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MOLECULAR EVALUATION OF GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES FOR SCLEROTIUM ROT RESISTANCE USING *AhTE* MARKERS

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Groundnut is one of the major economically important oilseed crops and is affected by multiple diseases, including rust, late leaf spot and stem rot. Stem rot, caused by *Sclerotium rolfsii* Sacc., has become a major constraint, leading to yield losses of up to 80 per cent in groundnut. In this study, 92 backcross lines and two parents including resistant parent to late leaf spot and rust, 44 elite genotypes, and 184 mini-core accessions of groundnut (collected from ICRISAT, Hyderabad), along with a resistant check (R 9227) and a susceptible check (TMV-2), were evaluated for stem rot resistance during *kharif* 2018 and 2019.

Field screening was conducted using artificial inoculation with mycelial culture containing sclerotial bodies, and disease incidence was recorded at 30-day intervals after inoculation until harvest. Most of the genotypes were susceptible; however, a few exhibited partial resistance.

ABSTRACT

For molecular screening, the backcross lines and elite genotypes were screened with 150 selected *AhTE* markers, and 50 *AhTE* markers were used to screen the 184 mini-core accessions. 22 markers showed polymorphism between the parents and were used to screen progenies to differentiate resistant and susceptible genotypes. Among the 150 *AhTE* markers, 61 showed polymorphism among elite genotypes, leading to the identification of 107 marker–trait associations. A total of 59 marker–trait associations were identified from the backcross lines screened with 22 markers, while screening the mini-core collection with 50 *AhTE* markers resulted in 95 marker–trait associations.

Key words : Groundnut, Disease incidence, Stem rot, *AhTE* markers, Marker-trait association.

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the major oilseed as well as supplementary food crops of the world (USDA, 2017). It is the fourth most important source of edible oil and the third most important source of vegetable protein (Krapovickas, 1973). Cultivated groundnut is an amphidiploid derived from the hybridization of two wild diploid species, *Arachis duranensis* × *Arachis ipaensis* (Krapovickas, 1969), with a chromosome number of 2n = 40. Peanut is primarily a rainfed crop and is subjected to several constraints affecting its normal growth. Stem and pod rot, caused by the soil-borne saprophytic pathogen *Sclerotium rolfsii*

(teleomorph: *Athelia rolfsii*), are among the major diseases responsible for significant annual yield losses.

The fungus *Sclerotium rolfsii* was first reported as the causal agent of tomato blight in Florida, United States of America, by P. H. Rolf in 1892 (Chauhan *et al.*, 1988; Mehan *et al.*, 1995). Stem rot symptoms generally appear 30–40 days after sowing (DAS), at various crop growth stages, and less frequently as pre-emergence seed rot (Asghari and Mayee, 1991). *S. rolfsii* infects groundnut seedlings and later spreads to other plant parts, causing severe stem infection (Wheeler, 1969; Aycok, 1966; Mathur and Sinha, 1970). The pathogen produces sclerotial bodies—specialized survival structures that

enable it to persist in soil for several years and infect over 500 host species (Weber, 1931; Garret, 1956). In irrigated groundnut-growing regions, particularly during the post-rainy (*rabi*) season, sclerotium wilt results in considerable yield losses (Punja, 1985).

Stem and pod rot occur in all groundnut-growing states of India, with varying levels of severity. The disease is widespread across the country but is comparatively more severe in Telangana, Madhya Pradesh, Karnataka, Andhra Pradesh, Odisha, Gujarat, Tamil Nadu, and Maharashtra, where it is estimated to affect more than 500,000 hectare of groundnut cultivation (Mayee and Datar, 1988). Yield losses range from 13% to 59%, and in severe cases, may reach up to 80% (Pande and Rao, 2000; Nautiyal, 2002). Popular varieties grown in Karnataka and Andhra Pradesh such as JL-24, KRG-1, Dh-40, and TMV-2 have recorded 20% to 60% pod yield reduction due to pod rot (Anonymous, 1992).

Many breeding approaches can be used to improve the disease resistance and molecular breeding in any crop is essentially aimed at accelerating the introgression of beneficial traits into preferred varieties and the availability of genetic linkage maps and molecular markers is a prerequisite for achieving this. Recent advancements in next-generation sequencing (NGS) in groundnut have generated extensive genomic resources that can be effectively utilized in genomics-assisted breeding. Nowadays, molecular markers are widely used to identify major genes/QTLs in various crop species and to introduce new traits into elite germplasm. Successful Marker-Assisted Selection (MAS) requires knowledge of the chromosomal location of genes and specific alleles associated with a particular trait. Molecular markers have become powerful genomic tools for characterizing genetic variation within populations. They not only differentiate genotypes but also aid in molecular breeding and trait-mapping programs (Nayak *et al.*, 2017).

Molecular breeding in groundnut has historically been hindered by a lack of polymorphic molecular markers due to its relatively narrow genetic base (Halward *et al.*, 1991 and Kochert *et al.*, 1991). To address this limitation, transposable element (TE) markers have been developed as an alternative and effective molecular marker system in groundnut (Bhat *et al.*, 2008). In many species, TEs represent a substantial and dynamic source of genetic variability. They are often referred to as “genomic parasites” because their association will increase genome size and contributes to genomic instability in both plants and animals (Kidwell and Lisch, 1997). There are two major types of transposable elements: Class I TEs, which use a copy-and-paste mechanism and migrate through

genomes via RNA intermediates and Class II TEs, which use a cut-and-paste mechanism to mobilize DNA transposons.

Both classes of TEs can be further subdivided into autonomous and non-autonomous elements, depending on whether the transposition-related enzyme is produced by the same element or a different one. One important group of non-autonomous elements is the miniature inverted-repeat transposable elements (MITEs), which are typically less than 600 bp in length (Shirasawa *et al.*, 2012) and contain terminal inverted repeats and flanking target site duplications. Since MITEs preferentially insert into gene-rich regions, they may influence gene expression (Zhang *et al.*, 2000). The “Kazusa Peanut Database” is a widely used resource for *Arachis hypogaea* transposable elements (*AhTEs*) (Shirasawa *et al.*, 2014). Bhat *et al.* (2008) recommended the utilization of transposon markers in groundnut, while Gowda *et al.* (2010) demonstrated the PCR-based validation for detecting the presence or absence of *AhTEs* at predefined genomic locations.

AhTE markers can be easily screened on agarose gels using electrophoresis, and because of this practical advantage, they have been successfully used in multiple trait-mapping studies (Bhat *et al.*, 2008; Gayathri *et al.*, 2018; Gowda *et al.*, 2010; Hake *et al.*, 2017a; Hake *et al.*, 2017b; Kolekar *et al.*, 2016; Mondal *et al.*, 2013; Shirasawa *et al.*, 2012). Previous research at the Department of Biotechnology, University of Agricultural Sciences, Dharwad, has contributed significantly to the development of a large number of *AhTE* markers in groundnut (Hake *et al.*, 2017a; Hake *et al.*, 2017b; Gayathri *et al.*, 2018; Bhat *et al.*, 2008), thereby facilitating marker–trait associations for several diseases, yield traits, and other agronomic characteristics. Utilizing *AhTE* markers for single-marker analysis of stem rot in the mini-core collection, interspecific populations, and elite genotypes of groundnut can help identify resistant genotypes, aiding efforts to manage this destructive soil-borne pathogen.

In this context, the present study was undertaken for field-based pathological screening of *S. rolfisii* resistance in backcross lines and elite genotypes of groundnut during *Kharif* 2018 and in the mini-core collection during *Kharif* 2019, as well as for molecular analysis of all genotypes using selected *AhTE* markers.

Materials and Methods

Seed Materials and Evaluation of population

A total of 325 genotypes—including 92 backcross lines derived from the cross ICGV 87846 × derivative of

ISATGR 278-18, 42 elite cultivars with two checks, and 184 mini-core accessions along with five checks were selected based on previous reports of resistance to rust and late leaf spot (LLS) (Sharma *et al.*, 2017; Gowda *et al.*, 1995; Adiver *et al.*, 1997; Naidu *et al.*, 2016) (Table 1, 2 and 3).

