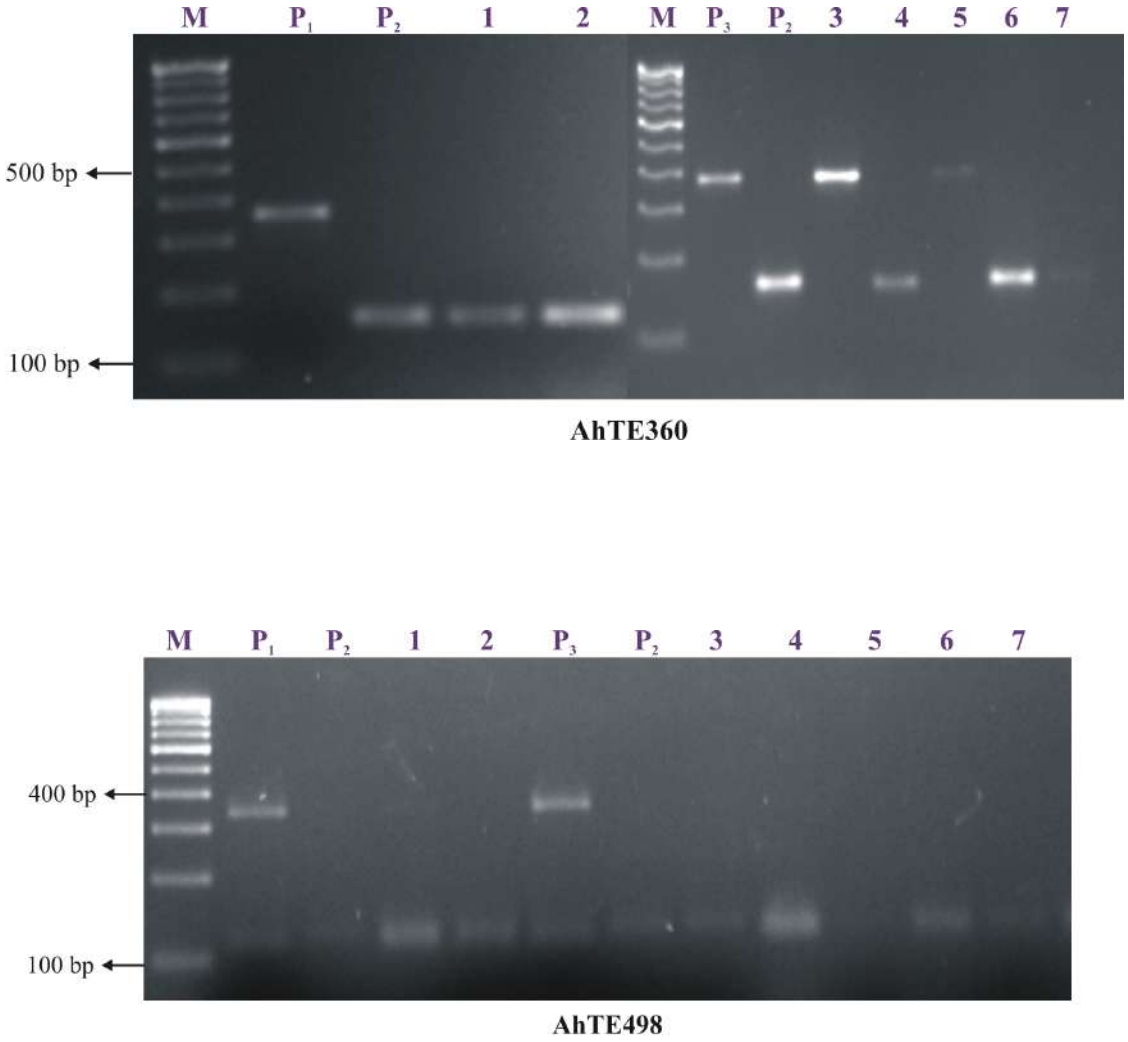
Genotypes	Marker					
	GO340445	AhTE360	AhTE498	AhTE621	AhTE487	AhTE618
ICGS 76 × ISATGR 278-18						
41 (19-4)	222	180	130	400	150	110
49 (22-3)	220	180	130	200	150	110
DH 86 × ISATGR 278-18						
4 (8-2)	222	380	130	200	150	110
12 (8-10)	220	180	130	400	150	110
5 (5-7)	222	380	130	400	150	110
8 (8-11)	222	180	130	400	150	110
9 (11-1)	222	180	130	400	150	110

Allele pattern is indicated in base pairs

Table 14b: Pod features of the superior introgression lines

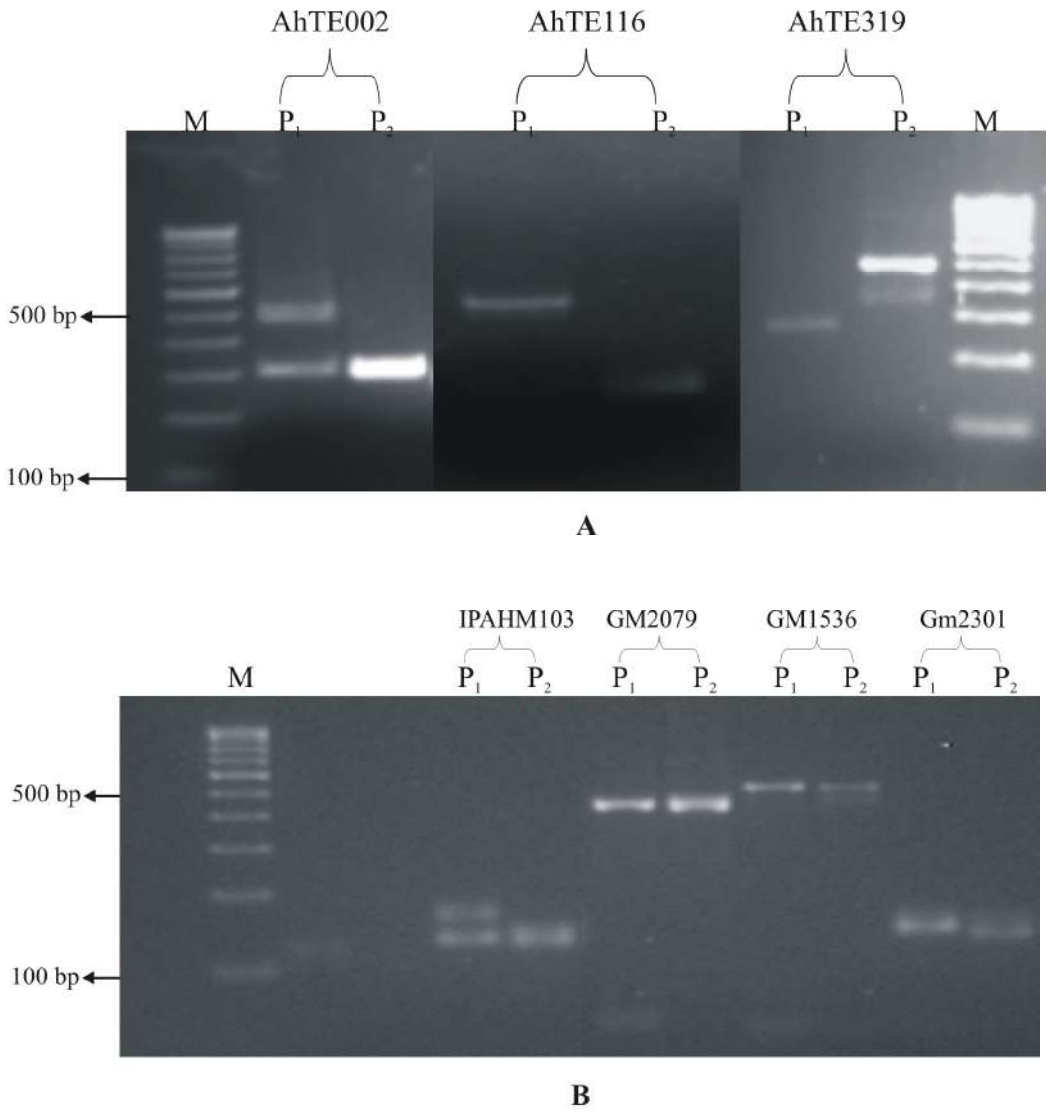
Genotypes	Pod constriction	Pod reticulation	Pod beak
ICGS 76 × ISATGR 278-18			
41 (19-4)	5	5	3
49 (22-3)	5	5	3
ICGS 76	5	5	3
DH 86 × ISATGR 278-18			
4 (8-2)	5	5	3
12 (8-10)	5	5	3
5 (5-7)	5	5	3
8 (8-11)	5	5	3
9 (11-1)	5	5	3
DH 86	5	5	5

