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Molecular Assessment of Late Leaf Spot Resistance in Germplasm Collections of Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) construed as one of the most dynamic oilseed crop, delivering high protein besides significantly meet country's major oil requirement. In India, 10.1 million tonnes of groundnut are produced over an area of around 5.7 million hectares at a productivity of 1777 kg/ha. Groundnut is always been a fascinating crop to small and marginal farmers but its yield was greatly challenged by foliar fungal diseases such as early leaf spot, late leaf spot and rust. Among these, late leaf spot (LLS) is a major foliar fungal disease which cause severe defoliation of diseased leaflets and reduce pod and fodder yield by 50 per cent besides the kernel quality.

Methods: The present investigation was conducted at Agricultural College and Research Institute, Killikulam during the year 2022-2024 involving 220 groundnut genotypes along with a resistant check (ICG 6066) and susceptible checks (TMV 2, TMV 7 and TMV 13) for screening against late leaf spot resistance under both field and artificial controlled conditions thereby assessment of the disease through molecular intervention.

Result: Molecular interpretation revealed five markers exhibiting polymorphism to LLS disease out of 30 markers validated. The PIC values ranged from 0.501 (TC7H11) to 0.570 (Ah3TC23H10). Molecular marker screening for disease resistance categorized ICG 978, ICG 1649, ICG 1703 and ICG 2280 genotypes as resistant to late leaf spot disease while ICG 8760, ICG 4598, ICG 5051, ICG 5891 and ICG 6057 exhibited moderately resistant for late leaf spot besides exhibited higher mean yield performance among the test genotypes. Hence, the genotypes ICG 8760, ICG 4598, ICG 5051, ICG 5891 and ICG 6057 may be utilized for developing ideal recombinants for LLS resistance breeding in Groundnut.

Key words: Genetic diversity, Groundnut, LLS resistance, SSR markers.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) construed as one of the most dynamic oilseed crop, delivering high protein besides significantly meet country's major oil requirement. Globally, groundnut is grown on 327 million hectares recording a production potential of 539 million tonnes and a productivity of 1648 kg/ha (FAOSTAT, 2021). India stands second largest producer of Peanut after China, followed by Nigeria and USA. In India, 10.1 million tonnes of groundnut are produced over an area of around 5.7 million hectares at a productivity of 1777 kg/ha (INDIASTAT, 2022). Groundnuts are majorly grown in Gujarat state followed by Rajasthan, Tamil Nadu, Andhra Pradesh and Karnataka during Kharif and Rabi seasons.

Groundnut is always been a fascinating crop to small and marginal farmers but its yield was greatly challenged by biotic and abiotic stresses. The most important and widely distributed diseases of groundnut are foliar fungal diseases such as early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (*Phaeoisariopsis personata*) and rust (*Puccinia arachidis* Speg.). Among these, late leaf spot is a major foliar fungal disease which cause severe defoliation of diseased leaflets and reduce pod and fodder yield by 50 per cent besides the kernel quality (Gujar *et al.,* 2025; Ndifon, 2022). When both rust and late leaf spot occur simultaneously, exhaustive damage results to pod yield and kernel quality to a prediction level of 70 per cent (Adorada *et al.*, 2025; Elmhirst, 2024). Groundnut exhibit an extensive genetic diversity and major share of groundnut germplasm have been maintained by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India. Therefore, the diverse groundnut germplasm collections can be sourced from ICRISAT for late leaf spot resistance and yield related traits to tailor desirable progenies for advanced breeding (Abadya *et al.*, 2021).

The genetic or molecular marker is referred to fragment of a gene or DNA sequence with a known chromosome location associated with particular trait. Phenotypic marker proclaimed as an initiative for trait based varietal assortment

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over greater decades as pointed by Sax (1923) who had proposed the idea of indirect selection using genetic markers. But now, new technologies have been developed that ensure the precise selection based on genetic variation at DNA level by crop breeders (Rathava *et al.,* 2025). During recent times, the molecular biologist are quenched on adoption of simple sequence repeats (microsatellites) markers to screen, identify and evaluate the crop genetic diversity. Microsatellites representing the simple repeated motifs of 1-6bp are usually co-dominant, reproducible and exhibit the higher level of polymorphism (Yadav *et al.,* 2023).

MATERIALS AND METHODS

Crop experiment

Field experiment was carried out at Department of Genetics and Plant Breeding, Agricultural College and Research Institute, Killikulam, Tamil Nadu during 2022 to 2024. Two hundred and twenty groundnut mini-core germplasm collections collected from ICRISAT, Hyderabad along with four check genotypes were evaluated for LLS resistance. The details of mini-core collections was given in Table 1.

Natural screening of mini-core collections for LLS resistance

During Rabi season of 2022-23, the screening was carried out on the groundnut field at two stages *viz*., 70th and 90th days after sowing (DAS) under natural field condition. Ten plants were selected at random from each genotype and observation on late leaf spot disease was recorded based on PDI scoring scale (Table 2). Disease reaction was assessed based on the disease severity level as such 0 per cent as highly resistant, 1-20 as resistant, 21-50 moderately resistant, 51-70 as susceptible and 71 -100 as highly susceptible with the respective scale of 1, 2-3, 4-5, 6-7 and 8-9 (Pooniya *et al*., 2020).