The BC_2F_6 population used in the present study was developed by crossing the derivative of the synthetic amphidiploids; ISATGR 278-18 (donor parent) [ICG 8138 (*Arachis duranensis* Krapov. & W.C. Greg., $2n = 2x = 40$)] with ICGV 87846 (recurrent parent) to introgress resistance against multiple biotic stresses (resistance traits contributed by the donor parent). Seeds of the amphidiploid-derived material along with the parents used in crossing were planted in the glasshouse at the University of Agricultural Sciences (UAS), Dharwad, India. The steps involved in the development of the BC_2F_7 population used in this study are illustrated in Plate 1.

Another set of genotypes used in the present study consisted of elite groundnut cultivars, including two checks (42 + 2), representing released varieties in Karnataka as well as advanced breeding lines developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. TAG 24 and TG 26 were developed and released by the Bhabha Atomic Research Centre (BARC), Bombay. GPBD 4, considered a national check for yield as well as LLS and rust resistance, released by UAS, Dharwad, was also included in the study. JL 24, an early-maturing and widely adapted variety imported and released by Mahatma Phule Krishi Vidyapeeth (MPKV), Jalgaon, was also part of the evaluation. TMV 2, a mutant variety released by Tamil Nadu Agricultural University (TNAU), Coimbatore, served as the national susceptible check for LLS, rust, and stem rot. Some advanced breeding lines developed by ICRISAT, such as ICGV 86699 and ICGV 99005, have shown resistance to LLS, rust, and stem rot (Hossain and Ali, 1990; Gowda *et al.*, 1995; Adiver *et al.*, 1997; Naidu *et al.*, 2016) were also included in the study.

A total of 184 mini-core genotypes, representing a subset of the core germplasm, were collected from ICRISAT, Patancheru, Hyderabad. These genotypes represented *fastigiata* (33), *vulgaris* (71), *peruviana* (2), *aequatoriana* (1), *hypogaea* runner (33), and *hypogaea* bunch (48) botanical types (Table 3).

All the 325 genotypes were evaluated for *Sclerotium rolfsii* resistance and yield-related traits during the rainy seasons of 2018 and 2019, respectively (Plates 2 and 3), using a randomized block design with two replications. Each replication consisted of two rows of 1.5 m length

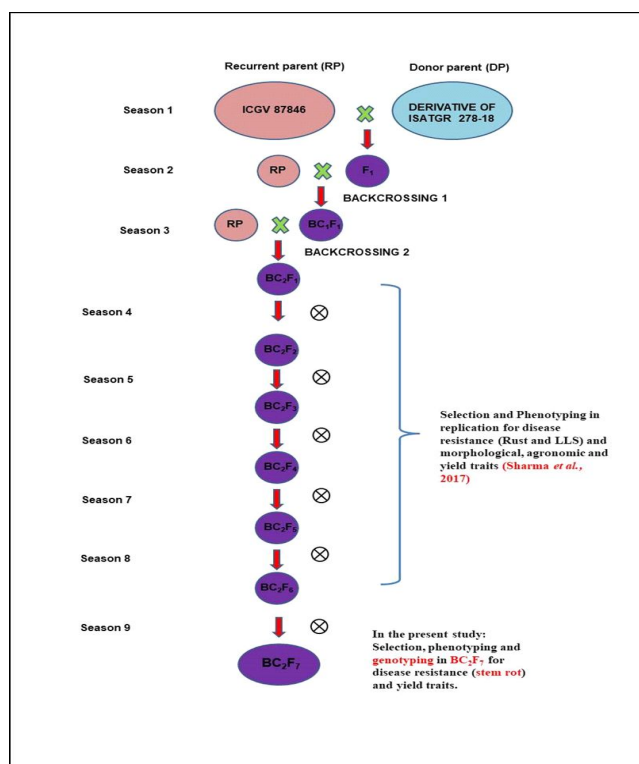


Plate 1 : Backcross population (BC_2F_7 lines) development flow chart.



Plate 2 : Field evaluation view of groundnut accessions carried out during *kharif* 2018 at IABT Garden (E115), MARS, UAS, Dharwad.



Plate 3 : Field evaluation view of groundnut accessions carried out during *kharif* 2019 at IABT Garden (E115), MARS, UAS, Dharwad.

with a spacing of 30 cm × 10 cm. Three representative plants were randomly selected from each genotype for recording data on productivity traits. Observations on pod weight per plant, number of pods per plant, shelling percentage, and test weight were recorded according to the groundnut descriptor (IBPGR/ICRISAT, 1992).

Artificial epiphytotic conditions for stem rot were created in the field using the “Spreader Row Technique” (Subrahmanyam *et al.*, 1995), in which disease-spreader plants (TMV 2) were planted at every 10th row in the experimental plot. The soil type was medium black (vertisol) with a pH range of 7.0 to 7.5, considered suitable for *S. rolfii* growth and development. The seedbed was prepared to a fine tilth prior to sowing. Cultural operations and plant protection measures were followed as per recommended agronomic practices to ensure a uniform and healthy crop stand. Disease observations were recorded at different growth stages—30 DAS, 60 DAS, 90 DAS, and at harvest. Harvesting was carried out according to pod maturity.

Collection, isolation and multiplication of fungus

The *Sclerotium rolfii*-infected plants were collected from the fields of the Main Agricultural Research Station (MARS), Dharwad, during the summers of 2018 and 2019. *S. rolfii* was isolated from the collar region of the affected plant tissues and stems.

Isolation of the fungus was carried out following the standard tissue isolation procedure described by Bagwan (2011), using Potato Dextrose Agar (PDA) medium. Small bits of root and collar tissues showing typical disease symptoms were surface-sterilized with a 1:1000 mercuric chloride solution, followed by rinsing with sterile distilled water to remove any traces of the chemical. The sterilized tissue pieces were then aseptically transferred to sterilized PDA plates and incubated at 27 ± 1°C for three days to induce fungal growth. The PDA culture of *S. rolfii* was maintained and used in the *kharif* 2018 and 2019 studies (Plate 4a).

Sand:corn meal medium was prepared in a 90:10 ratio to achieve maximum sclerotial body production (Abeygunwardhana and Wood, 1975). Four hundred grams of the sand:corn meal mixture was placed in 500 mL conical flasks, mixed with 30 mL of distilled water, and sterilized in an autoclave at 121°C and 1.1 kg cm⁻² pressure for 20 minutes. The pure culture isolate of *S. rolfii* was then inoculated into the flasks under aseptic conditions and incubated at 27 ± 1°C for 30 days (Plate 4b). The flasks were shaken periodically to facilitate uniform growth of the isolate. The mass culture obtained was used for artificial inoculation during *kharif* 2018 and

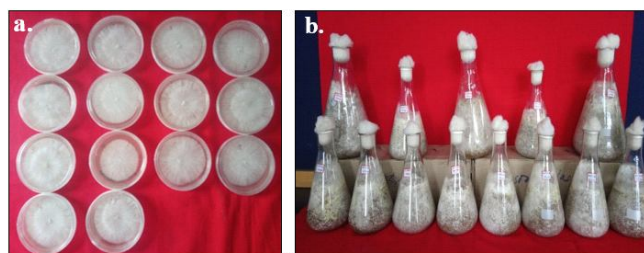


Plate 4: a. Growth of *S. rolfii* on Potato dextrose agar medium; b. Mass multiplication of *S. rolfii* on sand:corn meal medium.

2019.

Soil application and screening of groundnut accessions for resistance to *S. rolfii*

Inoculum containing mycelium and sclerotial bodies, along with corn meal and sand, was applied to the soil surface beside the sowing row at a rate of 100 g per 2.0 m row (ensuring a high inoculum load for uniform disease infection). The inoculum was covered with soil to create a suitable environment for fungal growth, and this was done a few days before sowing in both seasons. A second inoculation was carried out three weeks after sowing by applying the inoculum alongside each seed row. The field was irrigated as required until pod formation to promote stem rot development.