Pod feature score 5: medium and Pod feature score 3: slight



M: 100 bp DNA ladder; P_1: ICGS 76; P_2: ISATGR 278-18; P_3: DH 86; 1: 41 (19-4); 2: 49 (22-3); 3: 4 (8-2); 4: 12 (8-10); 5: 5 (5-7); 6: 8 (8-11) and 7: 9 (11-1)

Plate 2: Allele pattern at LLS and rust resistance-linked marker loci (AhTE360 and AhTE498) among the superior introgression lines



M: 100 bp DNA ladder; P_1: TMV 2 and P_2: GPBD 4

Plate 3: AhTE (A) and SSR (B) markers polymorphic between TMV 2 and GPBD 4

These markers were analyzed for their distribution across the 20 LGs of groundnut. Twenty six markers represented 12 linkage groups. Six markers represented LG III, three markers corresponded to LG XVII, two markers represented LG I, VII, X, XII, XV, and XVIII, and only one marker represented LG II, IV, XVIII and XIX

.

Table 15: AhTE and SSR markers polymorphic between TMV 2 and GPBD 4

Markers	Linkage group
AhTE0113, AhTE565	I
AhTE0245	II
AhTE0050, AhTE0194, AhTE0013, AhTE0634, AhTE0205, AhTE0498	III
AhTE0319	IV
AhTE0422, AhTE0073	VII
AhTE0520, AhTE0567, AhTE0010	IX
AhTE0372, AhTE0074	X
AhTE0599, AhTE0636, pPGPseq8D09	XII
AhTE0046, AhTE0600, GM2009, IPAHM103, GM1954, GM2079, GM1536, GM2301	XV
AhTE0116, AhTE0180, AhTE0191	XVII
AhTE0047	XVIII
AhTE0039	XIX
AhTE0048, AhTE0024, AhTE0044, AhTE0033, AhTE0045, AhTE0180 AhTE0467, AhTE0036, AhTE0098, AhTE0504, AhTE0040, AhTE0302, AhTE0043, AhTE0049, AhTE0002, AhTE0042	NM

NM: Not mapped

5. DISCUSSION

Late leaf spot and rust are the two major diseases in groundnut causing severe yield losses. Cultivated groundnut generally lacks resistance to these diseases. This has been attributed to its narrow genetic base (Kochert *et al.*, 1991) mainly due to a single hybridization event involving a “A” genome species (*A. duranensis* Krapov. et W.C. Gregory and a “B” genome species (*A. ipaensis* Krapov. et W.C. Gregory) and subsequent polyploidization. Hence, broadening of the genetic base could be vital in groundnut improvement. In this direction, closely related wild relatives prove to be potential resources in enriching the genetic base as well as providing novel alleles governing important traits (McCouch, 2004; Tanksley and Fulton, 2007). Use of wild relatives has helped in tomato, barley and rice to improve yield (Gur and Zamir, 2004; Fernie *et al.*, 2006; Fu *et al.*, 2010; Nevo and Chen, 2010).

Wild *Arachis* species exhibit desirable phenotype for resistance to diseases and insects in addition to drought tolerance. Deriving tetraploid groundnut from wild diploids and crossing them with cultivated groundnut might unravel considerable variability for many useful traits as observed in the wide crosses of wheat and rice (Hoisington *et al.*, 1999); McCouch *et al.*, 2005). Development of synthetics by combining the putative genome donors of the cultivated species in wheat and brassica has triggered the development of tetraploids/amphidiploids (synthetics) in groundnut. Three such amphidiploids were developed and successfully used in groundnut breeding. An amphidiploid originating from *A. cardenasii* Krapov. et W.C. Gregory, *A. diogoi* Hoehne and *A. batizocoi* Krapov. et W.C. Gregory was utilized in a backcross breeding to develop two groundnut cultivars namely Coan and NemaTAM (Simpson *et al.*, 1993). Recently, ICRISAT has developed a set of amphiploid and autotetraploid groundnuts by involving a number of *Arachis* species with A, B, K genome.

Arachis batizocoi and *A. cruziana* were very recently described as having a K genome based on FISH mapping of rDNA loci and heterochromatin detection (Robledo and Seijo, 2010). In the first step, diploid hybrids were developed by crossing program. In the second step, these diploid hybrids were treated with colchicine to develop tetraploids. Also, the diploid hybrids with 2n pollen were self

pollinated to obtain tetraploids. Since these tetraploids are not subjected for any kind of selection, they represent true genetic pool, which can be used to enhance the genetic base of the cultivated groundnut.