Extraction of genomic DNA and PCR amplification

Young leaves were collected from the mini-core germplasm lines and DNA was extracted adopting CTAB method developed by Saghai-Maroof *et al.* (1984). PCR reaction was performed with a total of five gene specific markers. The germplasm were surveyed utilizing the list of SSR markers as furnished in Table 3. PCR reaction was performed with a total volume of 10 µl. The thermal cycler programme as follows, step 1-Initial denaturation (94^{o}C for 5 minutes), step 2-Denaturation (94^{o}C for 30 seconds), step 3-Annealing (55^{o}C for 30 seconds), step 4-Extension (72^{o}C for 1 minute), steps 2-4 (40 cycles), step 5-final extension (72^{o}C for 10 minutes) and step 6 (Final Hold-4^{o}C until sample retrieval).

Polymorphism information content (PIC)

For critical estimation of genetic variation at DNA level among the groundnut germplasm, the gene specific markers were used to identify the polymorphism. The amplified DNA products were assessed as bands with clearly resolved, unambiguous polymorphic were scored using the standard 100 bp molecular weight ladder. The banding pattern was scored in binary digits as absence of band was indicated as '0' and presence of band was indicated as '1'. The polymorphic Information Content (PIC) was evaluated vide the equation 1-Σ $(Pi)^2$, whereas Pi hint the extent of samples carrying the ith allele.

Assessment of genetic diversity through molecular intervention

Molecular markers were employed to validate the polymorphism between groundnut germplasm at the DNA level. After the gel electrophoresis run, the gel was visualized to access the differences in the banding pattern of the amplified DNA products for each primer linked with disease resistance. Visually distinguishable polymorphic bands were assessed by comparing them to the standard 100bp ladder. A score of '0' indicated that no bands were seen in the respective lane, while the score of '1' indicated the presence of polymorphic bands. A two-way matrix was constructed by entering the genotype scores and allele sizes for a specific marker in rows and columns, respectively.

The amplified bands of the different groundnut genotypes in each lane were compared with bands of corresponding susceptible (TMV2, TMV7 and TMV 13) and resistant (ICG 6022) checks. A total of 224 genotypes were clustered using the software PAST version 4.03 by UPGMA method based on the dissimilarity matrix. Binary data generated during polymorphic survey has been taken as input and based on which a phylogenetic tree was constructed.

RESULTS AND DISCUSSION

Phenotypic Screening of groundnut genotypes for validation of late leaf spot resistance

Natural screening for LLS resistance

During *Rabi* 2022, 220 groundnut genotypes had been tested for their reaction to late leaf spot resistance along with the resistant check (ICG 6022) and susceptible checks (TMV 2, TMV 7 and TMV 13) at Agricultural College and Research institute, Killikulam. Scoring for the leaf spot disease was made at two stages *viz*., 75th and 90th days after sowing (DAS) which then interpreted using Percent Disease Index (PDI) of corresponding genotypes. On perusal of data, five groundnut genotypes *viz.,* ICG 532, ICG 2381, ICG 14179, ICG 15233 and ICG 6022 registered to be resistant while forty five genotypes had shown moderately resistant reaction (Table 4).

Controlled screening for LLS resistance

The groundnut germplasm along with the resistant check (ICG 6022) and susceptible checks (TMV 2, TMV 7 and TMV 13) were evaluated for late leaf spot resistance under controlled (glasshouse) condition adopting the inoculum spraying method. The results were pursued and found two genotypes as resistant namely ICG 15233, ICG 6022 with the PDI values at 75 DAS and 95 DAS as 16.33 and 19.37, 13.44 and 18.56 respectively.

Table 1: List of groundnut germplasm included in the study.

Genotypes	Source
ICG 1137, ICG 36, ICG 76, ICG 115, ICG 118, ICG 1519, ICG 1569, ICG 188, ICG 1973, ICG 2019, ICG 2031, ICG 2106, ICG 513, ICG 862, ICG 875, ICG 2777, ICG 11322, ICG 11386, ICG 11426, ICG 11457, ICG 14177, ICG 14179, ICG 12682, ICG 12697, ICG 12988, ICG 12991, ICG 15233, ICG 2925, ICG 3027, ICG 3053, ICG 3102, ICG 4798, ICG 4955, ICG 3343, ICG 3421, ICG 3584, ICG 3992, ICG 4343, ICG 4389, ICG 5779, ICG 5891, ICG 4538, ICG 4598, ICG 9666, ICG 7153, ICG 7181, ICG 7867.	India
ICG 1699, ICG 2381, ICG 4764, ICG 3775, ICG 6888, ICG 7190, ICG 14630, ICG 15287, ICG 15309, ICG 3746,	Brazil
ICG 1823, ICG 2857, ICG 3312, ICG 5221, ICG 11144	Argentina
ICG 1274, ICG 13603, ICG 15415	Indonesia
ICG 81, ICG 111, ICG 1487, ICG 1534, ICG 163, ICG 532, ICG 3140, ICG 4156, ICG 4543, ICG 6375, ICG 6394, ICG 6402, ICG 6643, ICG 6646, ICG 6654	Unknown
ICG 9987, ICG 8517, ICG 12235, ICG 12276, ICG 12509, ICG 12672	Bolivia
ICG 1668, ICG 311, ICG 397, ICG 434, ICG 442, ICG 2286, ICG 721, ICG 5016, ICG 5051, ICG 4111, ICG 4412, ICG 5827, ICG 6057, ICG 4684, ICG 9315,ICG 6667, ICG 6766, ICG 7243, ICG 7963, ICG 10185, ICG 8285.	USA
ICG 10010, ICG 10036, ICG 10053, ICG 8106, ICG 10890, ICG 10920, ICG 10950, ICG 11088, ICG 8751	Peru
ICG 14466, ICG 14475, ICG 14482	Nigeria
ICG 6407, ICG 10092 , ICG 7969	Zimbabwe
ICG 10479 , ICG 8567	Uruguay
ICG 14106, ICG 14118, ICG 10566	Congo
ICG 9249	Mauritius
ICG 9418	Martinique
ICG 9449	Burkina faso
ICG 9507	Philippines
ICG 6703	Paraguay
ICG 9777	Mozambique
ICG 9809	Mozambique
ICG 7883	Israel
ICG 7897	Venezuela
ICG 10384	Nigeria
ICG 10474	Cuba
ICG 8083	Russian federation
ICG 8490	Somalia
ICG 9037	Cote d'Ivoire
ICG 13491	Central african republic
ICG 11249	Tanzania
ICG 13787	Niger
ICG 11542	Tanzania
ICG 12000	Mali
ICG 12189	Israel
ICG 12625	Ecuador
ICG 14710	Cameroon
ICG 14834	Pakistan
ICG 12879	Myanmar
ICG 15190	Costa rica