Observations on stem and pod rot incidence were recorded visually for the parents, BC₂F₇ lines and elite genotypes in *kharif* 2018 and for the mini-core collection in *kharif* 2019, using the scale described by Ashok *et al.* (2004).

Genotyping with markers

Parents were screened with 351 *AhTE* markers and elite genotypes with 150 *AhTE* markers (Tables 4 and 5). Markers were selected randomly throughout the genome which covers the entire chromosomes of groundnut and 184 mini core collection were screened with 50 *AhTE* makers (Table 1).

Genotyping of the studied population

Genomic DNA was isolated from all the genotypes using healthy young leaves following the modified CTAB method (Mace *et al.*, 2003). *AhTE* markers were used to screen these genotypes. The PCR reaction volume was maintained at 10 µL, containing 50 ng of template DNA. PCR amplification was performed in 96-well plates using a Mastercycler-PCR (MC Nexus Gradient, Eppendorf AG, Germany) with the following temperature profile: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 53°C for 1 min and extension at 72°C for 1 min 30 s, with a final extension at 72°C for 5 min.

Table 1 : List of markers used in *kharif* 2019 studies.

<i>AhTE122</i>	<i>AhTE143</i>	<i>AhTE205</i>	<i>AhTE233</i>	<i>AhTE243</i>	<i>AhTE496</i>	<i>AhTE381</i>	<i>AhTE534</i>
<i>AhTE422</i>	<i>AhTE431</i>	<i>AhTE460</i>	<i>AhTE461</i>	<i>AhTE482</i>	<i>AhTE1348</i>	<i>AhTE1240</i>	<i>AhTE1450</i>
<i>AhTE1258</i>	<i>AhTE1266</i>	<i>AhTE1276</i>	<i>AhTE1277</i>	<i>AhTE1283</i>	<i>AhTE370</i>	<i>AhTE2010</i>	<i>AhTE1744</i>
<i>AhTE323</i>	<i>AhTE344</i>	<i>AhTE346</i>	<i>AhTE357</i>	<i>AhTE362</i>	<i>AhTE1092</i>	<i>AhTE1381</i>	<i>AhTE1777</i>
<i>AhTE556</i>	<i>AhTE614</i>	<i>AhTE615</i>	<i>AhTE1052</i>	<i>AhTE1087</i>	<i>AhTE2006</i>	<i>AhTE419</i>	<i>AhTE551</i>
<i>AhTE254</i>	<i>AhTE261</i>	<i>AhTE283</i>	<i>AhTE296</i>	<i>AhTE303</i>	<i>AhTE1379</i>	<i>AhTE1254</i>	<i>AhTE1522</i>
<i>AhTE1763</i>	<i>AhTE1907</i>						

AhTE: *Arachis hypogaea* transposable element

The amplicons were separated by electrophoresis on 1% agarose gels using a Bio-Rad gel electrophoresis unit in 1X TAE buffer at 80 V for 1 hour. Alleles differing by 205 bp (with *AhMITE1* insertion) amplified by *AhTE* markers were scored as “A”, while alleles without the *AhMITE1* insertion were scored as “B” and “H” (heterozygote).

Data analysis

The field data were analyzed to determine the Analysis of Variance (ANOVA), frequency distribution, and genetic variability for four diseases and nine yield-related traits for both populations across two seasons. Significance was assessed at alpha levels of 0.05 and 0.01 (5% and 1% probability). Correlation analysis was performed using SPSS 16.0 software. Genotypic data were used to estimate major allele frequency and Polymorphic Information Content (PIC) following Hildebrand *et al.* (1992).

To determine whether any of the *AhTE* markers screened in the selected populations were associated with the traits, Single Marker Analysis (SMA) was conducted to estimate marker significance and its contribution to disease and yield-related traits using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). Marker–Trait Association (MTA) analysis was carried out using SMA through single-factor ANOVA by calculating the F-statistic in MS-Excel data sheets. MTAs showing more than 10% R^2 (phenotypic variance explained, PVE%) were considered important for disease and yield-related traits. These markers associated with disease were further evaluated in the 184 mini-core collection.

Results and Discussion

Variability in disease and yield related traits

Most of the genotypes in the present study exhibited significant variation for yield and yield-related traits as well as disease-related traits. The mean sum of squares due to genotypes was significant for all traits at 1% probability in BC_2F_7 lines, elite genotypes, and the mini-core collection (Table 2). The significant differences

observed among genotypes for all the traits indicate the presence of considerable genetic variability and the potential for genetic improvement of the studied traits (Falconer, 1989).

The coefficient of variation (CV) in backcross lines for all traits ranged from 5.64% to 12.45% (Table 2). Pod yield per plant and disease incidence at 30, 60 and 90 DAS showed medium CV (10–20%) (Table 2) in BC_2F_7 genotypes. Similarly, in elite genotypes, CV values ranged from 7.59% to 12.55%, for the traits like, pod yield per plant, seeds per pod, shelling percentage and disease incidence at 30, 60 and 90 DAS (Table 2). In the mini-core collection, CV values ranged from 6.46% to 14.75%, for pod yield per plant and disease incidence at 20, 40 and 60 DAS and fell under medium CV (10–20%) category (Table 2). Korat *et al.* (2009), Savaliya *et al.* (2009) and Mukesh *et al.* (2014) reported similar results in groundnut for same traits.

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), range, mean, heritability in the broad sense (h^2) and predicted genetic advance (GA) as a percentage of the mean for all traits are presented in Table 3. These genetic variability parameters help estimate the relative contributions of genotype and environment in determining the phenotype and provide insight into the heritability of these traits. The mean is a measure of central tendency, while the range indicates the dispersion of data, reflecting the variability among the selected genotypes.

High PCV coupled with high GCV was observed for total disease incidence (48.34 and 47.85), pod yield per plant (33.25 and 31.23) and disease incidence at 20 DAS (74.50 and 72.20) (Table 3). These results are in agreement with the findings of Balaraju and Kenchanagoudar *et al.* (2016) for disease incidence at harvest (%) and with Pujer *et al.* (2013) for pod yield per plant. These findings indicate a substantial amount of genetic variability found among genotypes, which provides a good scope for genetic improvement.

All yield and yield-related traits exhibited moderate

Table 2 : ANOVA for disease incidence at various stages and yield related traits in the BC₂F₇ lines, elite genotypes, and mini core collection of groundnut.

BC ₂ F ₇ lines									
Source of variation	30PDI	60PDI	90PDI	TPDI	NPP	SP	TW	PYPP	SPP
S. Em. ±	0.92	1.73	3.15	1.42	0.79	3.83	1.88	1.41	0.29
F Value	16.29**	2.99**	3.69**	146.06**	29.13**	3.57**	5.08**	10.60**	1.74**
C.D. (5 %)	2.57	4.86	8.83	3.98	2.21	10.76	5.29	3.96	0.81
C.D. (1 %)	3.40	6.43	11.70	5.27	2.93	14.25	7.01	5.24	1.07
C.V.(%)	10.31	12.45	11.21	5.64	8.64	8.18	7.93	11.23	9.14
Elite genotypes									
Source of variation	30PDI	60PDI	90PDI	TPDI	NPP	SP	TW	PYPP	SPP
S. Em. ±	0.57	0.76	2.30	1.93	0.89	4.74	1.85	1.15	0.24
F Value	13.64**	7.91**	13.49**	86.72**	6.08**	4.09**	2.99**	5.91**	2.40**
C.D. (5 %)	1.63	2.17	6.57	5.51	2.53	13.53	5.27	3.27	0.70
C.D. (1 %)	2.19	2.90	8.78	7.37	3.38	18.09	7.05	4.37	0.93
C.V.(%)	12.55	11.25	10.45	7.95	8.33	10.47	7.39	10.41	12.18
Mini core collection									
Source of variation	20PDI	40PDI	60PDI	90PDI	CP	PYPP	NPP	SP	TW
S. Em. ±	0.46	0.47	0.47	0.48	1.13	0.31	0.18	0.34	0.50
F Value	27.72**	27.47**	29.44**	18.26**	21.20**	12.12**	24.58**	2.44**	21.24**
C.D. (5%)	3.37	3.48	3.43	4.36	9.43	3.44	1.43	8.42	4.17
C.D. (1%)	4.45	4.59	4.52	5.75	12.44	4.54	1.89	11.10	5.49
C.V.(%)	14.75	12.26	10.22	8.51	6.82	7.26	12.38	6.46	10.34