These synthetic tetraploids were evaluated for LLS and rust resistance at UAS, Dharwad (Varshakumari, 2013), and two amphidiploids [ISATGR 278-18 (*Arachis duranensis* × *Arachis batizocoi*) and ISATGR 5B (*Arachis magna* × *Arachis batizocoi*)] were found to be highly resistant. With an objective of transferring LLS and rust resistance, the two synthetics were crossed to two elite but disease susceptible varieties, ICGS 76 and DH 86. Two cycles of backcrossing with the recurrent parents resulted in the development of a large number of introgression lines (ILs). A large population consisting of such ILs is also useful in mapping the genomic regions contributing to late leaf spot and rust resistance, apart from simultaneous transfer of resistance. A population from ICGS 76 × ISATGR 278-18 was used to map the QTL for LLS and rust resistance in addition to a few productivity traits (Varshakumari, 2013). Two genomic regions; one on LG XV and the other on LG XII contributing towards LLS and rust resistance were identified. The region on LG XV governed both rust and LLS resistance, while the region on LG XII contributed only to LLS resistance. The same regions were also mapped in earlier studies using two RIL populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) for LLS and rust resistance with very high phenotypic variance explained (Khedikar *et al*., 2010; Sujay *et al*., 2012). The markers linked to the QTL among these genomic regions were identified. The markers were validated using a diverse genotypes including near isogenic lines. The markers were also successfully employed in a marker assisted backcrossing (MABC) programme to develop LLS and rust resistant genotypes from JL 24 (Varshney *et al*., 2014; Yeri *et al*., 2014a).

5.1 Initiative for MABC in TMV 2

Using the genomic resources (QTL and linked markers) developed in groundnut, an effort towards marker assisted backcross breeding in TMV 2 was initiated in this study. An exhaustive survey for polymorphic markers was attempted using TMV 2 and GPBD 4 (donor) to identify the markers for foreground

and background selection. For foreground selection, both LLS and rust resistance-linked markers such as GM2009, GM1954, GM2079, GM1536, GM2301, IPAHM103 belonging to LG XV, and only LLS resistance linked markers such as GM1009 and pPGPseq8D09 belonging to LG XII were employed for parental screening between TMV 2 and GPBD 4. All these markers, except GM1009, showed polymorphism between TMV 2 and GPBD 4. These polymorphic markers flank three QTL on LG XV and one QTL on LG XII. Therefore, an efficient foreground selection for all the four QTL can be attempted in marker assisted backcrossing of TMV 2 with GPBD 4. In addition to foreground selection for disease resistance-linked markers, a selection for the genome of the recurrent parent would enhance the efficiency of backcross breeding. A new class of markers, *AhMITE1*-speciifc markers (AhTE) was screened for use in background selection due to their high rate polymorphism over SSRs in groundnut (Shirasawa *et al*., 2012b). More than thousand such AhTE markers are available in groundnut (Shirasawa *et al*., 2012a; Shirasawa *et al*., 2012b).Of the 294 AhTE markers screened, 42 were polymorphic, indicating 14.28% polymorphism. This level of polymorphism is considerably higher than that of SSR markers (6-7%) (Shirasawa *et al*., 2012b) in groundnut. Identification of the markers for foreground and background selection would now make it possible to take up backcross breeding in TMV 2 as recurrent parent and GPBD 4 as the donor.

5.2 Identifying the superior introgression lines

In addition to the long term approach of developing LLS and rust resistant backcross lines in TMV 2, a short term approach in which the introgression lines available across three populations involving ICGS 76, DH 86, ISATGR 278-18 and ISATGR 5B were screened for LLS and rust resistance along with the productivity traits and pod features with an objective of identifying superior ILs. Sixty, fourteen and four ILs from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5, respectively were selected based on their performance (resistance) in the previous generations. These ILs were evaluated for disease resistance and productivity traits during the rainy season of 2013. Based on their phenotypic evaluation and allele type at LLS and rust resistance-linked marker loci, ILs combining high level of resistance and productivity along with desirable pod features were selected for further varietal release trials.

For selecting the superior ILs, an analysis of variation was attempted. Seventy eight ILs revealed significant genotypic differences for all the traits. The nature and magnitude of variation was assessed by phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). In general, high PCV and GCV were recorded for most of the traits. LLS score, rust score and pod yield (kg/ha) recorded high PCV and GCV, indicating the scope for selecting the superior ILs for these traits. Estimate of heritability serves as a useful guide; when the heritability of a character is very high, the selection for that character is fairly easy. Further, the estimate of genetic advance indicates the scope for the improvement of a trait through selection. Therefore, broad sense heritability, genetic advance (GA) and genetic advance over mean (GAM) were calculated. Again, majority of the traits recorded high heritability, GA and GAM. It was observed that the scores for late leaf spot and rust, and pod yield (kg/ha) recorded high heritability, GAM along with high PCV and GCV, indicating a great scope for selecting the superior lines based on these traits.

Selection of an IL based on a trait might influence its performance for other traits depending upon the extent of correlation between the traits under consideration. An effort was made to study the correlation between various productivity traits, morphological traits and disease resistance. Occurrence of LLS and rust were significantly and positively correlated. Pod yield (kg/ha) and a few productivity traits like pod yield per plant, number of pods per plant and test weigh had significantly negative correlation with the severity of LLS and rust. This could be due to the significant positive correlation between the pod yield (kg/ha) and other productivity traits. Majority of the ILs across the crosses showed moderate pod constriction and moderate pod reticulation along with slight pod beak, indicating that the ILs had acceptable pod features in addition to LLS and rust resistance. This observation could be significant considering the strong linkage between disease resistance and poor pod features as reported earlier (Singh, 1997).

An attempt was made to select the ILs that were superior to ICGS 76 and DH 86 for disease resistance as well as productivity traits. As expected, most of the ILs (76 out of 78) which were selected as resistant to LLS and rust in their previous generation (BC_2F_5), were resistant in the subsequent generation (BC_2F_6)

also, indicating their true breeding behavior for resistance. Genotypic data was used to select the superior introgression lines. Seventy eight introgression lines and their parents were genotyped with a LLS and rust resistance-linked SSR marker (GO340445). In addition, AhTE (AhTE360, AhTE498, AhTE621, AhTE618 and AhTE231) markers mapped on the genomic region on LG XV governing both LLS and rust resistance and those (AhTE487 and AhTE555) mapped on the genomic region on LG XII governing only LLS resistance were also used. First, these markers (GO340445, AhTE231, AhTE360, AhTE487, AhTE498, AhTE555, AhTE618 and AhTE621) were screened for polymorphism among parents, ICGS 76 versus ISATGR 278-18, DH86 versus ISATGR 278-18 and DH 86 versus ISATGR 5B. GO340445, AhTE360, AhTE498, AhTE621 and AhTE618 were polymorphic between all the three pairs of parents. However, AhTE487 was polymorphic between ICGS 76 and ISATGR 278-18 only. AhTE231 and AhTE555 were monomorphic between all the three pairs of parents. All introgression lines were found to be homozygous at all the polymorphic marker loci. The extent of co-segregation between the type of the allele and the phenotype was assessed using z test considering the introgression lines separately by the cross to which they belong. GO340445, AhTE360, AhTE621 and AhTE618 showed significant co-segregation with LLS as well as rust resistance among the ILs of ICGS 76 × ISATGR 278-18 and DH 86 × ISATGR 278-18, but not among the ILs of DH 86 × ISATGR 5B. AhTE498 showed co-segregation with LLS resistance among the ILs of both these crosses, while the ILs of ICGS 76 × ISATGR 278-18 showed co-segregation of AhTE498 with only rust resistance. AhTE487 also showed co-segregation with LLS resistance among the ILs of ICGS 76 × ISATGR 278-18. Thus majority of the markers could be validated for their association with LLS and rust resistance. The result was expected as the ILs used in this study were selected based on their superior performance for disease resistance in the previous generation. ILs of DH 86 × ISATGR 5B not showing significant co-segregation of any markers with LLS and rust resistance could due to small population size. These genotypic data were employed for selecting ILs resistant LLS and rust.