Table 2: Late leaf spot resistance scoring in groundnut germplasm.

Scale	Disease severity (%)	Description
1	0	Highly resistance
2-3	1-20	Resistance
4-5	21-50	Moderately resistance
6-7	51-70	Susceptible
8-9	71-100	Highly susceptible

Further, fourteen genotypes were adjudged as moderately resistant while 118 as susceptible and ninety as highly susceptible genotypes to late leaf spot disease (Table 5).

Molecular intervention for LLS resistance

Polymorphic information content (PIC) value assessment

Among the forty markers deployed, five molecular markers had shown polymorphism for the resistant and susceptible

Table 3: List of polymorphic markers associated with late leaf spot resistance.

Molecular markers	Sequence		Number of bases	Motif	Annealing (°C)	References
IPAHM 524	F	GCCATGGATAAGAACCTGAAA	21	(GA)20	55	(Khedikar *et al.*, 2010
	R	CAGTAAGCTGAGCTGGCAGA	20			and Zhao *et al.*, 2012)
TC7H11	F	AGGTTGGAACTATGGCTGATTG	22	(AG)18	55	(Khedikar *et al.*, 2010)
	R	CCAGTTTAGCATGTGTGGTTCA	22			
Ah3TC23H10	F	TCCCTTTGAGTCATTCATTGTG	22	(GA)19	55	(Zhao *et al.*, 2012)
	R	CATCAGAGCTCCTTTTCCCTAA	22			
Ah3TC24B05	F	ATTGATACCTCTTTGCTCTCGC	22	(TC)30	55	(Zhao *et al.*, 2012)
	R	TGAAACCCTAACTAGCTCGGAA	22			
Ah3TC28B01	F	ATTTATTGCCAAATCTGTCGCT	22	(AG)37	55	(Zhao *et al.*, 2012)
	R	CATTGCCAACTGTTACTACCCA	22			

Table 4: Grouping of groundnut genotypes based on field screening.