** : Significant at 1 % level of probability

df: Degrees of freedom; S. Em. ±: Standard error of mean; C.D.: Critical difference; C.V.: Coefficient of variation (%); 30PDI: Per cent disease incidence at 30 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod; 20PDI: Per cent disease incidence at 20 days(%); 40PDI: Per cent disease incidence at 40 days(%); CP: Colonization Percentage (%); NPP: Pods per plant.

to high heritability across both seasons (Table 3). Heritability estimates partition the environmental influence from the total variability, and their importance has been emphasized in plants by Johnson *et al.* (1955). In the present study, the highest heritability was observed for the number of pods per plant (84.23%) and pod yield per plant (84.76%), followed by colonization percentage (80.61%) (Table 3). High heritability suggests minimal environmental influence on these traits, indicating that they can be effectively improved through direct selection (Panse and Sukhatme, 1967).

The highest heritability coupled with high genetic advance as a percentage of mean (GAM) was observed for the number of pods per plant (54.12%), percent disease incidence at 30 DAS (39.22%), and pod yield per plant (74.17%) (Table 3). High heritability with high genetic advance is generally governed by additive gene action, suggesting that direct selection for these traits would be effective (Kadam *et al.*, 2016; John *et al.*, 2013; Savita *et al.*, 2014; Terkimbi *et al.*, 2014; Padmaja *et al.*,

2015; Vasanthi *et al.*, 2015).

Phenotypic correlation

Correlation coefficients measure the mutual relationships among various traits and help in designing efficient strategies for indirect selection using component traits, as well as simultaneous selection for multiple traits. Correlation between agronomic and disease-related traits are particularly useful for determining whether selection for one trait will affect another and highly heritable traits can be correlated with more complex traits in the selection process (Nair *et al.*, 1998).

In the present study, a total of 16 trait pairs in backcross lines and 17 in elite genotypes were found to be significantly correlated at 1% probability (Tables 4 and 5), while seven pairs were significantly correlated at 1% probability in the mini-core collection (Table 5).

As explained by Narashimulu *et al.* (2012) and Patil *et al.* (2004); shelling percentage, number of pods per plant and test weight showed a significant positive

Table 3 : Genetic components of variation for disease incidence at various stages and yield related traits in the BC₂F₇ lines, elite genotypes, and mini core collection of groundnut.

BC ₂ F ₇ lines							
Phenotypic traits	Range		Mean (%)	PCV (%)	GCV (%)	Heritability % of mean	Genetic advance as
	Maximum	Minimum					
30PDI	11.81	0.00	6.88	22.01	20.12	52.21	31.21
60PDI	24.26	0.00	16.28	23.45	20.14	65.14	24.09
90PDI	29.41	5.00	21.90	22.70	21.35	57.33	36.04
TDI	62.13	15.12	35.54	48.34	47.85	85.24	75.20
NPP	28.64	8.40	12.88	33.54	32.40	84.23	54.12
SP	78.41	53.34	66.21	12.37	10.23	56.21	26.35
TW	41.20	15.12	33.60	13.83	11.34	67.13	35.20
PYPP	29.80	6.10	13.10	35.56	32.35	70.23	60.62
SPP	3.38	2.07	2.78	17.19	14.23	45.89	19.23
Elite genotypes							
Phenotypic traits	Range		Mean (%)	PCV (%)	GCV (%)	Heritability % of mean	Genetic advance as
	Maximum	Minimum					
30PDI	12.14	0.00	6.37	27.60	24.10	76.20	39.22
60PDI	24.18	0.00	16.16	15.06	13.01	47.74	17.23
90PDI	31.18	5.13	20.90	23.12	21.13	66.20	28.21
TPDI	66.7	5.13	34.38	27.85	24.34	21.34	17.63
NPP	29.96	7.56	15.04	27.25	23.56	65.12	33.65
SP	79.32	39.89	64.03	16.71	13.02	54.83	20.89
TW	49.88	24.74	35.35	10.43	7.36	49.84	10.71
PYPP	39.40	8.30	15.57	33.25	31.23	80.24	36.25
SPP	3.43	2.21	2.84	15.88	10.18	41.10	13.44
Mini core collection							
Phenotypic traits	Range		Mean (%)	PCV (%)	GCV (%)	Heritability % of mean	Genetic advance as
	Maximum	Minimum					
20PDI	29.17	0.00	8.05	74.85	72.20	70.61	53.84
40PDI	30.87	0.00	8.27	69.81	68.20	72.04	60.47
60PDI	34.88	0.00	8.64	66.77	64.05	73.03	52.12
90PDI	38.18	5.90	14.92	48.54	46.16	78.12	66.45
CP	87.34	6.26	34.77	49.62	48.92	80.61	72.17
PYPP	26.56	6.09	10.52	42.48	41.23	84.76	74.17
NPP	14.17	4.33	7.00	37.11	35.63	64.76	44.79
SP	75.93	54.12	66.11	48.81	47.92	78.65	62.11
TW	62.18	26.03	37.94	37.16	35.86	74.08	63.93

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; h^2_{bs} : Heritability in broad sense; GA: Genetic advance; GAM: Genetic advance as percent of mean; 30PDI: Per cent disease incidence at 30 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod; 20PDI: Per cent disease incidence at 20 days (%); 40PDI: Per cent disease incidence at 40 days (%); CP: Colonization Percentage after harvest (%); NPP: Pods per plant.

Table 4 : Correlation between disease incidence at various stages and yield related traits in the backcross lines (BC₂F₁) of groundnut.

	30PDI	60PDI	90PDI	TPDI	NPP	TW	SP	PYPP	SPP
30PDI	1								
60PDI	0.371**	1							
90PDI	0.101	0.177	1						
TPDI	0.252**	0.138	0.208**	1					
NPP	-0.317**	-0.279	-0.197**	-0.234**	1				
TW	-0.456**	-0.293**	0.068	-0.113**	0.018	1			
SP	0.176	-0.011	-0.074	-0.12**	0.158**	0.236**	1		
PYPP	-0.216**	-0.308**	-0.008	-0.099**	0.343**	0.11	0.246**	1	
SPP	-0.046	0.111	-0.018	0.08**	-0.049	0.148**	0.005	-0.121	1

**: Significant at 1% level of probability

30PDI: Per cent disease incidence at 30 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod.

Table 5 : Correlation between disease incidence at various stages and yield related traits in the elite genotypes of groundnut.

	30PDI	60PDI	90PDI	TPDI	NPP	TW	SP	PYPP	SPP
30PDI	1.000								
60PDI	0.207**	1.000							
90PDI	0.167**	0.276	1.000						
TPDI	0.357**	0.366**	0.143**	1.000					
NPP	-0.218**	-0.252	-0.155	-0.388**	1.000				
TW	-0.041	-0.098	-0.022**	0.048	0.213**	1.000			
SP	-0.116**	-0.114	-0.071	-0.556**	0.251	0.135**	1.000		
PYPP	-0.273	0.045	-0.132**	-0.714**	0.139**	0.008	0.135**	1.000	
SPP	0.254	-0.086	-0.048	-0.158**	0.015	0.056	0.019	0.037	1.000

**: Significant at 1 % level of probability

30PDI: Per cent disease incidence at 30 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod.

correlation with pod yield per plant at both the phenotypic and genotypic levels (Tables 5, 7 and 8) and these trait correlations positively help to increase seed yield in groundnut. Percent disease incidence at harvest (colonization percentage) exhibited a negative correlation with number of pods per plant, test weight, and shelling percentage (Tables 4, 5 and 6), suggesting the possibility of identifying genotypes with higher pod yield and lower disease incidence (Pujer *et al.*, 2013).

