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Validation of Groundnut (*Arachis hypogaea* L.) Mini-core Genotypes for Phenotypic Extremities along with LLS Resistance Through Genetic and Molecular Intervention

S. Saravanan[1,2], G. Vaishali[1], M. Arumugam Pillai[1], J.R. Jerish[1]

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ABSTRACT

Background: The present investigation was conducted at Agricultural College and Research Institute, Killikulam, Tamil Nadu during the year 2022-2023 involving 220 groundnut genotypes along with a resistant and susceptible check towards validation of phenotypic extremities on yield and its contributing traits besides screening against late leaf spot resistance.

Methods: The data collected from two hundred and twenty germplasm along with check varieties for thirteen quantitative traits. The collected data were subjected to statistical analysis (Mahalanobis, 1936) besides interpreted by various genetic tools such as PCV, GCV, heritability and GAM. Further, ten plants were selected at random from each genotype and observation on late leaf spot disease was recorded based on modified 9 point scale.

Result: Molecular analysis for LLS resistance among the groundnut germplasm had shown the concurrent superiority in terms of yield and resistance to late leaf spot disease among the genotypes ICG 15233, ICG 8760, ICG 76, ICG 111, ICG 297, ICG 405, ICG 238, ICG 4598, ICG 5051, ICG 6057, ICG 8253, ICG 8285, ICG 10701 and ICG 15234. Hence, these genotypes can be exploited to develop a disease resistance and high yielding groundnut culture.

Key words: Conventional and molecular intervention, Genetic diversity, Genetic variability.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) construed as one of the most potent oilseed crop, delivering high protein besides meeting the larger share of country's oil requirement. Globally, India ranks first in Groundnut area under cultivation and is the second largest producer in the world with 101 lakh tonnes with productivity of 1863 kg per hectare in 2021-22. Tamil Nadu ranks first in terms of productivity (2980 kg/ha), followed by Gujarat (2751 kg/ha) and Telangana (2391 kg/ha). Peanut can also be eaten directly because of its greater food value glorified by the fact that it contains more protein (24.35 per cent), carbohydrate (21.26 per cent) and oil content (45-56 per cent) besides several minerals, antioxidants, flavonoids and vitamins such as niacin (12.1 mg/100 g) and choline (52.5 mg/100 g) (Bonku and Yu, 2020). Groundnut is always been a fascinating crop to small and marginal farmers but its yield was greatly challenged by Late leaf spot which cause severe defoliation of diseased leaflets during 30-50 days after sowing and reduce pod and fodder yield by 50 per cent besides the kernel quality (Damicone *et al*., 2010 and Ndifon, 2022). Though LLS is normally managed by application of fungicides, yet the incorporation of host resistance gene in susceptible cultivar has always been an eco-friendly and sustainable approach in crop improvement (Wankhade *et al*., 2021). Plant breeders exploit the genetic diversity to choose superior genotypes with the estimation of heritability because of good indicator of transmission of traits from parents to progeny (Gangadhara *et al*., 2023).

[1]Department of Genetics and Plant Breeding, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam-628 252, Tamil Nadu, India.
[2]Rice Research Station, Ambasamudram-627 416, Tamil Nadu, India.

Corresponding Author: S. Saravanan, Rice Research Station, Ambasamudram-627 416, Tamil Nadu, India.
Email: saravananspbg@tnau.ac.in

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MATERIALS AND METHODS

Experimental sites and source of materials

The field trials were laid at Agricultural College and Research Institute, Killikulam, Tamil Nadu during *Kharif* and *Rabi* seasons of 2021-23 in 'D' block farm. The experimental materials consisted of 220 groundnut mini-core collections made from ICRISAT, Hyderabad besides the four check varieties availed from Genetics and Plant Breeding, Killikulam. Biometrical data on yield and its contributing traits *viz*., days to fifty per cent flowering, plant height, number of primary branches per plant, number of secondary branches per plant, kernel length, kernel breadth, number of seeds per plant, 100 seed weight,

shelling per cent and pod yield per plant were recorded on five plants of each genotype. Oil content on the genotypes was assessed on Near Infra-red spectroscopy instrument adopting non-destructive method. The collected data were interpreted for genetic diversity utilizing the D^2 statistical analysis (Mahalanobis, 1936) besides the genetic variability parameters such as PCV and GCV, heritability and GAM were also estimated according to Burton *et al*. (1953), Lush (1940) and Johnson *et al*. (1955) respectively. Further, ten plants were selected at random from each genotype and observation on late leaf spot disease was recorded based on modified 9 point scale (Subrahmanyam *et al*., 1995).