Scale	Category	No. of genotypes	Germplasm
1	Highly resistant	-	-
2-3	Resistant	4	ICG 532, ICG 2381, ICG 14179, ICG 15233, ICG 6022
4-5	Moderately resistance	45	ICG 8760, ICG 76, ICG 111, ICG 115, ICG 163, ICG 188, ICG 297, ICG 332, ICG 405, ICG 513, ICG 875, ICG 1668, ICG 2511, ICG 3027, ICG 3992, ICG 4156, ICG 4343, ICG 4527, ICG 4598, ICG 5016, ICG 5051, ICG 5891, ICG 6057, ICG 6402, ICG 6407, ICG 6646, ICG 6667, ICG 6703, ICG 6766, ICG 7153, ICG 7883, ICG 8083, ICG 8253, ICG 8285, ICG 9249, ICG 10701, ICG 10890, ICG 10920, ICG 11386, ICG 11426, ICG 12235, ICG 13787, ICG 14705, ICG 15234, ICG 15236
6-7	Susceptible	126	ICG 36, ICG 118, ICG 311, ICG 334, ICG 397, ICG 434, ICG 442, ICG 721, ICG 862, ICG 928, ICG 1399, ICG 1415, ICG 1487, ICG 1519, ICG 1534, ICG 1569, ICG 1699, ICG 1703, ICG 2286, ICG 2772, ICG 2777, ICG 2857, ICG 2925, ICG 3053, ICG 3102, ICG 3140, ICG 3240, ICG 4389, ICG 4412, ICG 4538, ICG 4670, ICG 4746, ICG 4798, ICG 4906, ICG 4955, ICG 4998, ICG 5327, ICG 5475, ICG 5609, ICG 5827, ICG 6201, ICG 6394, ICG 6643, ICG 6654, ICG 6888, ICG 7181, ICG 7190, ICG 7243, ICG 7867, ICG 7897, ICG 7963, ICG 7969, ICG 8106, ICG 8490, ICG 8567, ICG 8751, ICG 9037, ICG 9449, ICG 9507, ICG 9666, ICG 9777, ICG 9809, ICG 9842, ICG 9961, ICG 9987, ICG 10010, ICG 10036, ICG 10053, ICG 10185,ICG 10474, ICG 10479, ICG 10566, ICG 10950, ICG 11088, ICG 11109, ICG 11144, ICG 11219, ICG 11249, ICG 11322, ICG 11457, ICG 11515, ICG 11542, ICG 11651, ICG11687, ICG 12000, ICG 12189, ICG 12276, ICG 12509, ICG 12625, ICG 12665, ICG 12672, ICG 12682, ICG 12988, ICG 13099,ICG 13491, ICG 13603, ICG 13856, ICG 13858, ICG 13895, ICG 14106, ICG 14118, ICG 14127, ICG 14177, ICG 14466, ICG 14475, ICG 14482, ICG 14523, ICG 14710, ICG 14834, ICG 14985, ICG 15042, ICG 15190, ICG 15232, ICG 15287, ICG 15309, ICG 15379, ICG 15380, ICG 15384, ICG 15385, ICG 15390, ICG 15395, ICG 15396, ICG 15398, ICG 15401, ICG 15415, ICG 15419
8-9	Highly susceptible	45	ICG 3746, ICG 81, ICG 1137, ICG 1142, ICG 1274, ICG 1711, ICG 1823, ICG 1834, ICG 1973, ICG 2019, ICG 2031, ICG 2106, ICG 3312, ICG 3343, ICG 3421, ICG 3584, ICG 3673, ICG 3775, ICG 4111, ICG 4543, ICG 4684, ICG 4729, ICG 4750, ICG 4764, ICG 4911, ICG 5195, ICG 5221, ICG 5236, ICG 5494, ICG 5779, ICG 6263, ICG 6375, ICG 8517, ICG 9315, ICG 9362, ICG 9418, ICG 10092, ICG 10384, ICG 12697, ICG 12879, ICG 12921, ICG 12991, ICG 13982, ICG 14630, ICG 15397, TMV 2, TMV 7, TMV 13.

checks and hence these markers were used for the detection of genetic background of 220 groundnut genotypes along with the resistant check (ICG 6022) and susceptible checks (TMV 2, TMV 7 and TMV 13) for late leaf spot resistance. The assessment of association existing between the molecular markers used was made through the interpretation on Polymorphic Information Content (PIC) value. Upon perusal of the PIC values, it was inferred that the markers had a significant level of polymorphism with PIC value >0.5 (Table 6). The maximum allele size was

Table 5: Grouping of groundnut genotypes based on glasshouse screening.

Sclae	Category	No. of genotypes	Germplasm
1	Highly resistant	-	-
2-3	Resistant	1	ICG 15233, ICG 6022
4-5	Moderately resistant	14	ICG 8760, ICG 76, ICG 111, ICG 297, ICG 405, ICG 2381, ICG 4598, ICG 5051, ICG 5891, ICG 6057, ICG 8253, ICG 8285, ICG 10701, ICG 15234
6-7	Susceptible	118	ICG 36, ICG 115, ICG 118, ICG 163, ICG 188, ICG 332, ICG 434, ICG 442, ICG 513, ICG 532, ICG 721, ICG 862, ICG 875, ICG 928, ICG 1487, ICG 1519,ICG 1668, ICG 1699, ICG 2286, ICG 2511, ICG 2857, ICG 2925, ICG 3027, ICG 3053, ICG 3992, ICG 4156, ICG 4343, ICG 4389, ICG 4412, ICG 4527, ICG 4746, ICG 4906, ICG 4998, ICG 5016, ICG 5475, ICG 5827, ICG 6201, ICG 6402, ICG 6643, ICG 6667, ICG 6703, ICG 6766, ICG 6888, ICG 7153, ICG 7181, ICG 7190, ICG 7243, ICG 7867, ICG 7883, ICG 7897, ICG 7963, ICG 7969, ICG 8083, ICG 8106, ICG 8490, ICG 8567, ICG 8751, ICG 9449, ICG 9507, ICG 9666, ICG 9777, ICG 9809, ICG 9842, ICG 9961, ICG 9987, ICG 10010, ICG 10036, ICG 10053, ICG 10185, ICG 10474, ICG 10479, ICG 10566, ICG 10890, ICG 10920, ICG 10950, ICG 11088, ICG 11109, ICG 11144, ICG 11219, ICG 11249, ICG 11322, ICG 11386, ICG 11426, ICG 11457, ICG 11687, ICG 12000, ICG 12189, ICG 12235, ICG 12276, ICG 12509, ICG 12625, ICG 12665, ICG 12672, ICG 13099, ICG 13491,ICG 13603, ICG 13787, ICG 13856, ICG 13858, ICG 14106, ICG 14127, ICG 14177, ICG 14179, ICG 14466, ICG 14475, ICG 14482, ICG 14705, ICG 14710, ICG 14834, ICG 15190, ICG 15236, ICG 15379, ICG 15385, ICG 15390, ICG 15395, ICG 15398, ICG 15401, ICG 15419
8-9	Highly susceptible	87	ICG 3746, ICG 81, ICG 311, ICG 334, ICG 397, ICG 1137, ICG 1142, ICG 1274, ICG 1399, ICG 1415, ICG 1534, ICG 1569, ICG 1703, ICG 1711, ICG 1823, ICG 1834, ICG 1973, ICG 2019, ICG 2031, ICG 2106, ICG 2772, ICG 2777, ICG 3102, ICG 3140, ICG 3240, ICG 3312, ICG 3343, ICG 3421, ICG 3584, ICG 3673, ICG 3775, ICG 4111, ICG 4538, ICG 4543, ICG 4670, ICG 4684, ICG 4729, ICG 4750, ICG 4764, ICG 4798, ICG 4911, ICG 4955, ICG 5195, ICG 5221, ICG 5236, ICG 5327, ICG 5494, ICG 5609, ICG 5779, ICG 6263, ICG 6375, ICG 6394, ICG 6407, ICG 6646, ICG 6654, ICG 8517, ICG 9037, ICG 9249, ICG 9315, ICG 9362, ICG 9418, ICG 10092, ICG 10384, ICG 11515, ICG 11542, ICG 11651, ICG 12682, ICG 12697, ICG 12879, ICG 12921, ICG 12988, ICG 12991, ICG 13895, ICG 13982, ICG 14118, ICG 14523, ICG 14630, ICG 14985, ICG 15042, ICG 15232, ICG 15287, ICG 15309, ICG 15380, ICG 15384, ICG 15396, ICG 15397, ICG 15415, TMV 2, TMV 7, TMV 13