Furthermore, number of pods per plant, shelling percentage, and test weight were not only positively and significantly associated with pod yield per plant but also showed significant positive associations among themselves. Disease incidence at 30, 60 and 90 DAS, as well as at harvest (colonization percentage), exhibited negative significant associations with pod yield per plant and significant positive associations among themselves (Tables 4, 6, and 7).

Motagi *et al.* (1997) also showed a negative association of late leaf spot with yield in Spanish bunch groundnut mutants and Iroume and Knauff (1987) reported a negative association between biotic stress response and pod yield in advanced breeding lines.

Grouping of the groundnut lines into different classes of resistance

Based on the range of total per cent disease incidence at harvest (colonization percentage), all genotypes were classified into five categories: immune (0%), resistant (0.1–10%), moderately resistant (10–20%), moderately susceptible (20–30%) and susceptible (>30%) (Ashok *et al.*, 2004).

Under field conditions, although the artificial inoculum load (~100 g per row per genotype) was distributed equally, variability in disease incidence among genotypes (Plate 5A & 5B) was clearly observed. To quantify this



Plate 5 : Appearance of (A) Recurrent parent; (B) Donor parent, at the time of harvesting.

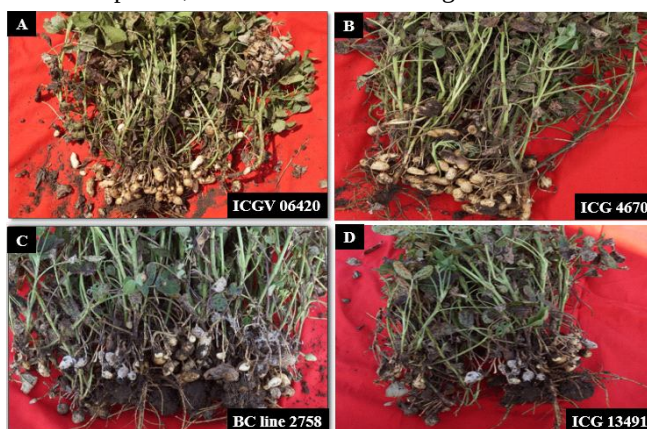


Plate 6 : Appearance of (A) resistant genotype ICGV 06420 (*kharif* 2018); (B) resistant genotype ICG 4670 (*kharif* 2019); (C) susceptible genotype (BC line 2758) (*kharif* 2018); (D) susceptible genotype ICG 13491 at harvesting time (*kharif* 2019).

variability, genotypes were grouped into different classes based on disease incidence (DI). The results showed 11 moderately resistant, 73 moderately susceptible and 43 susceptible genotypes. A few genotypes exhibited partial

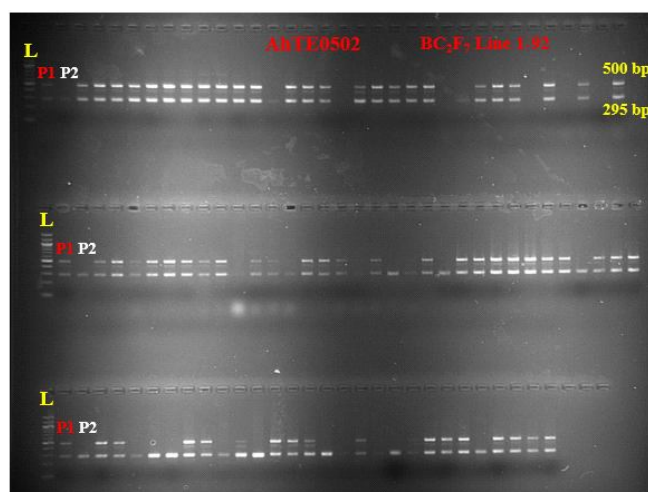


Plate 7 : PCR results for genotyping of 92 backcross lines (BC_2F_7) with *AhTE0502* marker. L = DNA ladder, P1 = Parent one (ICGV 87846), P2 = Parent two (derivative of ISATGR 278-18) and bp = base pairs.

resistance, including derivatives of ISATGR 278-18 (2552, 2584, 2854, and 2951), ICGV 06420, Mutant III, R 9227, TG 38, ICG 2773, ICG 14008, ICG 5891, ICG 12988, ICG 4670 and ICG 4543 (Plate 6A & 6B; Table 6, 7, and 8).

Most genotypes were susceptible to the selected *Sclerotium rolfii* isolate (Plate 6C & 6D) and resistant reactions were observed only in a few genotypes (ICGV 06420 and ICG 4670) that showed no systemic infection symptoms, such as yellowing or wilting (Farooq *et al.*, 2011; Eslami *et al.*, 2015).

Genotyping of all the groundnut genotypes

The parental genotypes (ICGV 87846 × derivative of ISATGR 278-18) were screened with 351 *AhTE* markers (Shirasawa *et al.*, 2012). Among these, 25 markers showed polymorphism, indicated by a band

Table 6 : Phenotypic correlations among various yield and yield related traits in groundnut mini-core collection.

	20PDI	40PDI	60PDI	90PDI	CP	PYPP	NPP	SP	TW
20PDI	1								
40PDI	0.989**	1							
60PDI	0.992**	0.981**	1						
90PDI	0.064	0.095	0.068	1					
CP	0.095	0.116	0.098	0.331**	1				
PYPP	-0.054	-0.056	-0.047*	-0.734**	-0.163*	1			
NPP	-0.080	-0.082	-0.073	-0.130*	-0.090	0.879**	1		
SP	-0.084	-0.091	-0.084*	-0.033	-0.111*	0.132	0.260	1	
TW	-0.015	-0.005	-0.015	-0.087*	-0.060	0.895*	0.149	0.635**	1

** and *: Significant at 1% and 5% level of probability.

20PDI: Per cent disease incidence at 20 days (%); 40PDI: Per cent disease incidence at 40 days(%); 60PDI: Per cent disease incidence at 60days(%); 90PDI: Per cent disease incidence at 90 days(%); CP: Colonization Percentage (%); PYPP: Pod yield per plant; NPP: Pods per plant; SP: Shelling percentage; TW: Test weight (g).

Table 7 : Marker trait association for disease incidence at various stages and yield related traits in the backcross lines (BC₂F₇) of groundnut.

S. no.	Chromosome No.	Marker	Trait	F value	P-Value	PVE %
1	A01	<i>AhTE119</i>	90PDI	4.56	0.03	4.72
2			NPP	7.89	0.006	7.9
3		<i>AhTE1052</i>	NPP	6	0.01	6.12
4			TW	5.12	0.02	5.27
5		<i>AhTE1258</i>	60PDI	3.7	0.02	7.52
6			NPP	3.22	0.004	6.62
7	A03	<i>AhTE504</i>	30PDI	12.33	0.0006	11.81
8			60PDI	14.74	0.02	13.81
9			90PDI	10.42	0.0001	10.17
10			NPP	7.79	0.006	7.81
11			SP	15.07	0.0001	14.08
12			YP	13.48	0.0003	12.86
13		<i>AhTE534</i>	30PDI	5.72	0.01	5.85
14			60PDI	4.8	0.03	4.95
15			SP	30.59	0.006	24.95
16			TW	5.4	0.02	5.55
17			YP	17.12	0.0001	15.69
18	A04	<i>AhTE1253</i>	NPP	5.43	0.02	5.57
19			SPP	23.64	0.002	20.43
20			YP	13.6	0.0003	12.88
21	A05	<i>AhTE1266</i>	90PDI	9.6	0.002	9.23
22			TPDI	19.36	0.001	17.38
23			SP	4	0.04	4.17
24	A05	<i>AhTE1276</i>	SPP	21.55	0.000001	18.97
25			30PDI	9.81	0.002	9.63
26			90PDI	7.73	0.006	7.75
27			TDI	9.67	0.002	9.51
28			NPP	6.39	0.01	6.5
29			YP	7.47	0.007	7.51
30		<i>AhTE1277</i>	30PDI	5.56	0.02	5.7
31			90PDI	12.47	0.0006	11.94
32			NPP	7.82	0.006	7.84
33			YP	11.69	0.0003	11.27
34		<i>AhTE1283</i>	TPDI	5.17	0.02	5.32
35	A06	<i>AhTE1348</i>	NPP	18.31	0.0001	16.6
36			SPP	15.77	0.0001	14.63
37			YP	5	0.02	5.15

Table 7 continued...