Molecular analysis-DNA extraction and PCR amplification

The extracted DNA pellets vide modified CTAB method as suggested by Kumari *et al*. (2020) were purified by ethanol wash and the purified DNA was treated with RNase enzyme to remove RNA contamination and the DNA was quantified using gel electrophoresis unit on a 0.8 per cent agarose gel and loading dye. DNA samples were amplified in a 10 µl reaction mixture consisting of 2.5 µl of template DNA, 0.75 µl of each forward primer and reverse primer, 3.5 µl of PCR master mix and 2.5 µl of sterile distilled water. PCR amplification was carried out using thermal cycler, programmed for an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 1 minute and a final extension at 72°C for 20 minutes. PCR products were analyzed in 3% agarose gels, visualized and documented using BIORAD gel documentation system. The gel score as interpreted as '0' for no bands and as '1' for polymorphic bands were further fitted to the equation as given by Anderson *et al*. (1993) and the polymorphism information content (PIC) value was computed.

$$PIC = 1 - \Sigma P_{ij}^{2}$$

Where,

P_{ij} = Frequency of the jth allele for the ith marker.

RESULTS AND DISCUSSION

Two hundred and twenty groundnut genotypes received from ICRISAT, Hyderabad were evaluated for their late leaf spot resistance along with yield superiority through phenotypic and genotypic intervention. The phenotypic screening for LLS resistance was carried out in groundnut genotypes using Percent Disease Index (PDI) score (Subrahmanyam *et al*., 1995 and Zanjare *et al*., 2023). The genetic diversity was assessed for two hundred and twenty four groundnut genotypes through the D^2 analysis and Principal Component Analysis (PCA) besides clustering of genotypes was also performed based on molecular data for late leaf spot resistance.

The extent of genetic variability and the degree of its heritability available in the germplasm determines the effective superior genotype (Johnson *et al*., 1955). The present investigation had shown that the phenotypic coefficient of variation (PCV) value for all thirteen biometrical traits was higher than that of genotypic coefficient of variation (GCV) suggesting the role of environment on the phenotype of the crop and presented in Table 1. Similar results was also reported by Mubai *et al*. (2019) and Sab *et al*. (2018). In this study, the higher estimates of PCV and GCV was expressed for plant height, primary branches per plant,

Table 1: Genetic variability parameters for thirteen quantitative traits.

Traits	Range	Mean	PCV (%)	GCV (%)	h^2 (%)	GAM (%)
PH	9.18-44.25	22.53	24.41	22.61	85.78	43.14
DFL	26.00-52.00	40.83	10.36	10.27	98.44	21.01
PB	2.25-23.50	8.94	54.88	53.66	95.58	108.07
SB	0-55.25	10.74	101.67	100.07	96.88	202.92
KL	0.85-1.93	1.30	17.51	15.00	73.35	26.47
KW	0.49-1.08	0.78	12.79	8.23	41.39	10.91
HSW	15.25-59.6	36.35	22.42	21.82	94.76	43.77
NP	31-67.75	44.66	16.93	15.86	87.67	30.59
NMP	28.25-64.25	42.23	16.99	16.05	89.18	31.22
SN	1.50-3.50	2.01	15.24	12.22	64.36	20.21
SH	33.00-84.00	67.26	13.95	13.59	95.00	27.00
OC	43.25-56.22	49.09	3.60	-1.07	-8.89	-0.60
SPY	5.70-28.33	16.26	28.51	27.46	92.77	54.49

PH- Plant height (cm), DFL- Days to 50% flowering, PB- Number of primary branches per plant, SB- Number of secondary branches per plant, KL- Kernel length (cm), KW- Kernel width (cm), HSW- Hundred seed weight (g), NP- Number of pods per plant, NMP- Number of mature pods per plant, SN- Number of seeds per pod, SH- Shelling percentage, OC- Oil content (%), SPY- Pod yield per plant (g), PCV- Phenotypic coefficient of variation (%), GCV- Genotypic coefficient of variation (%), h^2- Heritability (%), GAM- Genetic advance as per cent of mean (%).

secondary branches per plant and hundred seed weight. Patil *et al*. (2014) and Rao *et al*. (2014) have also reported high GCV and PCV for hundred seed weight. Further, Patil *et al*. (2014) reported high GCV and PCV for primary and secondary branches per plant. Higher heritability and genetic advance as a per cent of mean indicating greater non-additive gene action was observed for all traits except kernel width and oil content wherein the lower h^2, GCV and GAM were reported suggesting more of additive gene action. The results were akin to the reports given by Sab *et al*. (2018) and Patil *et al*. (2014).