Table 6: Determination of Polymorphic Information Content (PIC) and Allele Size (bp) for markers.

Molecular Markers	Total no. of alleles	Allele size (bp)		PIC Value
		Maximum	Minimum	
IPAHM 524	5	300	260	0.570
TC7H11	5	400	280	0.509
Ah3TC23H10	5	220	140	0.501
Ah3TC24B05	5	240	160	0.502
Ah3TC28B01	5	260	180	0.530

observed for the genetic marker TC7H11 while minimum was observed for Ah3TC23H10. As such the PIC values for the molecular markers IPAHM 524 (Plate 1), TC7H11 (Plate 2), Ah3TC23H10 (Plate 3), Ah3TC24B05 (Plate 4) and Ah3TC28B01 (Plate 5) were recorded as 0.570, 0.509, 0.501, 0.502 and 0.530 respectively. The results are akin with the reports made by Mace *et al*. (2006) and Khedikar *et al*. (2010).

Molecular assessment of genetic diversity on groundnut germplasm

The studies on genetic diversity made by assessment of phenotypic extremities of 220 genotypes along with checks employing the validation of banding pattern differences generated by five molecular markers. The molecular data were elucidated in PAST 4.03 software following UPGMA

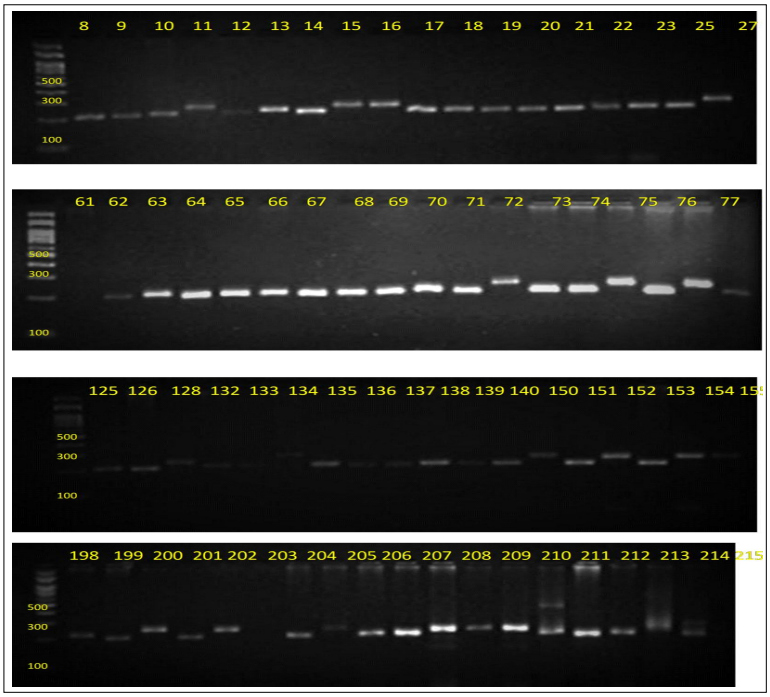

Plate 1: Molecular profile of SSR marker Ah3Tc28B01.