Table 7 continued...

38	A09	<i>AhTE</i> 121	30PDI	9.18	0.003	9.07
39			60PDI	13.51	0.0003	12.8
40			90PDI	9.68	0.002	9.52
41			TPDI	7.559	0.007	7.62
42			NPP	15.7	0.0001	14.57
43	A10	<i>AhTE</i> 314	PYPP	6.08	0.0003	11.79
44			NPP	8.75	0.0003	16.14
45			SP	2.97	0.05	6.14
46			YP	25.01	0.00001	21.37
47	B02	<i>AhTE</i> 296	60PDI	5.64	0.01	5.77
48	B07	<i>AhTE</i> 190	30PDI	14.87	0.0002	13.91
49			NPP	13.52	0.0003	12.82
50	B09	<i>AhTE</i> 254	60PDI	3.79	0.02	7.7
51			90PDI	5.03	0.008	9.97
52			TPDI	3.17	0.04	6.52
53			TW	11.45	0.003	20.1
54	Unknown	<i>AhTE</i> 256	60PDI	4.64	0.01	9.26
55			PYPP	11.8	0.001	20.6
56		<i>AhTE</i> 551	30PDI	4043	0.03	4.6
57			TW	22.54	0.0001	19.68
58		<i>AhTE</i> 556	30PDI	10.96	0.001	10.65
59			NPP	20.16	0.01	17.97

AhTE: *Arachis hypogaea* transposable element; 30PDI: Per cent Disease Incidence at 30 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod; YP: Yield per plant.

difference of 205 base pairs between the parents, while 335 markers were monomorphic, producing bands of similar size. The 25 polymorphic markers were further used to screen 92 backcross lines and 22 markers were able to differentiate the population into homozygous and heterozygous types relative to the parents (Plate 7, Table 9).

Similarly, 150 *AhTE* markers were screened for polymorphism in 42 elite genotypes. Of these, 61 markers were polymorphic and 76 were monomorphic (Plate 8) (Table 10). Based on high phenotypic variance explained (PVE) and polymorphic information content (PIC) values, 52 *AhTE* markers were selected for screening the 184 mini-core genotypes, including checks. Among these 52 markers, 50 showed polymorphism within the mini-core collection (Plate 9, Table 11).

Marker-trait association analysis in BC₂F₇ lines, elite genotypes and mini core collections

Marker-Trait Associations (MTAs) helps in identification of genetic markers that contribute significantly to the genetic variation of a specific trait. In the present study, marker-trait associations were analyzed using Single Marker Analysis (SMA). SMA integrates molecular marker data with conventional genetic analysis to identify MTAs (Halward *et al.*, 1991). Unlike linkage mapping, SMA does not require the construction of a linkage map, which is often tedious and time-consuming.

SMA estimates the contribution of each marker to the total phenotypic variance of a trait. A high percentage of phenotypic variance explained (PVE) by a marker suggests that it may behave similarly across different sets of genotypes, while a low P-value indicates high accuracy and precision in the experiment. In a previous

Table 8 : Marker trait association for disease incidence at various stages and productivity traits in the elite genotypes of groundnut.

S. no.	Chromosome	Marker	Trait	F value	P-Value	PVE %
1	A01	<i>AhTE1052</i>	NPP	10.53	0.002	20.44
2		<i>AhTE233</i>	TW	12.33	0.001	23.15
3		<i>AhTE363</i>	TPDI	4.25	0.04	9.4
4			TW	9.82	0.0083	19.33
5		<i>AhTE365</i>	30PDI	5.04	0.03	10.96
6			60PDI	4.45	0.04	9.8
7			90PDI	4.61	0.03	10.12
8	A02	<i>AhTE1087</i>	30PDI	5.1	0.02	11.06
9			60PDI	5.19	0.02	11.23
10			90PDI	4.64	0.03	10.17
11			NPP	6.17	0.01	13.08
12		<i>AhTE1092</i>	SPP	6.87	0.01	14.36
13			YP	5.2	0.02	11.26
14	A03	<i>AhTE504</i>	90PDI	4.2	0.04	9.3
15			NPP	4.5	0.03	9.96
16			SPP	4.05	0.05	9
17		<i>AhTE334</i>	YP	8.12	0.006	16.53
18	A04	<i>AhTE1240</i>	TPDI	9.78	0.003	19.26
19			SPP	4.14	0.04	9.18
20		<i>AhTE336</i>	SP	5.32	0.02	11.48
21		<i>AhTE422</i>	SPP	4.96	0.03	10.79
22		<i>AhTE419</i>	TW	5.34	0.02	11.53
23	A05	<i>AhTE1283</i>	NPP	7.8	0.007	16.03
24			SP	11.2	0.001	21.57
25		<i>AhTE370</i>	SP	12.36	0.0001	23.16
26		<i>AhTE460</i>	60PDI	4.75	0.03	10.39
27	A06	<i>AhTE1351</i>	60PDI	4.87	0.03	10.62
28			TPDI	9.33	0.003	18.53
29			NPP	11.58	0.001	22.03
30		<i>AhTE1381</i>	SP	5.7	0.02	12.29
31			TW	3.75	0.05	8.39
32			TPDI	9.57	0.003	18.92
33		<i>AhTE344</i>	60PDI	5.35	0.02	11.54
34			NPP	4.23	0.04	9.35
35		<i>AhTE472</i>	30PDI	4.13	0.04	9.16
36	A07	<i>AhTE392</i>	60PDI	4.51	0.03	4.07
37			TW	12.84	0.0008	23.85

Table 8 continued...

Table 8 continued...

38	A08A08	<i>AhTE</i> 1423	30PDI	4.63	0.03	10.16
39			60PDI	5.43	0.02	11.7
40			90PDI	4.58	0.03	10.05
41			TPDI	4.94	0.03	10.75
42		<i>AhTE</i> 1450	NPP	5.7	0.02	12.28
43		<i>AhTE</i> 1431	30PDI	4.62	0.03	10.13
44			90PDI	4.2	0.04	9.3
45			SP	5.01	0.03	10.89
46			SPP	4.21	0.04	9.31
47		<i>AhTE</i> 216	YP	9.74	0.003	19.2
48	A09	<i>AhTE</i> 303	SPP	7.45	0.009	15.37
49		<i>AhTE</i> 122	SP	6.36	0.01	13.43
50		<i>AhTE</i> 346	SPP	9.96	0.002	18.45
51			TW	12.46	0.001	23.31
52		<i>AhTE</i> 362	SPP	3.89	0.05	8.67
53			90PDI	4.87	0.03	10.63
54		<i>AhTE</i> 499	SPP	3.91	0.05	8.71
55		<i>AhTE</i> 491	90PDI	4.49	0.04	9.87
56			TPDI	5.93	0.01	12.64
57			YP	4.21	0.04	9.33
58	A10	<i>AhTE</i> 1522	SPP	6.02	0.01	12.8
59			60PDI	4.77	0.03	10.42
60		<i>AhTE</i> 283	SPP	4.81	0.03	10.5
61		<i>AhTE</i> 357	SPP	13.49	0.0006	24.7
62		<i>AhTE</i> 374	YP	4.42	0.04	9.73
63			TPDI	8.51	0.005	17.19
64		<i>AhTE</i> 314	SPP	4.18	0.04	9.25
65			TW	6.22	0.01	13.17
66			YP	6.45	0.01	13.6
67	B02	<i>AhTE</i> 296	TW	6.86	0.01	14.34
68	B04	<i>AhTE</i> 261	NPP	7.8	0.07	16.09
69		<i>AhTE</i> 1744	90PDI	4.25	0.04	9.39
70			NPP	5.4	0.02	11.7
71			30PDI	6.39	0.01	13.49
72		<i>AhTE</i> 1763	YP	5.01	0.03	10.89
73		<i>AhTE</i> 1777	30PDI	4.97	0.03	10.81
74	B05	<i>AhTE</i> 119	NPP	10.4	0.002	20.2
75		<i>AhTE</i> 323	TPDI	9.12	0.004	18.19
76			SPP	4.93	0.01	19.77

Table 8 continued...