Clustering is also used to summarize information on relationships between objects by grouping similar units so that the relationship may be easily understood and communicated. All the two hundred and twenty four genotypes were evaluated based on Mahalanobis D^2 method for thirteen traits and the analysis revealed the presence of wide genetic diversity as they formed seven different group constellations and cluster I comprised of maximum number of genotypes (79), followed by cluster III (68), cluster VI (67) and presented in Table 2. The maximum intra cluster distance was observed for cluster VII (22.171) which represented the presence of diverse nature of genotypes within the cluster followed by cluster III (21.533). The inter cluster distance between cluster VI and VII was maximum (31.795), followed by the clusters III and VII (31.174). The maximum inter cluster distance exists between the clusters having highly diverse genotypes.

In this study, 220 groundnut genotypes had also 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) and presented in Fig 1. On perusal of data, five groundnut genotypes *viz*., ICG 532, ICG 2381, ICG 14179, ICG 15233, ICG 6022 were registered to be resistant while forty five genotypes had shown moderately resistant reaction. Susceptible and highly susceptible reaction was exhibited by 126 and 48 genotypes. The groundnut mini-core germplasm were also evaluated for late leaf spot resistance under glasshouse condition adopting the inoculum spraying method. The results were pursued and found two genotypes as resistant namely ICG 15233 and ICG 6022. Further, fourteen genotypes were adjudged as moderately resistant while one hundred and eighteen as susceptible and ninety as highly susceptible to late leaf spot.



Fig 1: Field evaluation against LLS in groundnut genotypes.

Table 2: Composition of D^2 cluster for groundnut genotypes.

Cluster no.	No. of genotypes	Genotypes
I	79	ICG 3746, ICG 8760, ICG 36, ICG 76, ICG 81, ICG 111, ICG 115, ICG 118, ICG 163, ICG 188, ICG 297, ICG 311, ICG 332, ICG 334, ICG 397, ICG 405, ICG 434, ICG 442, ICG 513, ICG 532, ICG 721, ICG 862, ICG 875, ICG 928, ICG 1137, ICG 1142, ICG 1274, ICG 1399, ICG 1415, ICG 1487, ICG 1519, ICG 1534, ICG 1569, ICG 1668, ICG 1699, ICG 1703, ICG 1711, ICG 1823, ICG 1834, ICG 1973, ICG 2019, ICG 2031, ICG 2106, ICG 2286, ICG 2381, ICG 2511, ICG 2772, ICG 2777, ICG 2857, ICG 2925, ICG 3027, ICG 3053, ICG 3102, ICG 3140, ICG 3240, ICG 3312, ICG 3343, ICG 3421, ICG 3584, ICG 3673, ICG 3775, ICG 3992, ICG 4111, ICG 4156, ICG 4343, ICG 4389, ICG 4412, ICG 4527, ICG 4538, ICG 4543, ICG 4598, ICG 4670, ICG 4684, ICG 4729, ICG 4746, ICG 4750, ICG 4764, ICG 4798, ICG 11687
II	2	ICG 9362, ICG 11651
III	68	ICG 4906, ICG 4911, ICG 4955, ICG 4998, ICG 5016, ICG 5051, ICG 5195, ICG 5221, ICG 5236, ICG 5327, ICG 5475, ICG 5494, ICG 5609, ICG 5779, ICG 5827, ICG 5891, ICG 6057, ICG 6201, ICG 6263, ICG 6375, ICG 6394, ICG 6402, ICG 6407, ICG 6643, ICG 6646, ICG 6654, 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 8253, ICG 8285, ICG 8490, ICG 8517, ICG 8567, ICG 8751, ICG 9037, ICG 9249, ICG 9315, ICG 9418, ICG 9449, ICG 9507, ICG 9666, ICG 9777, ICG 9809, ICG 9842, ICG 9961, ICG 9987, ICG 10010I, CG 10036, ICG 10053, ICG 10092, ICG 10185, ICG 10384, ICG 10474, ICG 13856, ICG 13858, ICG 13895, ICG 13982, ICG 14106
IV	2	ICG 11515, 15401
V	2	ICG 11542, ICG 15386
VI	67	ICG 10479, ICG 10566, ICG 10701, 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 12000, ICG 12189, ICG 12235, ICG 12276, ICG 12509, ICG 12625, ICG 12665, ICG 12672, ICG 12682, ICG 12697, ICG 12879, ICG 12921, ICG 12988, ICG 12991, ICG 13099, ICG 13491, ICG 13603, ICG 13787, ICG 13858, ICG 13895, ICG 13982, ICG 14118, ICG 14127, ICG 14177, ICG 14179, ICG 14466, ICG 14475, ICG 14482, ICG 14523, ICG 14630, ICG 14705, ICG 14710, ICG 14834, ICG 14985, ICG 15042, ICG 15190, ICG 15232, ICG 15233, ICG 15234, ICG 15236, ICG 15287, ICG 15309, ICG 15379, ICG 15380, ICG 15384, ICG 15385, ICG 15390, ICG 15395, ICG 15397, ICG 15398, ICG 15415, ICG 15419
VII	4	TMV 2, TMV 7, TMV 13, ICG 6022