Despite pod yield (kg/ha) being significantly and negatively correlated with the severity of the disease, two ILs from ICGS 76 × ISATGR 278-18, and five from DH 86 × ISATGR 278-18 were selected as superior to respective recurrent parent for pod yield as well as LLS and rust resistance. Apart from resistance to LLS and rust, and pod yield (kg/ha), significant superiority for other productivity traits like number of pods per plant, test weight and shelling percentage were also checked among the selected lines. IL 41 (19-4) from ICGS 76 × ISATGR 278-18 exhibited significant superiority for shelling percentage to ICGS 76. IL 49 (22-3) was marginally superior for number of pods per plant, test weight and shelling percentage to ICGS 76. Selected superior lines were checked for the type of allele at LLS and rust resistance-linked marker loci. Line 41 (19-4) superior to ICGS 76 possessed resistant allele at all six marker loci. However, IL 49 (22-3) carried resistant allele at four markers (out of total 6).

Of the five ILs that were superior to DH 86 for LLS and rust resistance and pod yield (kg/ha), IL 4 (8-2) exhibited significant superiority for test weight over DH 86. Similarly, IL 5 (5-7) was significantly superior for number of pods per plant, and IL 8 (8-11) was significantly superior for shelling percentage to DH 86. Line 8-10 was significantly superior to DH 86 for test weight. Line 9 (11-1) recorded marginally superiority for number of pods per plant, test weight and shelling percentage. Similarly, from the lines superior to DH 86, IL 8 (8-11) and 9 (11-1) showed resistant allele at all the five marker loci. IL 12 (8-10) and 5 (5-7) carried resistant allele at four marker loci. IL 4 (8-2) exhibited resistant allele at three marker loci. Pods of all the selected seven superior lines showed medium constriction, reticulation and slight beak, which were in the acceptable category (Table 14b) (Plate 4).

GPBD 4, being an improved variety and national check for late leaf spot and rust resistance with high productivity, an attempt was made to compare the performance of selected ILs with GPBD 4. Three lines, IL 4 (8-2), 12 (8-10) and 5 (5-7) (all from the cross DH 86 × ISATGR 278-18) were significantly superior for LLS and rust resistance, number of pods per plant, pod yield (kg/ha). They were marginally superior for test weight and shelling percentage to GPBD 4.

ICGS 76 41 (19-4) 49 (22-3)

DH 86 4 (8-2) 12 (8-10)

5 (5-7) 8 (8-11) 9 (11-1)

Plate 4: Pod and kernel features of the superior introgression lines

Future lines of investigation

The introgression lines used in this study represented a genetic resource with broad genetic base since they involved synthetic groundnut developed from various diploid species. Considering the existence of significant variability in these ILs, they can be employed as source of resistance to LLS and rust in groundnut breeding. Evaluation of these ILs could identify seven superior genotypes for disease resistance as well as productivity traits. They also possessed acceptable pod features. These superior lines can be considered for further testing towards commercialization. Introgression lines differing only for resistance to LLS can be crossed to develop a mapping population for mapping LLS resistance as the inheritance pattern of LLS is complex, and those ILs contrasting only for LLS response would provide ideal genetic backgrounds to identify the genomic regions. Further, the SSR markers polymorphic between TMV 2 and GPBD 4 can be used for foreground selection in marker assisted backcross breeding to improve TMV 2. Also, the large number of transposon markers (42) identified as polymorphic between TMV 2 and GPBD 4, covering various regions of the genome, can be used for background selection.

6. SUMMARY AND CONCLUSIONS

Based on the high level of resistance to LLS and rust in the previous generation, seventy eight BC_2F_5 introgression lines (ILs) derived from the crosses involving cultivated varieties (ICGS 76 and DH 86) and synthetic tetraploids were selected. The progenies (BC_2F_6) of these ILs were evaluated for agronomic, disease resistance and productivity traits during the rainy season of 2013 at IABT Garden, Main Agriculture Research Station, UAS, Dharwad. In addition, an initiative towards marker assisted backcrossing of TMV 2 with GPBD 4 was undertaken. The results of these experiments are summarized below.

- Of the 78 ILs, 60 belonged to ICGS 76 × ISATGR 278-18, 14 to DH 86 × ISATGR 278-18 and four lines belonged to DH 86 × ISATGR 5. They were evaluated for agronomic, disease resistance and productivity traits in a randomized block design during the rainy season of 2013.
- Introgression lines exhibited significant variation for most of the traits studied.
- LLS score, rust score and pod yield (kg/ha) recorded high PCV and GCV. These traits also showed high heritability, GA and GAM.
- Correlation studies revealed significant positive association between the occurrence of LLS and rust. Pod yield (kg/ha) and a few productivity traits like pod yield per plant, number of pods per plant and test weight had significantly negative correlation with the severity of LLS and rust. However, these productivity traits were significantly and positively correlated with each other.
- Majority of the ILs across the crosses showed medium pod constriction and medium pod reticulation along with slight pod beak, indicating that the ILs had acceptable pod features in addition to LLS and rust resistance.
- Introgression lines along with their parents were genotyped with both LLS and rust (GO340445, AhTE360, AhTE498, AhTE621, AhTE618 and AhTE231) and only LLS (AhTE487 and AhTE555) resistance-linked markers with an objective of validating them and also select the resistant ILs. z test showed significant co-segregation of most of these with LLS and rust resistance, thus validating the markers.