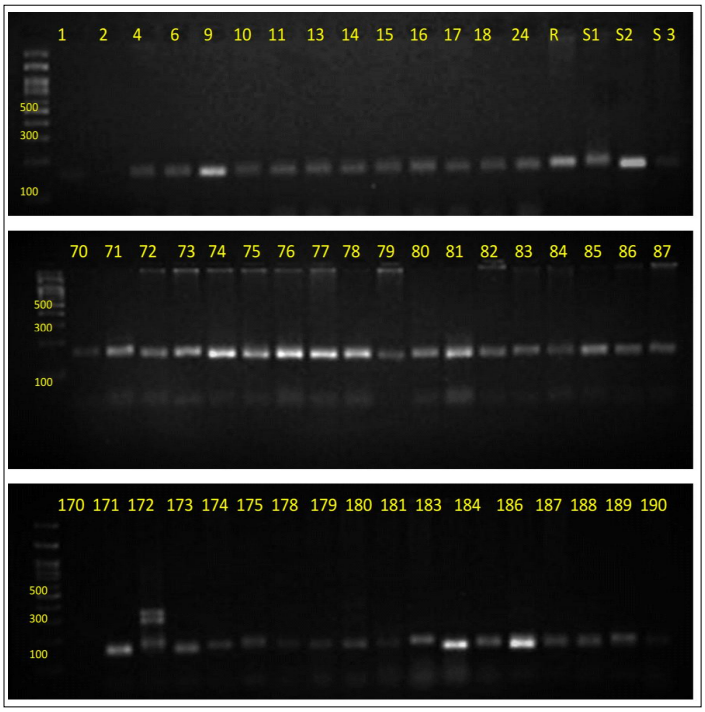

Plate 2: Molecular profile of SSR marker Ah3Tc23H10.

(unweighted pair group method with arithmetic mean) pattern and thereby further clustering of 224 groundnut germplasm were made (Table 7; Fig 1). Henceforth, the groundnut genotypes were grouped into five clusters namely I, II, III, IV and V accommodating 9, 3, 168, 12 and 32 genotypes respectively. The susceptible (TMV 2, TMV 7, TMV 13) and Resistant (ICG 6022) checks were assigned in cluster III. The principal co-ordinate analysis was also performed using GenA1Ex 6.5 software. Similar studies have been reported by Sathees *et al.* (2019). The result had shown that the total variation in the genotypes was 12.78 per cent, 11.21 per cent and 10.12 per cent for PC1, PC2 and PC3 respectively and had given in Fig 2.

Molecular validation of groundnut germplasm for late leaf spot resistance

Five polymorphic molecular markers such as IPAHM 524, TC7H11, Ah3TC23H10, Ah3TC24B05 and Ah3TC28B01 were deployed to identify the late leaf spot resistant genotype among groundnut germplasm. The results had

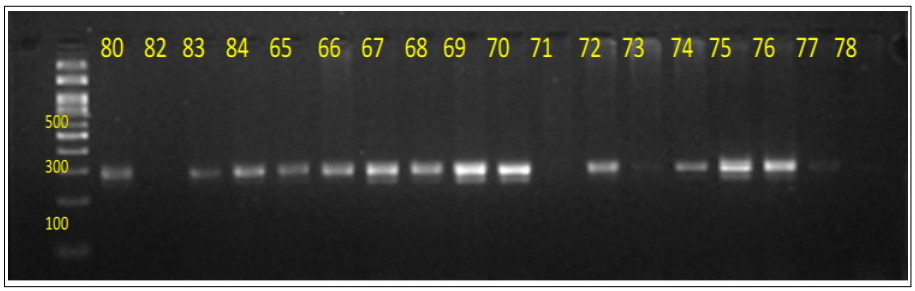

Plate 3: Molecular profile of SSR marker IPAHM 524.

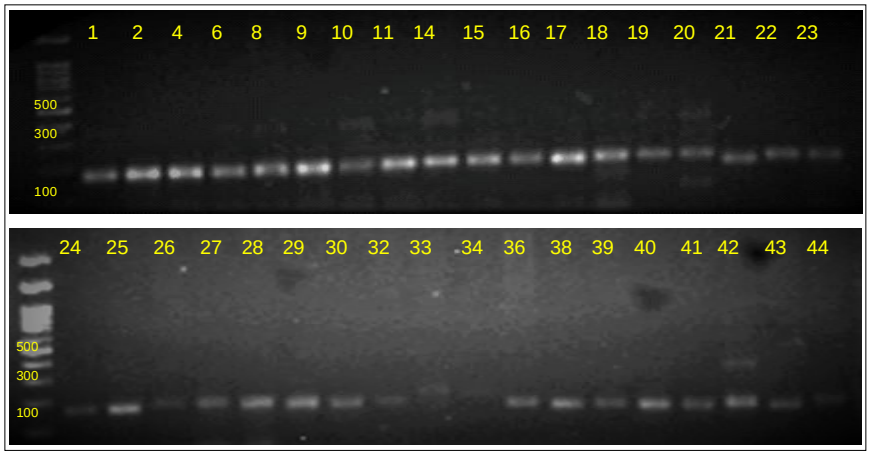

Plate 4: Molecular profile of SSR marker Ah3Tc24B05.