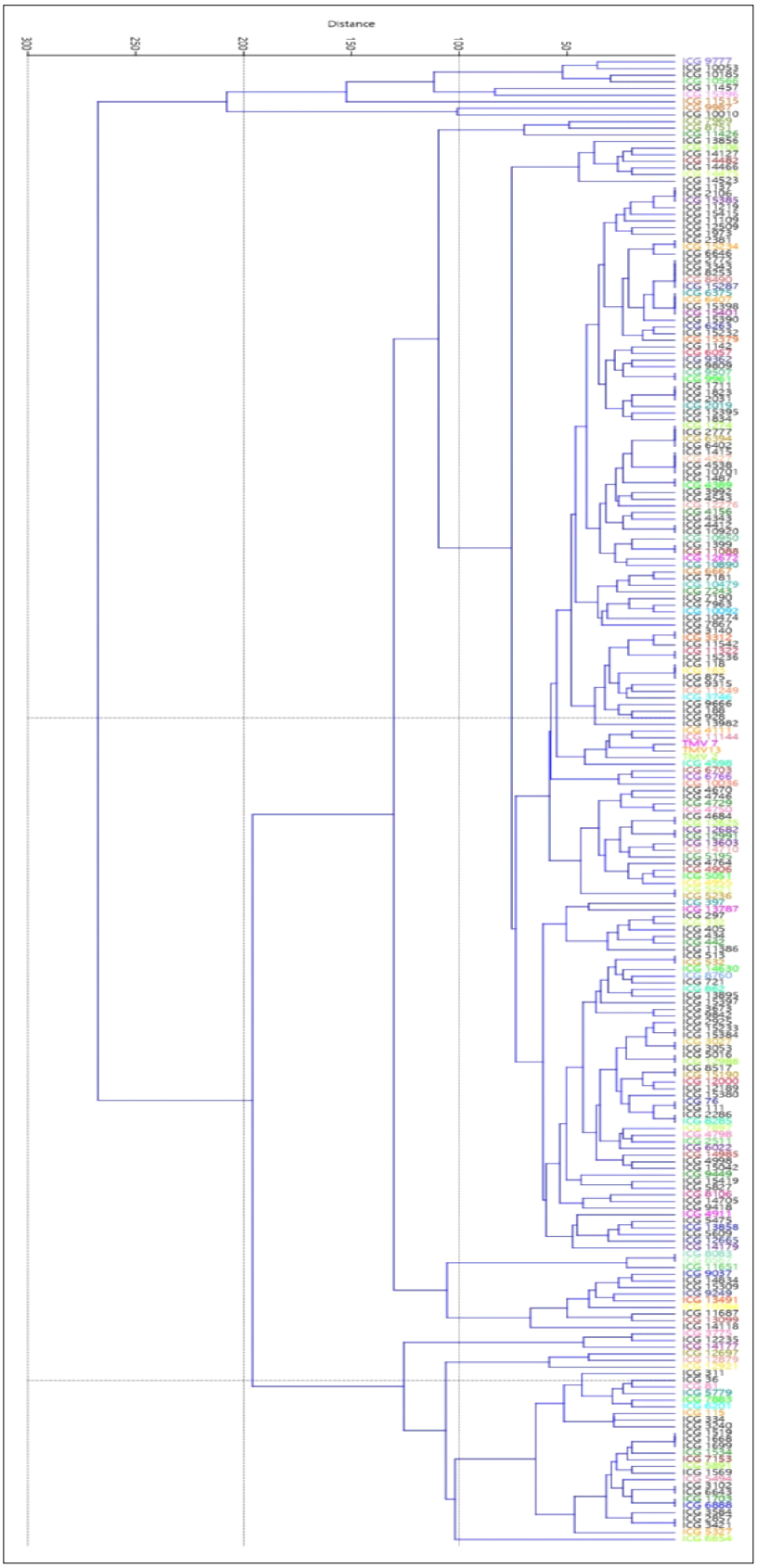

Fig 2: UPGMA dendogram constructed based on molecular data.