- Two ILs from ICGS 76 × ISATGR 278-18 and five from DH 86 × ISATGR 278-18 were selected as superior to respective recurrent parent for pod yield as well as LLS and rust resistance. Apart from resistance to LLS and rust, and pod yield (kg/ha), significant superiority for other productivity traits like number of pods per plant, test weight and shelling percentage were also checked among the selected lines. IL 41 (19-4) from ICGS 76 × ISATGR 278-18 exhibited significant superiority for shelling percentage to ICGS 76. IL 49 (22-3) was marginally superior for number of pods per plant, test weight and shelling percentage to ICGS 76. Selected superior lines were checked for the type of allele at LLS and rust resistance-linked marker loci. Line 41 (19-4) superior to ICGS 76 possessed resistant allele at all six marker loci. However, IL 49 (22-3) carried resistant allele at four markers (out of total 6).
- Of the five ILs that were superior to DH 86 for LLS and rust resistance and pod yield (kg/ha), IL 4 (8-2) exhibited significant superiority for test weight to DH 86. Similarly, IL 5 (5-7) was significantly superior for number of pods per plant, and IL 8 (8-11) was significantly superior for shelling percentage to DH 86. Line 12 (8-10) was significantly superior over DH 86 for test weight. Line 9 (11-1) recorded marginally superiority for number of pods per plant, test weight and shelling percentage. Similarly, from the lines superior to DH 86, IL 8 (8-11) and 9 (11-1) showed resistant allele at all the five marker loci. IL 12 (8-10) and 5 (5-7) carried resistant allele at four marker loci. IL 4 (8-2) exhibited resistant allele at three marker loci. Pods of all the selected seven superior lines showed medium constriction, reticulation and slight beak, which were in the acceptable category.
- GPBD 4, being an improved variety and national check for late leaf spot and rust resistance with high productivity, an attempt was made to compare the performance of selected ILs with GPBD 4. Three lines, IL 4 (8-2), 12 (8-10) and 5 (5-7) (all from the cross DH 86 × ISATGR 278-18) were significantly superior for LLS and rust resistance, number of pods per plant, pod yield (kg/ha). They were marginally superior for test weight and shelling percentage over GPBD 4.

- With an objective of initiating MABC in TMV 2, both LLS and rust (GM2009, IPAHM103, GM1954, GM2301, GM2079 and GM1536) and only LLS (GM1009 and pPGPseq8D09) resistance-linked SSR markers were screened for polymorphism between TMV 2 and GPBD 4. All these markers, except GM1009, showed polymorphism between TMV 2 and GPBD 4. These markers can be used for foreground selection.
- In order to find a set of markers for background selection, TMV 2 and GPBD 4 were screened using 294 AhTE markers. Of them, 42 AhTE markers were found polymorphic. They represented 12 LGs.
- A new class of markers, *AhMITE1*-specific markers (AhTE) was screened for use in background selection. Of the 294 AhTE markers screened, 42 were polymorphic, indicating 14.28 % polymorphism. Of the 42 markers, 26 were mapped in an earlier study on 12 linkage groups. Identification of the markers for foreground and background selection would now make it possible to take up backcross breeding in TMV 2 as recurrent parent and GPBD 4 as the donor.

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Appendix I: Mean performance of the introgression lines for agronomic, disease and productivity traits

Sl. No.	Genotypes	PH	NPB	HPB	LL	LW	LLS	RUST	PY	PW	PL	PYP	NPP	TW	SP
1	TMV 2	21.90	5.50	16.00	6.90	3.35	7.19	6.27	993.00	1.19	2.35	10.76	13.55	43.50	54.00
2	JL 24	25.95	6.00	12.85	7.08	3.65	6.00	5.15	549.83	1.11	2.03	9.35	13.65	43.50	55.25
3	GPBD 4	29.80	6.75	15.25	5.43	2.63	2.99	3.00	2872.90	1.17	2.29	15.80	17.40	43.50	74.25
4	TG 26	18.83	6.25	14.00	4.38	2.28	7.00	7.00	853.33	1.31	1.98	10.18	9.10	40.00	73.50
5	ICGS 76	21.72	7.50	25.90	4.99	2.71	5.00	5.50	2410.00	2.35	2.95	12.35	28.05	40.50	62.50
6	DH 86	17.17	5.70	20.80	4.15	2.73	8.50	4.50	2786.68	1.15	2.65	15.50	25.50	44.00	71.00
7	ISATGR 278-18	89.75	4.00	72.00	3.33	2.53	3.00	3.00	978.89	1.10	2.65	18.35	29.67	27.50	30.25
8	IASTGR-5B	92.40	4.00	24.95	2.45	2.15	3.00	3.50	603.33	0.90	1.70	13.18	28.49	29.75	48.50
9	11 (7-3)	16.80	5.90	20.92	4.62	2.55	3.00	3.00	2026.67	1.25	3.50	12.00	31.60	33.75	55.00
10	14 (9-1)	22.47	5.50	23.73	4.56	2.51	3.00	3.00	2606.67	1.10	2.50	18.47	31.50	34.50	64.00
11	20 (14-1)	23.84	6.40	23.85	4.42	2.52	3.00	3.00	2683.33	1.15	3.15	21.58	24.50	39.50	59.00
12	39 (16-2)	20.31	6.00	18.84	3.92	2.30	3.50	3.00	2290.00	1.20	2.30	12.48	26.00	28.75	58.00
13	24 (16-3)	17.62	5.90	20.01	4.31	2.40	3.00	3.50	1766.67	1.15	2.55	9.53	25.50	27.00	45.50
14	25 (16-4)	17.18	6.50	21.86	4.46	2.55	3.00	3.00	2190.00	1.20	2.50	13.91	24.55	30.00	71.50
15	27 (16-6)	20.13	5.30	19.01	4.35	2.54	3.00	3.00	1566.67	1.15	2.55	16.79	24.65	34.00	70.50
16	28 (16-7)	19.50	6.30	21.74	4.14	2.53	3.00	3.00	2133.33	1.15	2.65	12.81	31.50	39.00	73.00
17	29 (16-8)	19.01	5.20	21.05	4.49	2.95	3.00	3.00	2460.00	1.20	2.25	16.04	33.70	33.00	71.50
18	30 (16-9)	21.14	6.10	24.18	4.36	2.74	3.00	3.00	2640.00	1.25	2.50	14.78	33.75	34.50	71.50
19	31 (16-10)	21.73	5.30	23.35	3.90	3.11	3.00	3.00	2601.67	1.15	2.65	16.29	34.55	33.00	73.50
20	32 (17-1)	19.51	4.50	21.57	4.08	3.85	3.00	3.00	1730.00	1.15	2.40	13.89	33.55	32.00	70.00