Table 8 continued...

77	B06	<i>AhTE496</i>	NPP	8.38	0.0006	16.9
78			SP	9.3	0.003	18.49
79			YP	3.75	0.05	8.39
80		<i>AhTE1908</i>	30PDI	5.04	0.03	10.95
81			90PDI	5.58	0.02	11.98
82			TPDI	9.27	0.004	18.44
83			NPP	13.87	0.004	25.2
84			SP	10.3	0.002	20.08
85	B07	<i>AhTE190</i>	30PDI	4.13	0.04	9.17
86			60PDI	5.14	0.02	11.15
87			90PDI	4.53	0.03	9.94
88			TPDI	4.75	0.03	10.38
89			TW	9.05	0.004	18.09
90			YP	9.54	0.003	18.87
91		<i>AhTE143</i>	TW	8.22	0.006	16.7
92	B08	<i>AhTE2006</i>	30PDI	4.1	0.04	9.09
93			60PDI	8.36	0.006	16.94
94			TPDI	5.21	0.02	11.29
95			TW	4.83	0.03	10.54
96		<i>AhTE2010</i>	30PDI	5.77	0.02	12.35
97			60PDI	4.32	0.04	9.53
98			90PDI	4.99	0.03	10.86
99			SP	13.6	0.006	24.99
100	Unknown	<i>AhTE256</i>	SPP	4.04	0.02	16.49
101		<i>AhTE614</i>	NPP	11.18	0.001	21.4
102		<i>AhTE558</i>	TPDI	8.69	0.005	17.49
103		<i>AhTE556</i>	SP	7.3	0.009	15.28
104		<i>AhTE551</i>	60PDI	5.04	0.03	10.95
105			TPDI	8.94	0.004	17.91
106			TW	4.06	0.05	9.01

AhTE: *Arachis hypogaea* transposable element; 30PDI: Per cent disease incidence at 30 days (%); 60PDI: Per cent Disease Incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); TPDI: Total Per cent disease incidence (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g); SPP: Seeds per pod; YP: Yield per plant (g).

study on LLS and rust resistance, the *AhTE0491* marker for rust at 90 DAS showed 10.52% PVE and *AhTE0121* for LLS at 90 DAS showed 12.96% PVE. In the present study, these same markers showed 12.64% and 7.62% PVE for total disease incidence of stem rot, respectively.

In the BC₂F₇ lines, out of 22 *AhTE* markers, 19 markers (representing 59 MTAs) showed significant

association at the 5% and 1% probability levels (Table 7). The 59 MTAs in backcross lines were categorized based on PVE: 32 MTAs showed <10% PVE, 22 MTAs showed 10–20% PVE, and 5 MTAs showed >20% PVE, with a range of 4.17% to 24.95%. The corresponding P-values ranged from 0.0001 to 0.05.

In backcross lines, the highest R² values, indicating

Table 9 : Marker-trait association for disease incidence at various stages and yield related traits in mini core collection of groundnut.

S. no.	Marker	Chromosome No.	Trait	F Value	P-Value	PVE %
1	<i>AhTE0122</i>	A09	CP	5.60	0.004	7.59
2			PYPP	4.23	0.005	7.35
3			NPP	6.49	0.03	5.61
4	<i>AhTE0143</i>	B07	SP	7.63	0.02	5.69
5			NPP	5.12	0.0001	9.45
6	<i>AhTE0205</i>	A03	90PDI	3.67	0.001	5.05
7			PYPP	6.56	0.001	7.90
8			NPP	7.09	0.0008	10.43
9			SP	10.30	0.0001	15.75
10			TW	5.85	0.03	5.20
11	<i>AhTE0233</i>	A01	90PDI	3.25	0.002	7.71
12			SP	4.21	0.02	5.46
13			TW	12.40	0.0002	16.11
14	<i>AhTE0243</i>	B01	PYPP	4.91	0.03	6.28
15			NPP	3.71	0.001	6.67
16	<i>AhTE0254</i>	B09	NPP	6.28	0.005	7.56
17			TW	13.19	0.0006	12.40
18	<i>AhTE0261</i>	A09	90PDI	4.65	0.007	7.66
19			PYPP	4.30	0.02	6.49
20			NPP	3.83	0.001	7.56
21	<i>AhTE0283</i>	B02	60PDI	4.51	0.04	5.45
22			90PDI	4.55	0.08	5.42
23			CP	8.35	0.002	9.12
24			SP	4.59	0.002	8.07
25	<i>AhTE0296</i>	B02	60PDI	3.64	0.04	5.35
26			90PDI	5.43	0.02	5.57
27	<i>AhTE0296</i>	B02	SP	4.85	0.04	5.12
28			TW	3.92	0.03	6.14
29	<i>AhTE0344</i>	A06	NPP	5.16	0.0002	10.93
30	<i>AhTE0346</i>	A06	CP	3.89	0.01	5.42
31			SP	5.16	0.009	8.27
32	<i>AhTE0357</i>	A10	90PDI	4.13	0.009	7.67
33			NPP	5.70	0.001	8.77
34	<i>AhTE0362</i>	A09	NPP	6.89	0.0007	13.10
35			TW	5.98	0.007	6.15
36	<i>AhTE0370</i>	A09	PYPP	4.27	0.05	6.23
37			NPP	4.11	0.002	6.44

Table 9 continued...

Table 9 continued...

38	AhTE0381	A10	90PDI	4.64	0.04	6.21
39			NPP	4.01	0.003	7.13
40	AhTE0419	A04	NPP	4.36	0.002	7.31
41	AhTE0422	A04	TW	5.70	0.009	7.29
42	AhTE0431	A04	PYPP	4.39	0.0008	10.39
43	AhTE0460	A05	90PDI	5.32	0.0001	13.16
44			CP	4.10	0.07	5.89
45	AhTE0482	B04	NPP	6.48	0.008	7.53
46	AhTE0499	A01	CP	4.28	0.02	5.10
47	AhTE0504	A03	40PDI	8.16	0.006	8.23
48			60PDI	6.08	0.0007	11.55
49			90PDI	9.28	0.0005	10.71
50			CP	7.57	0.02	5.25
51	AhTE0556	A03	NPP	10.22	0.0002	13.83
52	AhTE0614	A10	CP	9.43	0.05	6.85
53	AhTE0614	A10	NPP	7.57	0.04	6.89
54			SP	5.19	0.09	5.57
55			TW	6.97	0.003	7.30
56	AhTE0615	A10	NPP	10.66	0.0001	17.36
57			SP	7.44	0.03	9.56
58	AhTE1087	A02	SP	5.54	0.04	5.11
59	AhTE1240	A03	CP	12.50	0.0001	11.58
60	AhTE1254	A04	60PDI	11.01	0.001	7.47
61			CP	9.20	0.02	6.34
62			NPP	8.32	0.005	7.09
63			TW	8.75	0.001	7.10
64	AhTE1258	A04	60PDI	8.35	0.002	8.03
65			90PDI	10.13	0.001	8.75
66			SP	8.44	0.002	7.99
67	AhTE1266	A05	CP	13.28	0.0001	14.50
68			NPP	6.41	0.005	5.98
69	AhTE1283	A05	90PDI	11.75	0.0001	10.84
70	AhTE1348	A06	60PDI	9.31	0.0001	10.38
71			CP	7.33	0.02	5.12
72	AhTE1379	A06	NPP	9.82	0.02	5.54
73	AhTE1381	A06	90PDI	7.30	0.003	6.26
74			SP	4.15	0.004	7.00

Table 9 continued...

Table 9 continued...