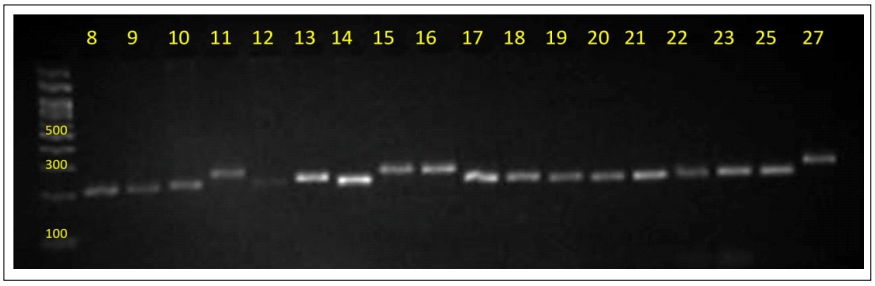

Fig 3: Molecular profile of SSR marker Ah3Tc28B01.

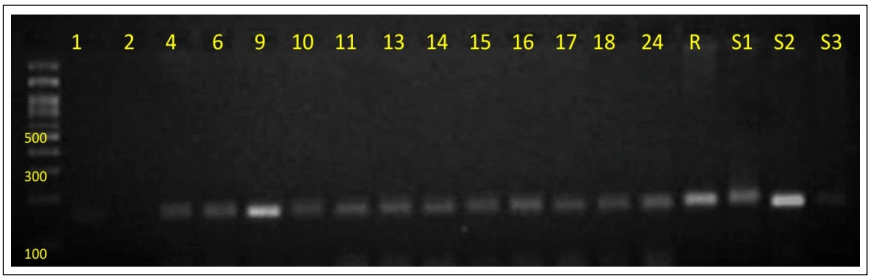

Fig 4: Molecular profile of SSR marker Ah3Tc23H10.

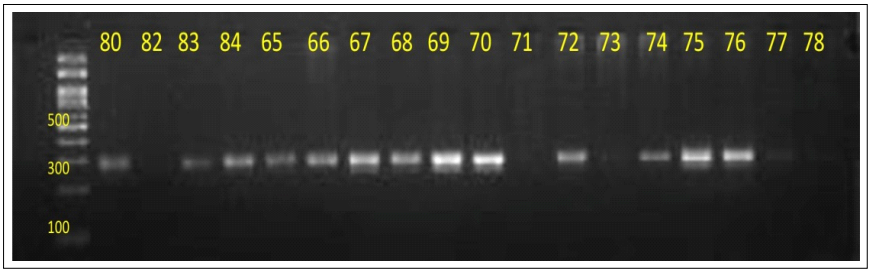

Fig 5: Molecular profile of SSR marker IPAHM 524.

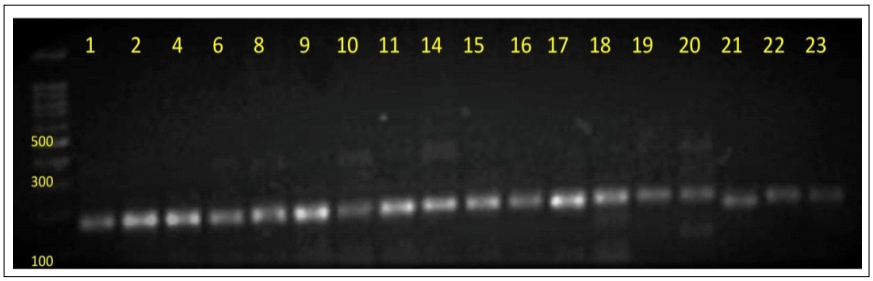

Fig 6: Molecular profile of SSR marker Ah3Tc24B05.