Contd…

21	38 (19-1)	21.57	5.20	22.58	4.23	3.92	3.00	3.00	2488.33	1.15	2.60	17.73	36.50	35.00	62.00
22	39 (19-2)	24.34	5.80	23.66	4.57	4.06	3.00	3.00	2170.00	1.20	2.50	13.66	23.50	33.50	65.00
23	40 (19-3)	23.37	5.00	21.52	4.09	2.42	3.00	3.00	2910.00	1.10	2.30	17.45	26.80	34.00	71.50
24	41 (19-4)	21.07	4.80	21.49	4.29	2.44	3.00	3.00	3306.67	1.10	2.45	17.75	25.20	31.75	72.00
25	45 (20-2)	19.76	5.50	22.43	3.99	2.29	3.00	3.00	2480.00	1.15	2.45	14.90	28.95	34.00	67.00
26	46 (20-3)	20.29	4.90	24.57	4.42	2.59	3.00	3.00	2140.00	1.55	2.70	13.35	30.05	35.50	64.00
27	49 (22-3)	23.11	5.70	25.75	4.09	2.49	3.00	3.00	3568.33	1.15	2.45	19.28	31.35	43.50	65.00
28	53 (26-1)	24.86	5.40	26.19	4.40	2.49	3.00	3.00	2500.00	1.10	2.00	15.88	26.10	40.00	52.00
29	62 (27-3)	21.95	5.00	23.29	3.84	2.25	3.00	3.00	1641.67	1.20	2.70	19.05	28.75	31.50	61.00
30	63 (27-4)	22.40	6.40	22.40	4.15	2.36	3.00	3.00	2733.33	1.25	2.85	16.04	23.25	35.50	70.00
31	64 (30-2)	23.18	6.00	22.72	3.81	2.38	3.00	3.00	2743.33	1.10	2.30	20.02	25.50	35.00	61.50
32	66 (32-3)	19.42	5.40	23.59	3.94	2.29	3.00	3.00	2546.67	1.20	2.50	15.27	32.50	35.50	69.50
33	69 (39-2)	22.19	5.80	27.04	4.44	2.53	3.00	3.00	2660.00	1.20	2.30	14.55	33.05	38.50	68.00
34	70 (39-3)	26.34	5.50	24.13	4.23	2.34	3.00	3.00	2840.00	1.25	2.75	14.14	34.30	36.00	69.50
35	71 (39-4)	21.25	5.30	25.64	4.01	2.40	3.00	3.00	2113.33	1.15	2.35	13.17	33.50	34.50	71.50
36	72 (39-5)	23.26	5.60	22.16	4.26	2.19	3.00	3.00	2076.67	1.10	2.50	11.76	29.05	33.50	70.00
37	73 (39-6)	25.13	6.30	28.82	4.18	2.26	3.00	3.00	1476.67	1.20	2.80	19.69	28.15	32.00	68.50
38	74 (39-7)	26.07	5.60	25.82	4.25	2.41	3.00	3.00	1973.33	1.15	2.80	14.61	31.40	30.50	69.00
39	75 (40-1)	26.06	5.40	25.72	3.83	2.18	3.00	3.00	1950.00	1.10	2.90	14.08	30.45	34.50	68.50
40	78 (42-1)	22.15	4.90	22.28	3.80	2.38	3.00	3.00	2405.00	1.15	2.70	17.69	27.95	35.75	65.50
41	84 (42-7)	19.92	5.80	22.46	4.30	2.57	3.00	3.00	2326.67	1.15	2.70	16.17	27.25	33.00	63.50
42	88 (43-3)	21.94	6.20	23.67	4.23	2.26	3.00	3.00	2166.67	1.25	2.80	15.57	30.00	45.50	67.00
43	90 (43-3)	22.28	5.40	20.42	3.40	3.19	3.00	3.00	2860.00	1.15	2.20	16.43	31.70	43.50	68.50

Contd…

44	94(44-1)	19.14	5.30	23.30	3.55	2.96	3.00	3.00	2473.33	1.15	2.40	12.28	23.00	42.00	64.50
45	103 (50-3)	21.50	5.40	24.47	3.66	2.26	3.00	3.00	1736.67	1.75	2.40	11.25	32.55	40.00	73.50
46	109 (52-1)	22.37	6.00	22.83	3.95	2.22	3.00	3.00	2943.33	1.20	2.25	20.95	23.50	41.00	74.00
47	110 (52-2)	21.56	6.10	23.22	4.34	2.38	3.00	3.00	2403.33	1.10	2.45	16.81	28.00	42.00	71.50
48	112 (52-4)	25.17	5.00	25.77	4.79	2.10	3.00	3.00	2690.00	1.20	2.45	15.01	26.50	38.50	77.00
49	113 (53-4)	22.24	5.90	22.23	4.40	2.27	3.00	3.00	2520.00	1.20	2.35	17.06	27.50	37.50	71.50
50	118 (60-1)	19.00	5.00	23.60	4.19	2.41	3.00	3.00	2273.33	1.20	2.50	14.82	24.00	40.50	59.50
51	120 (92-2)	24.16	5.60	26.48	4.06	2.26	3.00	3.00	2486.67	1.00	2.30	15.22	27.55	38.50	71.00
52	121 (92-3)	26.57	4.80	27.46	4.11	2.42	3.00	3.00	2123.33	1.55	2.50	13.20	32.05	36.50	74.00
53	122 (92-4)	21.23	6.00	23.62	3.94	2.33	3.00	3.00	2863.33	1.10	2.60	14.32	32.25	34.50	66.50
54	123 (92-5)	23.80	6.00	23.43	4.29	2.31	3.00	3.00	2453.33	1.15	2.70	14.82	32.65	35.50	70.50
55	124 (98-1)	25.95	6.00	24.96	3.85	2.21	3.00	3.00	2863.33	1.20	2.55	15.94	33.75	35.50	69.50
56	126 (99-5)	22.06	6.50	26.04	3.91	2.32	3.00	3.00	2173.33	1.20	2.70	14.88	33.20	34.50	69.00
57	127 (99-6)	25.19	5.70	25.47	4.44	2.63	3.00	3.00	2971.67	1.30	2.85	14.39	23.50	43.00	70.50
58	128 (100-1)	21.15	5.60	26.67	3.90	2.31	3.00	3.00	1418.33	1.70	2.80	13.54	29.50	40.00	59.00
59	131 (101-3)	23.61	6.80	26.58	4.13	2.15	3.00	3.00	1213.33	1.25	2.90	13.02	30.00	41.50	64.50
60	132 (102-3)	21.87	6.30	24.36	3.82	2.35	3.00	3.00	2660.00	1.05	2.90	14.51	24.12	44.50	73.50
61	134 (105-3)	20.31	5.70	20.22	3.66	2.14	3.00	3.00	1831.67	1.05	2.80	13.04	26.45	37.00	73.50
62	135 (105-4)	18.61	5.70	22.04	3.92	2.28	3.00	3.00	1870.00	1.10	2.60	12.47	28.10	34.50	69.50
63	136 (105-5)	21.39	6.30	20.63	3.55	2.15	3.00	3.00	2066.67	1.15	2.70	14.18	26.30	32.00	67.00
64	137 (105-6)	18.14	5.80	21.69	4.31	2.30	3.00	3.50	2203.33	1.15	2.50	16.57	29.55	36.50	70.50
65	138 (105-7)	20.82	6.10	21.94	4.17	2.29	3.00	3.00	2270.00	1.20	2.65	14.72	24.55	39.50	71.00
66	140 (106-1)	17.88	6.10	19.28	3.98	2.29	3.00	3.00	2395.00	1.10	2.65	16.30	31.05	36.50	73.00

