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Evaluation of rust and late leaf spot mapping RILs population in groundnut using *AhMITE1* Specific PCR

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ABSTRACT

VL 1 is resistant to rust but susceptible to late leaf spot while its mutant derivative, M 110 is resistant to LLS but susceptible to rust and they exhibited polymorphism for *AhMITE* and hence it will be interesting to examine its usefulness as a marker for resistance to rust/LLS. Recombinant Inbred Line (RIL) population derived from their cross (VL 1 x Mutant 110) was phenotyped for these two diseases and selected lines were examined for *AhMITE* polymorphism to examine its association with resistance. Since the magnitude of variation was more and heritability was high for rust compared to LLS, an attempt was made to assess segregation of the maker *vis-à-vis* resistance to rust. 20 lines exhibiting extremely high resistance (10 RILs) and susceptibility (10 RILs) were selected and examined for *AhMITE* polymorphism. It was present in 8 out of 10 (80%) susceptible RILs; while absent in 6 out of 10 (60%) rust resistant RILs indicating a strong association with rust susceptibility.

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INTRODUCTION

The cultivated groundnut (*Arachis hypogaea* L.) originated from hybridization between diploid female species *A. duranensis* with the A genome and *A. ipaensis* with the B genome (Kochert *et al.,* 1996). Groundnut (*A. hypogaea*) is classified into two subspecies, *viz.* ssp. *hypogaea* (Krap. and Rig.) and ssp. *fastigiata* (Wald.) based on variation in morphology. Further, the ssp. *hypogaea* is bifurcated into var. *hypogaea* (Virginia bunch/runner) and var. *hirsuta* (Peruvian runner), and likewise ssp. *fastigiata* into var. *fastigiata* (Valencia), Peruviana, aequatoriana and var. *vulgaris* (Spanish bunch) (Stalker and Simpson, 1995). Only four botanical types namely, Virginia bunch (VB), Virginia runner (VR), Valencia (VL), and Spanish bunch (SB) are exclusively cultivated by the farmers owing to their agronomic attributes and market value. Kochert *et al*. (1996) suggested that *A. hypogaea* might have arisen as the result of single polyploidization event and the dramatic shifts in the morphology of plant organ arose as a result of changes in one or two major genes and a few modifier loci. Late leaf spot (LLS), caused by *Phaeoisariopsis personata* and rust caused by *arachis* is a serious disease leading to significant yield loss in groundnut (Subrahamanyam *et al.,* 1980). In particular, most popular and widely cultivated early maturing Spanish bunch types are highly susceptible to LLS. Several fungicides can effectively control them, but cost and environmental considerations limit their use. Some of the Valencia landraces and introgression lines from wild species are resistant. But they are associated with several undesirable attributes such as late maturity, thick shell, low productivity and poor adaptation making them unacceptable for direct utilization (Reddy *et al.,* 1991). Also the breeding programmes employing such lines have not been completely successful in breaking the undesirable associations. Miniature inverted-repeat transposable elements (*MITEs*) belonging to non-autonomous class II type (Osborne *et al.,* 2006), and commonly distributed in animal and plant genomes (Feschotte *et al.,* 2002), are activated by chemical mutagen treatment (Patel *et al*., 2004)

and in vitro tissue culture stresses (Kikuchi *et al.,* 2003). A simple PCR using primers specific to FST and *AhMITE1* would indicate whether *AhMITE1* is transposed from this predetermined site, thus allowing understanding its role in peanut mutations. In this study, an effort was made to evaluate RIL population for rust and LLS and find their association with *AhMITE1*.

MATERIALS AND METHODS

Recombinant inbred lines (RILs) were developed at the University of Agricultural Sciences, Dharwad from the cross (VL 1×110). VL1 is a peanut mutant with resistance to rust and susceptibility to late leaf spot (LLS). Upon ethyl methane sulphonate mutagenesis it generated high frequency of independent, but morphologically similar mutants, M 110 which were resistant to LLS and susceptible to rust.F1s were selfed to produce F2s and advanced through Single Seed Descent (SSD) till F6 generation, now it is in F12 generation. 114 lines were sown for two replication and scored for rust and LLS at 80 days and 90 days. Statistical analysis performed were analysis of variance (ANOVA), coefficient of variance and correlation analysis. Selected lines (Table A) were also subjected for *AhMITE* specific PCR to check polymorphism. For *AhMITE1*-specifc PCR, genotypes were grown in pots and genomic DNA was extracted from young leaves using cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984). A final volume of 20 ll containing 100 ng genomic DNA, 19 PCR buffer, 0.5 mM dNTPs, 10 pmol of each primer, and one unit of Taq DNA polymerase (Genei, Bangalore, India) was used for PCR. The amplification reaction was carried out in a Mastercycler (Eppendorf, Germany) by setting the conditions for one cycle of pre-denaturation (94^{0}C for 5 min), 35 cycles of denaturation (94^{0}C for 1 min), annealing (60^{0}C for 1 min), and extension (72^{0}C for 1 min). One cycle of final extension (72^{0}C for 1 min) was included before the PCR product was stored at 4^{0}C until further use. Presence of the PCR product was checked on 1.2% agarose gel by electrophoresis. The PCR amplification would give a band of 242 bp when *AhMITE1* is inserted at FST1-linked site. Absence of 242 bp product with the same primers indicates the excision of *AhMITE1* from FST1-linked site.