75	<i>AhTE</i> 1450	A08	40PDI	8.13	0.01	5.19
76			60PDI	7.57	0.001	6.77
77			90PDI	7.16	0.003	5.07
78	<i>AhTE</i> 1522	A10	60PDI	4.23	0.0006	10.60
79	<i>AhTE</i> 1522	A10	NPP	8.34	0.01	5.06
80	<i>AhTE</i> 1744	B04	60PDI	8.43	0.003	7.01
81			90PDI	7.10	0.02	5.33
82			PYPP	8.63	0.006	9.03
83			SP	8.61	0.01	5.61
84	<i>AhTE</i> 1763	B04	PYPP	9.63	0.002	8.30
85	<i>AhTE</i> 1907	B06	90PDI	8.11	0.0004	10.85
86			PYPP	6.93	0.0001	11.96
87	<i>AhTE</i> 2006	B08	40PDI	8.39	0.003	7.96
88			60PDI	7.52	0.0002	11.15
89			90PDI	11.86	0.0001	18.07
90			NPP	8.65	0.001	9.12
91			SP	8.85	0.003	7.68
92	<i>AhTE</i> 2010	B08	40PDI	9.59	0.02	5.15
93			60PDI	10.90	0.001	9.78
94			90PDI	12.20	0.0004	10.98
95			SP	8.52	0.005	7.21

AhTE: *Arachis hypogaea* transposable element; 40PDI: Per cent disease incidence at 40 days (%); 60PDI: Per cent disease incidence at 60 days (%); 90PDI: Per cent disease incidence at 90 days (%); CP: Colonization percentage (%); NPP: Number of pods per plant; SP: Shelling percentage (%); TW: Test weight (g); PYPP: Pod yield per plant (g).

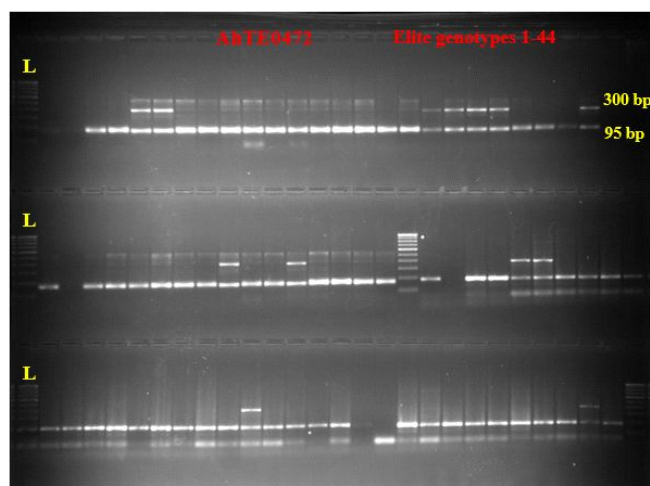


Plate 8 : PCR results for genotyping of elite genotypes with *AhTE*0472 marker. L= DNA ladder and bp= base pairs.

the greatest contribution of a marker to phenotypic variance for stem rot resistance, were observed for *AhTE*1266 (17.38%) for total disease incidence at harvest (colonization) and *AhTE*0504 (13.81%) for disease

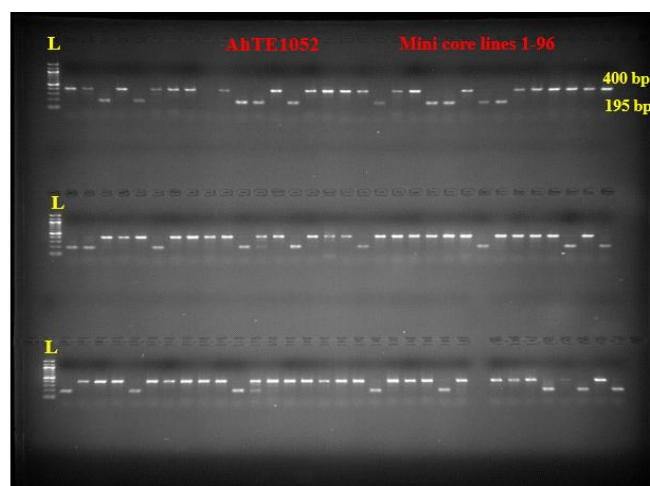


Plate 9 : PCR results for genotyping of mini core lines 1 to 96 with *AhTE*1052 marker. L= DNA ladder and bp= base pairs.

incidence at 60 DAS (Table 7).

Similarly, in elite genotypes, out of 61 *AhTE* markers, a total of 53 markers (corresponding to 107 MTAs)

showed significant associations at the 5% and 1% probability levels (Table 8). Genotypic data from 52 polymorphic *AhTE* markers across 189 genotypes, along with phenotypic data on nine traits—including five disease-related traits and four yield-related traits—were subjected to single marker analysis (SMA) to study marker-trait associations.

The 106 MTAs identified by 61 markers in elite genotypes were categorized based on PVE (%): 25 MTAs showed <10%, 68 MTAs were between 10–20%, and 13 MTAs showed >20% PVE, with a range of 4.07% to 25.2%. The P-values ranged from 0.0001 to 0.07. The highest R^2 values were observed for *AhTE*1240 (19.26%), *AhTE*2010 (12.35%), *AhTE*2006 (16.94%) and *AhTE*1908 (11.98%) for total disease incidence at harvesting (colonization), disease incidence at 30 DAS, disease incidence at 60 DAS, and disease incidence at 90 DAS, respectively (Table 8) in *kharif* 2018.

In the mini core collection, out of 50 *AhTE* markers, a total of 44 markers (corresponding to 95 MTAs) showed significant associations at the 5% and 1% probability levels (Table 9). These markers were categorized based on PVE (%), in which 73 MTAs showed <10%, and 22 MTAs were between 10–20%, with a PVE range of 5.05% to 18.07% and P-values ranging from 0.0001 to 0.09. The highest R^2 values were observed for *AhTE*2006 (18.07%), *AhTE*1266 (14.50%) and *AhTE*0460 (13.16%) for disease incidence at 90 DAS, and total disease incidence at harvesting (colonization), respectively (Table 9) in *kharif* 2019.

Comparable results were reported by Kolekar *et al.* (2017), who identified *AhTE* markers (*AhTE*0498 and *AhTE*0621) associated with rust resistance in RIL populations (TG 26 × GPBD 4). The association of *AhTE*0498 with rust resistance was also reported by Mondal *et al.* (2013) in a different population (VG 9514 × TAG 24).

It is well noted that, 2 resistant, 11 moderately resistant and 73 moderately susceptible genotypes were identified in the field condition for sclerotium disease in groundnut genotypes as mentioned above. Totally more than 500 *AhTE* markers were evaluated for single marker association for disease resistance across all the studied genotypes. The highest R^2 values were recorded for 9 markers (as mentioned above) for disease incidence at 90DAS and at harvesting stage disease incidence across all the genotypes, which can be utilized for SMA association for sclerotium rot resistance in different groundnut genotypes across the globe.

Conclusion

Overall, this study contributed to the characterization of different groundnut populations in response to artificial *S. rolfisii* infection. Two resistant lines (ICGV 06420 and ICG 4670) were identified for sclerotium rot disease in groundnut which can be further utilized in groundnut crop improvement program. And for the identification of MTAs for these genotypes using *AhTE* markers for stem rot resistance, the MAS program in BC₂F₇ populations, elite genotypes, and the mini-core collection, utilizing *AhTE* markers, facilitated the establishment of associations for disease resistance. *AhTE*1266, *AhTE*0504, *AhTE*1240, *AhTE*2010, *AhTE*2006, *AhTE*1908, *AhTE*2006, *AhTE*1266 and *AhTE*0460 markers showed highest R^2 values for disease resistance especially at 90DAS and at harvest stage. These results were further validated to select stem rot-resistant genotypes and markers with high PIC and PVE values. This study represents the first attempt to generate genetic resources for stem rot resistance in groundnut and will be valuable for improving groundnut disease screening. Furthermore, these markers need to be validated in different populations, and developing a linkage map for *Sclerotium rolfisii* resistance is strongly recommended for future research.

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