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67	141 (107-1)	15.95	7.30	20.02	3.94	3.78	3.00	3.50	1516.67	1.70	2.85	16.25	29.30	34.50	58.00
68	145 (107-5)	22.07	5.20	20.93	4.79	2.66	5.50	3.50	2636.65	1.10	2.60	16.98	29.25	46.50	65.50
69	4 (8-2)	16.05	6.87	24.15	4.57	2.25	3.00	3.00	3881.67	1.05	2.45	15.50	23.65	48.50	56.50
70	5 (8-3)	22.55	6.70	24.37	4.40	2.76	3.00	3.00	3416.67	1.15	2.60	14.50	28.05	43.50	68.50
71	6 (8-4)	23.15	5.93	23.27	4.25	2.51	3.00	3.00	3313.33	1.10	2.70	14.00	25.70	40.50	68.00
72	7 (8-5)	20.62	5.60	22.55	4.50	2.65	3.00	3.50	3043.33	1.25	2.60	14.00	25.80	42.50	75.50
73	9 (8-7)	22.94	6.83	27.55	4.57	3.48	3.00	3.00	3380.00	1.15	2.40	16.50	24.05	44.50	66.00
74	10 (8-8)	23.06	6.70	27.80	4.99	2.59	3.00	3.00	3080.00	1.25	2.80	14.50	24.05	45.50	66.50
75	11 (8-9)	22.98	6.37	24.49	4.60	2.56	3.00	3.50	3233.78	1.15	2.60	14.00	28.95	44.50	65.50
76	12 (8-10)	20.68	5.95	24.25	3.87	2.80	3.00	3.00	3720.00	1.25	2.85	15.53	25.60	47.50	63.50
77	4 (4-3)	25.31	7.37	25.90	4.86	3.60	3.00	3.00	2946.67	1.60	2.60	15.50	25.80	41.00	69.50
78	5 (5-7)	21.07	7.17	29.25	5.14	2.41	3.00	3.00	3653.33	1.20	2.45	16.11	30.05	42.00	68.00
79	6 (6-11)	25.70	5.80	26.92	5.28	3.23	3.00	3.00	3210.00	1.10	2.50	15.83	27.50	43.50	69.50
80	8 (8-11)	23.30	6.83	30.30	6.04	3.02	3.00	3.50	3456.67	1.15	2.25	17.43	26.00	41.00	76.50
81	9 (11-1)	26.80	7.61	30.98	5.68	3.06	3.00	3.50	3376.67	1.25	2.75	13.99	27.70	45.00	70.50
82	32 (47-11)	21.64	6.60	20.56	3.54	2.26	3.00	3.00	1186.67	1.15	2.70	15.17	25.80	42.00	71.50
83	9 (17-1)	20.89	6.70	31.77	4.29	2.37	5.50	3.00	2403.33	1.20	2.45	15.50	25.88	43.75	73.00
84	10 (17-2)	16.85	7.40	22.91	4.66	2.62	3.50	3.50	2126.67	1.10	2.35	14.94	27.95	35.50	71.50
85	12 (17-4)	23.43	6.00	27.53	4.64	2.58	3.00	3.00	3093.33	1.05	2.50	15.49	26.70	39.00	70.50
86	12 (2-4)	20.51	6.35	22.28	4.51	2.64	3.00	3.00	2150.00	1.05	2.30	17.13	28.70	42.50	68.50

PH: Plant height (cm); NPB: Number of primary branches; PW: Pod width (cm); LL: Leaf length (cm); LLS: Late leaf spot PY: Pod yield (kg/ha); LW: Leaf width (cm); HPB: Height of primary branches (cm); TW: Test weight (g) PYP: Pod yield per plant; PL: Pod length (cm); NPP: Number of pods per plant and SP: Shelling percentage

Appendix II: Pod features of the introgression lines

Sl. No.	Genotypes	PC	PR	PB	Sl. No.	Genotypes	PC	PR	PB
1	ICGS 76	5	5	3	41	110 (52-2)	5	3	3
2	DH 86	5	5	5	42	112 (52-4)	5	5	3
3	11 (7-3)	5	5	3	43	113 (53-4)	5	5	3
4	14 (9-1)	5	5	3	44	118 (60-1)	5	5	3
5	20 (14-1)	5	5	3	45	120 (92-2)	5	5	3
6	39 (16-2)	7	5	5	46	121 (92-3)	5	3	3
7	24 (16-3)	5	5	3	47	122 (92-4)	5	5	3
8	25 (16-4)	5	5	3	48	123 (92-5)	5	5	3
9	27 (16-6)	5	3	5	49	124 (98-1)	5	5	3
10	28 (16-7)	5	5	3	50	126 (99-5)	3	3	3
11	29 (16-8)	5	5	3	51	127 (99-6)	5	5	3
12	30 (16-9)	5	5	5	52	128 (100-1)	5	5	3
13	31 (16-10)	5	5	3	53	131 (101-3)	3	5	3
14	32 (17-1)	5	5	3	54	132 (102-3)	5	5	3
15	38 (19-1)	5	5	3	55	134 (105-3)	5	5	3
16	39 (19-2)	5	5	3	56	135 (105-4)	5	5	3
17	40 (19-3)	5	5	3	57	136 (105-5)	5	5	3
18	41 (19-4)	5	5	3	58	137 (105-6)	5	5	3
19	45 (20-2)	5	5	3	59	138 (105-7)	5	5	5
20	46 (20-3)	5	5	3	60	140 (106-1)	5	5	5
21	49 (22-3)	5	5	3	61	141 (107-1)	5	5	5
22	53 (26-1)	5	5	3	62	145 (107-5)	5	5	5
23	62 (27-3)	5	5	3	63	4 (8-2)	5	5	3
24	63 (27-4)	5	5	3	64	5 (8-3)	5	5	3
25	64 (30-2)	5	5	3	65	6 (8-4)	5	5	3
26	66 (32-3)	3	5	3	66	7 (8-5)	5	5	5
27	69 (39-2)	5	5	3	67	9 (8-7)	5	5	3
28	70 (39-3)	5	5	3	68	10 (8-8)	5	5	3
29	71 (39-4)	5	5	3	69	11(8-9)	5	5	5
30	72 (39-5)	5	5	3	70	12 (8-10)	5	5	3
31	73 (39-6)	5	5	3	71	4 (4-3)	5	5	3
32	74 (39-7)	5	5	3	72	5 (5-7)	5	5	3
33	75 (40-1)	5	5	3	73	6 (6-11)	5	5	3
34	78 (42-1)	5	5	3	74	8 (8-11)	5	5	3
35	84 (42-7)	5	5	3	75	9 (11-1)	5	5	3
36	88 (43-3)	5	5	3	76	32 (47-11)	5	5	3
37	90 (43-3)	5	5	5	77	9 (17-1)	5	5	3
38	94 (44-1)	5	3	5	78	10 (17-2)	5	5	3
39	103 (50-3)	5	5	7	79	12 (17-4)	5	5	3
40	109 (52-1)	5	5	3	80	12 (2-4)	5	5	3