Table A : List of selected RILs from (VL1×110) populations for testing polymorphism with AhMITE primer

Sr. No.	RILs resistant to LLS and susceptible to Rust	RILs resistant to Rust and susceptible to LLS
1.	M 110(Parent)	VL1(Parent)
2.	1a	1b
3.	15a	2c
4.	17	3a
5.	18a	4b
6.	20c	5
7.	21b	7
8.	1-5a	19
9.	1-7a	1-1c
10.	3-7b	1-6b
11.	3-13b	1-13a

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) for rust (80days), rust (90 days), LLS (80 days) and LLS (90 days) revealed highly significant differences among the RILs (Table 1). The Component of variances was assessed. The nature and magnitude of the variation was assessed by phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H2%), genetic advance (GA) and genetic advance over mean (GAM) for disease score (Table 2). The estimates of GCV, PCV, heritability and genetic advance were high for rust but low to moderate for LLS at both stages (80 and 90 days). Rust (80 days) showed strong and positive association with rust (90 days) but not with LLS at both the stages. But LLS (80days) showed strong and positive association with LLS (90 days) (Table 3a, b). RIL population (VL1 X 110) segregated for both LLS and rust and frequency distribution of RILs was continuous and within the range of

Table 1 : ANOVA for VL1×110 population for disease traits

SV	D.F	Rust(80days)	Rust(90days)	LLS(80days)	LLS(90days)
Repl (rmss)	1	0.109	0.043	3.688	39.580
Treat(tmss)	113	6.296**	7.029**	1.372**	0.965**
Error(emss)	113	0.260	0.526	0.431	0.388
C.D. (P=0.05)		0.845	1.203	1.089	1.033
C.D. (P=0.01)		1.202	1.710	1.549	1.469
S.E.±		0.360	0.513	0.464	0.440
CV		14.51	15.71	10.63	9.53

* and **: indicate significance of values at P=0.05 and 0.01, respectively
CD = critical difference CV = Coefficient of variances SEM = Standard error of mean Rmss = Replication mean sum of square
Tmss = Treatment mean sum of square Emss = Error mean sum of square SV = Source of variances DF = Degree of freedom

Table 2 : Component of variances for (VL1×110) population for disease traits

Sr. No.	Characters	MEAN	RANGE		GCV	PCV	H^2%	GA	GAM
1.	Rust(80 days)	3.51	2	7	49.45	51.54	92.1	3.43	97.72
2.	Rust(90 days)	4.61	3	9	39.04	42.09	86.1	3.45	74.03
3.	LLS(80 days)	6.17	3.5	7	11.10	15.37	52.1	1.02	16.53
4.	LLS(90 days)	6.53	3.5	7.5	8.22	12.58	42.1	0.72	11.02

GCV = Genotypic coefficient of variances PCV = Phenotypic coefficient of variances H^2% = Heritability percentage
GA= Genetic advance GAM = Genetic advance mean

Table 3a : Genotypic correlation of RUST with LLS in VL1×110 populations

Sr. No.	Characters	Rust(80 days)	Rust(90 days	LLS(80 days)	LLS(90 days)
1.	Rust(80 days)	1.000	0.990**	0.143	0.294**
2.	Rust(90 days		1.000	0.107	0.225*
3.	LLS(80 days			1.000	0.936**
4.	LLS(90 days)				1.000

Table 3b : Phenotypic correlation of RUST with LLS in VL1×110 populations

Sr. No.	Characters	Rust(80 days)	Rust(90 days	LLS(80 days)	LLS(90 days)
1.	Rust(80 days)	1.000	0.948**	0.096	0.169
2.	Rust(90 days		1.000	0.097	0.162
3.	LLS(80 days			1.000	0.797**
4.	LLS(90 days)				1.000

* and ** indicate significance of values at P=0.05 and 0.01, respectively

the parents indicating quantitative nature of the traits (Fig. 1a, b; 2a, b). Out of 114 RILs, 43 lines were resistant to rust and some RILs were resistant to both rust LLS indicating segregation for both the diseases.