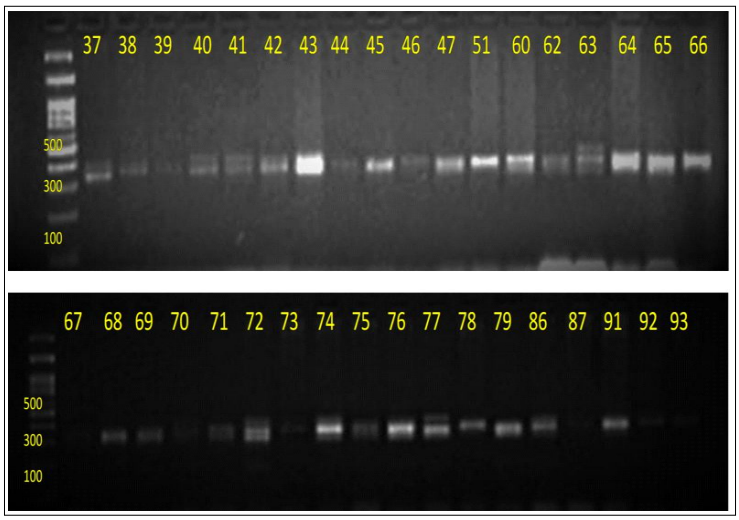

Plate 5: Molecular profile of SSR marker TC7H11.

revealed that 35 genotype had shown that the same allele size (bp) as the resistant check at 280 bp for IPAHM 524 marker and 36 genotype out of 220 showed allele size (bp) as the resistant check at 360 bp for the marker TC7H11. Also, the genetic markers Ah3TC23H10, Ah3TC24B05, Ah3TC28B01 had identified 44, 24, 67 genotypes with similar allele size as resistant check at 160 bp, 160 bp, 220 bp, respectively (Table 8). The genotype, ICG 15419 had shown the exact band size as the resistant check for late leaf spot disease and was confirmed by all five polymorphic markers whereas ICG 2511 had exhibited band size similar as the resistant check which was confirmed

Table 7: Distribution pattern of groundnut genotypes on the basis of phylogenetic tree constructed based on molecular data.

Cluster Number	No. of genotypes	Genotypes
I	9	ICG 9777, ICG 10053, ICG 10185, ICG 10566, ICG 11457, ICG 15396, ICG 11515, ICG 9987, ICG 10010
II	3	ICG 7969, ICG 8751, ICG 11426
III	168	ICG 13856, ICG 14106, ICG 14127, ICG 14482, ICG 14466, ICG 14475, ICG 14523, ICG 1137, ICG 2106, ICG 15385, ICG 11219, ICG 15415, ICG 11109, ICG 12509, ICG 1973, ICG 2381, ICG 15234, ICG 6646, ICG 2772, ICG 3343, ICG 8253, ICG 8490, ICG 15287, ICG 6375, ICG 6407, ICG 15398, ICG 15401, ICG 15390, ICG 6263, ICG 15232, ICG 15379, ICG 1142, ICG 6057, ICG 9362, ICG 9809, ICG 9507, ICG 9961, ICG 1711, ICG 1823, ICG 2031, ICG 2019, ICG 15395, ICG 1834, ICG 1274, ICG 2777, ICG 6394, ICG 6402, ICG 1415, ICG 4527, ICG 4538, ICG 10701, ICG 1487, ICG 4389, ICG 3992, ICG 4543, ICG 12276, ICG 4156, ICG 4343, ICG 4412, ICG 10920, ICG 10950, ICG 1399, ICG 11088, ICG 12672, ICG 10890, ICG 6667, ICG 7181, ICG 10479, ICG 7243, ICG 7190, ICG7963, ICG 10092, ICG 10474, ICG 7867, ICG 3140, ICG 3312, ICG 11542, ICG 11322, ICG 15236, ICG 118, ICG 163, ICG 875, ICG 9315, ICG 11249, ICG 3746, 9666, ICG 188, ICG 928, ICG 13982, ICG 4111, ICG 11144, ICG 4598, TMV 2, TMV 7, TMV 13, ICG 6703, ICG 6766, ICG 10036, ICG 4670, ICG 4746, ICG 4729, ICG 4750, ICG 4684, ICG 12625, ICG 12682, ICG 12991, ICG 13603, ICG 14710, ICG 5195, ICG 4764, ICG 4906, ICG 5051, ICG 4955, ICG 5221, ICG 5236, ICG 397, ICG 13787, ICG 297, ICG 332, ICG 405, ICG 434, ICG 442, ICG 11386, ICG 513, ICG 532, ICG 14630, ICG 8760, ICG721, ICG 862, ICG 13895, ICG 15397, ICG 3673, ICG 9842, ICG 2925, ICG 15233, ICG 15384, ICG 3027, ICG 3053, ICG 5016, ICG 12988, ICG8517, ICG 15190, ICG 12000, ICG 12189, ICG15380, ICG 76, ICG 111, ICG 2286, ICG 8285, ICG 7897, ICG 4798, ICG 2511, ICG 6022, ICG 14985, ICG 4998, ICG 15042, ICG 9449, ICG 15419, ICG 5827, ICG 8106, ICG 14705, ICG 9418, ICG4911, ICG 5475, ICG 13858, ICG 5609, ICG 12665, ICG 14179
IV	12	ICG 8083, ICG 8567, ICG 11651, ICG 9037, ICG 14834, ICG 15309, ICG9249, ICG 13491, ICG 10384, 11687, ICG 13099, ICG 14118
V	32	ICG 3775, ICG 12235, ICG 14177, ICG 12697, ICG 12879, ICG 12921, ICG 311, ICG 36, ICG 81, ICG 5779, ICG 7883, ICG 6201, ICG 115, ICG 334, ICG 3240, ICG 1519, ICG 1668, ICG 1699, ICG 1534, ICG 7153, ICG 5891, ICG 1569, ICG 5494, ICG 3102, ICG 6643, ICG 1703, ICG 6888, ICG 3584, ICG 2857, ICG 3421, ICG 5327, ICG 6654