PC: Pod constriction; PB: Pod beak and PR: Pod reticulation

Appendix III: Allele pattern for rust and LLS resistance-linked marker loci among the introgression lines

Sl. No.	Genotypes	GO340445	AhTE360	AhTE498	AhTE621	AhTE618	AhTE487
1	ICGS 76	1	1	1	1	1	1
2	DH 86	1	1	1	1	1	M
3	ISATGR 278-18	2	2	2	2	2	2
4	IASTGR 5B	2	2	2	2	2	M
5	11 (7-3)	1	2	2	2	1	2
6	14 (9-1)	1	2	2	2	2	1
7	20 (14-1)	1	2	2	2	2	2
8	39 (16-2)	2	2	2	2	2	2
9	24 (16-3)	2	2	2	2	1	2
10	25 (16-4)	2	1	2	2	2	2
11	27 (16-6)	2	2	2	2	2	2
12	28 (16-7)	2	2	2	2	2	2
13	29 (16-8)	2	2	2	2	2	2
14	30 (16-9)	2	2	2	2	2	2
15	31 (16-10)	2	2	2	2	2	2
16	32 (17-1)	2	2	2	2	2	2
17	38 (19-1)	2	2	2	2	2	2
18	39 (19-2)	2	2	2	2	2	2
19	40 (19-3)	2	2	2	2	2	2
20	41 (19-4)	2	2	2	2	2	2
21	45 (20-2)	2	2	2	2	2	2
22	46 (20-3)	1	2	2	1	2	2
23	49 (22-3)	2	2	2	2	2	2
24	53 (26-1)	1	2	2	2	2	2
25	62 (27-3)	1	2	2	2	2	2
26	63 (27-4)	1	2	2	2	2	2
27	64 (30-2)	2	2	2	2	2	2
28	66 (32-3)	1	2	2	2	2	2
29	69 (39-2)	1	2	2	2	2	2
30	70 (39-3)	1	2	2	2	2	2
31	71 (39-4)	2	2	1	2	2	2
32	72 (39-5)	2	2	2	2	2	2
33	73 (39-6)	2	1	2	2	2	2
34	74 (39-7)	2	2	2	2	2	2
35	75 (40-1)	2	2	2	2	2	2
36	78 (42-1)	2	1	2	2	2	2
37	84 (42-7)	2	2	1	2	2	2
38	88 (43-3)	2	2	2	1	2	2
39	90 (43-3)	1	2	2	1	2	2
40	94 (44-1)	1	2	2	2	2	2
41	103 (50-3)	1	2	2	2	2	2
42	109 (52-1)	2	2	2	2	2	2
43	110 (52-2)	1	2	2	2	2	2
44	112 (52-4)	2	2	2	2	2	2
45	113 (53-4)	2	2	2	2	2	2
46	118 (60-1)	2	2	2	1	2	2

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47	120 (92-2)	2	2	2	1	2	2
48	121 (92-3)	2	2	2	1	2	2
49	122 (92-4)	2	2	2	2	2	2
50	123 (92-5)	2	2	2	2	2	2
51	124 (98-1)	1	2	2	2	2	2
52	126 (99-5)	2	1	2	2	2	2
53	127 (99-6)	2	2	2	2	2	2
54	128 (100-1)	2	2	2	2	2	2
55	131 (101-3)	1	2	2	1	2	2
56	132 (102-3)	2	2	2	1	2	2
57	134 (105-3)	2	2	2	1	2	2
58	135 (105-4)	2	2	2	1	2	2
59	136 (105-5)	2	2	2	2	2	2
60	137 (105-6)	2	2	2	2	2	2
61	138 (105-7)	2	2	2	2	2	2
62	140 (106-1)	2	2	2	2	2	2
63	141 (107-1)	2	2	2	2	2	2
64	145 (107-5)	2	2	2	2	2	2
65	4 (8-2)	1	1	2	1	1	M
66	5 (8-3)	2	2	2	2	2	M
67	6 (8-4)	2	1	2	2	2	M
68	7 (8-5)	2	2	2	2	2	M
69	9 (8-7)	2	2	2	2	2	M
70	10 (8-8)	2	2	2	2	2	M
71	11 (8-9)	2	2	1	2	2	M
72	12 (8-10)	1	2	2	2	2	M
73	4 (4-3)	2	2	1	2	2	M
74	5 (5-7)	1	1	2	2	2	M
75	6 (6-11)	2	2	2	2	2	M
76	8 (8-11)	2	2	2	2	2	M
77	9 (11-1)	2	2	2	2	2	M
78	32 (47-11)	1	1	2	2	2	M
79	9 (17-1)	2	2	1	2	2	M
80	10 (17-2)	2	2	1	2	2	M
81	12 (17-4)	2	2	1	2	2	M
82	12 (2-4)	1	2	1	2	2	M

Allele pattern 1: susceptible; 2: Resistance and M: Monomorphic

EVALUATION OF INTROGRESSION LINES FOR PRODUCTIVITY TRAITS AND RESISTANCE TO LATE LEAF SPOT AND RUST AND AN INITIATIVE TOWARDS MARKER ASSISTED BACKCROSSING IN TMV 2 IN GROUNDNUT (*Arachis hypogaea* L.)

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Major Advisor

ABSTRACT

Seventy eight introgression lines (ILs) from three populations (ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B) involving late leaf spot (LLS) and rust susceptible varieties (ICGS 76 and DH 86) and disease resistant synthetic allotetraploids (ISATGR 278-18 and ISATGR 5B) were screened for LLS and rust resistance and productivity traits during 2013 *kharif*. ILs differed significantly for most of the disease resistance and productivity traits. High variability, heritability and genetic advance over mean were recorded for LLS score, rust score and pod yield (kg/ha). Significant positive association was found between the occurrence of LLS and rust. Pod yield (kg/ha), pod yield per plant, number of pods per plant and test weight showed significant negative correlation with the severity of LLS and rust. Productivity traits were significantly and positively correlated with each other. Majority of the ILs showed medium pod constriction and medium pod reticulation along with slight pod beak. Two and five lines superior to ICGS 76 and DH 86, respectively selected. Of the seven, three ILs [4 (8-2), 12 (8-10) and 5 (5-7)] were also superior over GPBD 4. The selected superior lines carried resistance allele at majority of the LLS and rust resistance-linked marker loci. These ILs with high level of resistance to LLS and rust and high productivity can be considered for variety release trials or as donor in breeding programmes.

An effort towards marker assisted backcross breeding (MABC) was initiated in TMV 2 for improving LLS and rust resistance by screening the markers for polymorphism. TMV2 and GPBD 4 (donor) showed polymorphism for both LLS and rust (GM2009, IPAHM103, GM1954, GM1536, GM2301 and GM2079) resistance-linked SSR markers, which can be used for foreground selection. Of the 294 background AhTE markers, 42 (14.28%) were polymorphic, which can be employed for background selection in MABC of TMV 2.