***AhMITE*-specific primer:**

VL 1 and M 110 were polymorphic with this primer. A RIL population (VL1 X 110) segregating for rust and LLS was utilized to investigate its association with the diseases. For this purpose 20 RILs representing resistance (10) and susceptibility (10). Ah MITE-specific fragment of 242 bp was present in 8 out of 10 (80%) susceptible RILs; while absent in 6 out of 10 (60%) rust resistant RILs, indicating strong association with rust susceptibility. This was further confirmed through test of independence (χ2 value, 1.875, table value 3.89 and 6.64 at 5% and 1% level of probability, respectively)

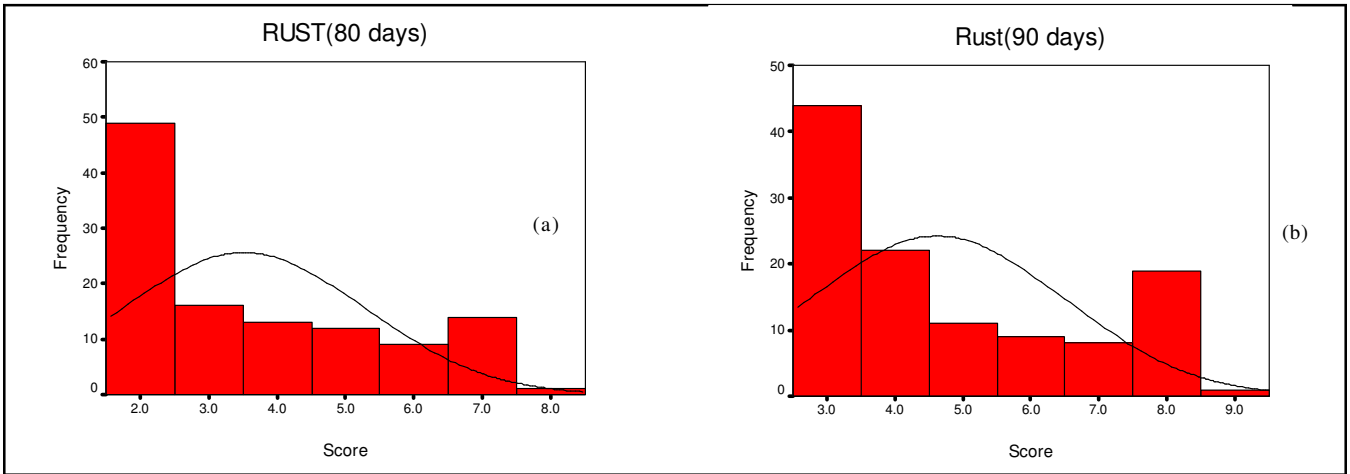

Fig. 1 : Pattern of distribution of Rust (80 days) and RUST (90 days) in (VL1×110) segregating population

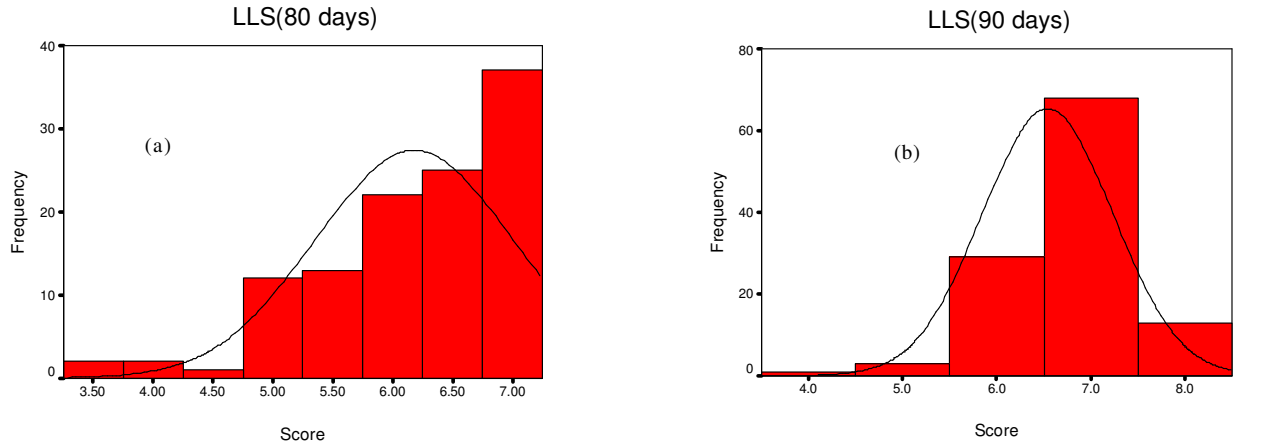

Fig. 2 : Pattern of distribution of LLS (80 days) and LLS (90 days) in (VL1×110) segregating population; RUST and LLS Score (1-9 scale)