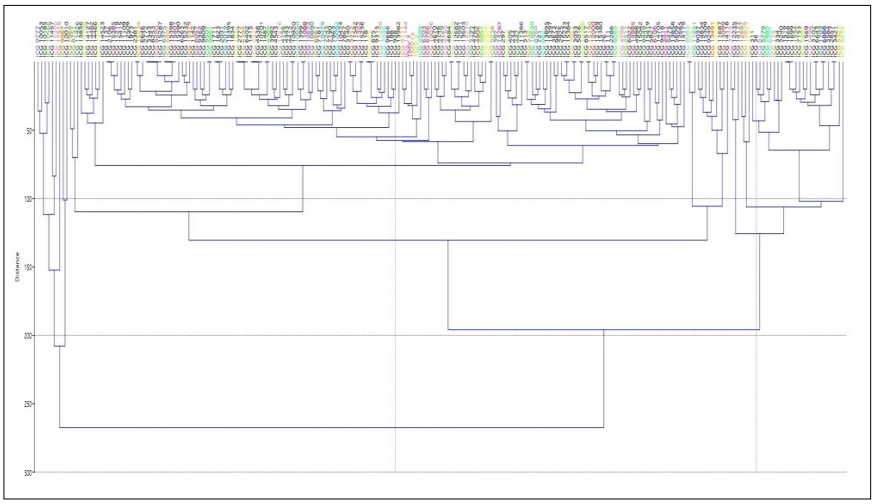

Fig 1: Phylogenetic tree constructed based on molecular data.

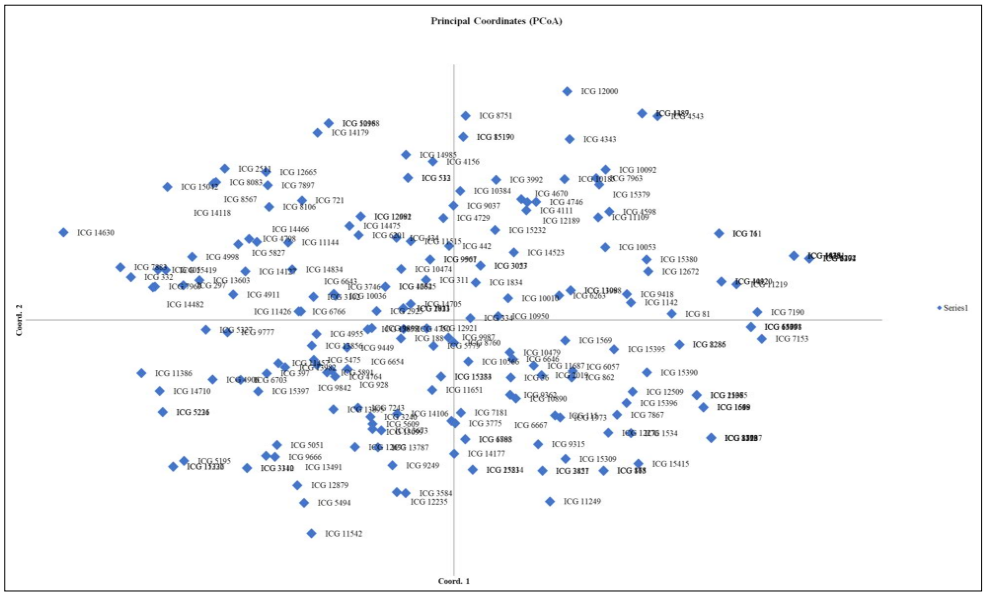

Fig 2: Principal coordinates of 224 accessions based on SSR markers.

Table 8: Band size (bp) of the resistant and susceptible checks for the molecular markers used in this study.

Molecular markers	Band size			
	ICG 6022 (Resistant check)	TMV 2 (Susceptible check)	TMV 11 (Susceptible check)	TMV 13 (Susceptible check)
IPAHM 524	280	300	300	300
TC7H11	360	340	340	340
Ah3TC23H10	160	180	150	150
Ah3TC24B05	160	150	140	150
Ah3TC28B01	220	200	200	200

by four SSR polymorphic markers. The results were akin with the findings of Saravanan *et al.,* (2024). Further, confirmation of groundnut genotypes *viz.,* ICG 405, ICG 721, ICG 14118, ICG 11144 for LLS resistance was made with record of similar band size using three SSR polymorphic markers as the resistant check for late leaf spot resistance.

CONCLUSION

Groundnut germplasm *viz*., ICG 978, ICG 1649, ICG 1703 and ICG 2280 were categorized as resistant genotypes for late leaf spot through molecular validation while ICG 8760, ICG 4598, ICG 5051, ICG 5891 and ICG 6057 had shown moderately resistant to LLS disease besides exhibited higher mean yield performance among the test genotypes. Hence, the groundnut germplasm *viz*., ICG 8760, ICG 4598, ICG 5051, ICG 5891 and ICG 6057 may be utilized for developing ideal recombinants for LLS resistance breeding in Groundnut.

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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