Table 4a : Distribution of 242 bp band in (VL1×110) segregating population for MITEs primer

Sr. No.	Genotypes		RUST		LLS	
		Distribution of 242 bp band	80 Days	90 Days	80 Days	90 Days
1.	M 110(Parent)	+	6.0	7.0	4.0	5.5
2.	1a	+	6.5	8.0	7.0	6.5
3.	15a	+	6.5	8.0	6.0	6.0
4.	17	+	6.5	7.5	7.0	7.5
5.	18a	+	7.0	8.0	7.0	7.5
6.	20c	+	6.5	7.5	6.0	6.5
7.	21b	+	5.0	7.5	4.5	5.5
8.	1-5a	_	6.5	8.0	6.5	7.5
9.	1-7a	_	6.5	8.0	6.0	6.5
10.	3-7b	+	7.0	8.0	7.0	7.0
11.	3-13b	+	6.5	7.5	7.0	7.0
12.	VL1 (Parent)	-	2.5	5.0	7.0	7.0

Table 4b: Distribution of 242 bp band in (VL1×110) segregating population for MITEs primer

Sr. No.	Genotypes		RUST		LLS	
		Distribution of 242 bp band	80Days	90Days	80 Days	90 Days
1.	VL1(Parent)	_	2.5	5.0	7.0	7.0
2.	1b	_	2.0	3.0	7.0	7.5
3.	2c	+	2.0	3.0	7.0	6.5
4.	3a	_	2.0	3.0	7.0	7.0
5.	4b	+	2.0	3.0	6.5	6.5
6.	5	_	2.0	3.0	7.0	7.0
7.	7	_	2.0	3.0	7.0	7.0
8.	19	_	2.5	3.5	7.0	7.0
9.	1-1c	+	2.0	3.0	7.0	7.0
10.	1-6b	+	2.0	3.0	6.5	6.5
11.	1-13a	_	2.0	3.0	7.0	7.0
12.	M110(Parent)	+	6.0	7.0	4.0	5.5

+ Presence of 242 bp band - Absence of 242 bp band

(Table 4a, b and Fig. 3).

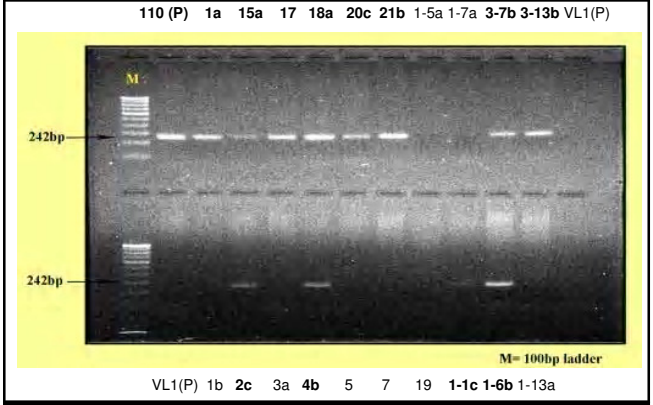

Fig. 3 : Frequency of *AhMITE* – specific primer in RILs

Phenotyping of RIL for disease reaction:

A population comprising 114 RILs was evaluated in the field for LLS and rust incidence at two stages under natural epiphytic condition. ANOVA revealed significant variation for LLS and rust at both the stages. The phenotypic and genotypic coefficients of variation, heritability and genetic advance revealed very high magnitude of heritable variation for rust but it was moderate for LLS. Correlation analysis revealed strong association between stages in each disease but rust and LLS were found to be independent. RIL population of VL 1 x M 110 exhibited segregation for rust and LLS, but heritable variation was more for rust than LLS. Some RILs combined resistance to both the diseases.

Association of *AhMITE* with rust resistance:

Among the mutants, VL 1 is resistant to rust but susceptible to LLS while its mutant derivative M 110 is resistant to LLS but susceptible to rust and they exhibited polymorphism for *AhMITE* and hence it will be interesting to examine its usefulness as a marker for resistance to rust/LLS. In the present study, a recombinant inbred line (RIL) population derived from their cross (VL 1 x Mutant 110) was phenotyped for the two diseases and selected lines were examined for *AhMITE* polymorphism to examine its association with resistance.

***AhMITE* polymorphism in the selected RILs**:

Since the magnitude of variation was more and heritability was high for rust compared to LLS, an attempt was made to assess the segregation of the maker *vis-à-vis* resistance to rust. For this purpose, 20 lines exhibiting extremely high resistance (10 RILs) and susceptibility (10 RILs) were selected and examined for *AhMITE* polymorphism. It was present in 8 out of 10 (80%) susceptible RILs; while absent in 6 out of 10 (60%) rust resistant RILs indicating a strong association with rust susceptibility.

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