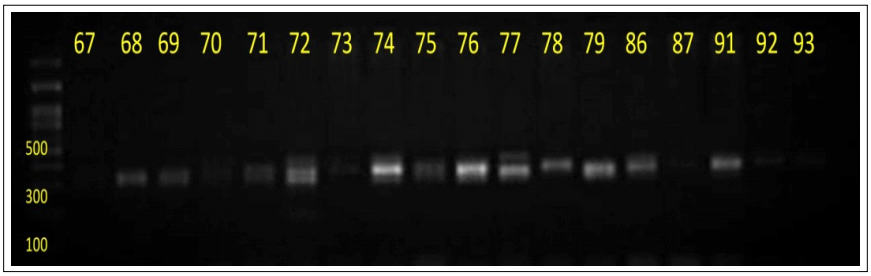

Fig 7: Molecular profile of SSR marker TC7H11.

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

Molecular markers		Sequence	Number of bases	Motif	Annealing (°C)	Total no. of alleles	PIC value	References
IPAHM 524	F	GCCATGGATAAGAACCTGAAA	21	(GA) 20	55	5	0.570	(Khedikar *et al.*, 2010 and Zhao *et al.*, 2012)
	R	CAGTAAGCTGAGCTGGCAGA	20					
TC7H11	F	AGGTTGGAACTATGGCTGATTG	22	(AG) 18	55	5	0.509	(Khedikar *et al.*, 2010)
	R	CCAGTTTAGCATGTGTGGTTCA	22					
Ah3TC23H10	F	TCCCTTTGAGTCATTCATTGTG	22	(GA)19	55	5	0.501	(Zhao *et al.*, 2012)
	R	CATCAGAGCTCCTTTTCCCTAA	22					
Ah3TC24B05	F	ATTGATACCTCTTTGCTCTCGC	22	(TC) 30	55	5	0.502	(Zhao *et al.*, 2012)
	R	TGAAACCCTAACTAGCTCGGAA	22					
Ah3TC28B01	F	ATTTATTGCCAAATCTGTCGCT	22	(AG) 37	55	5	0.530	(Zhao *et al.*, 2012)
	R	CATTGCCAACTGTTACTACCCA	22					

Among the twenty two markers deployed, five molecular markers had shown polymorphism for the resistant and susceptible checks (IPAHM524 at 280 bp; TC7H11 at 360 bp; Ah3TC23H10, Ah3TC24B05 at 160 bp and Ah3TC28B01 at 220 bp) and hence these markers were used for the detection of genetic background of 224 groundnut genotypes for late leaf spot resistance and presented in Table 3 and Fig 2. The assessment of level of association existing between the molecular markers used was made through the interpretation on Polymorphic Information Content (PIC) value and this study inferred that all markers had expressed significant level of polymorphism. The maximum allele size was observed for the genetic marker TC7H11 while the minimum allele size was observed for Ah3TC23H10. As such the PIC values for the molecular markers IPAHM 524, TC7H11, Ah3TC23H10, Ah3TC24B05 and Ah3TC28B01 were recorded as 0.57, 0.509, 0.501, 0.502 and 0.53 respectively (Fig 3 to 7) and the result akin with findings of (Khedikar *et al*., 2010).

The studies on genetic diversity made by assessment of phenotypic extremities of 220 genotypes along with checks validated banding pattern differences generated by five molecular markers and thereby further clustering of 220 genotypes were made following UPGMA pattern (Fig 2). Based on amplification pattern, the genotypes were investigated for genetic divergence with SSR markers. The dendrogram constructed using molecular data had grouped the groundnut genotypes into five clusters. The maximum number of genotypes were presented in cluster III (168), followed by cluster V (32), cluster IV (12), cluster I (9) and cluster II (3). The susceptible (TMV 2, TMV 7, TMV 13) and resistant (ICG 6022) checks had exhibited very low dissimilarity index and were assigned in cluster VII. The results highlighted the cluster III for inclusion of maximum genotypes for yield and LLS resistance.

Molecular intervention of LLS resistance among 220 groundnut germplasm had revealed that the genotypes *viz*., ICG 15233, ICG 8760, ICG 297, ICG 405, ICG 15234 had shown the exact band size as the resistant check attributing resistance to late leaf spot disease. Further, these genotypes also had proven their resistance to LLS under field and controlled condition.

CONCLUSION

In this investigation, two hundred and twenty groundnut germplasm were interpreted for the phenotypic extremities besides expression of late leaf spot tolerance through *in vitro* and *in vivo* protocols. The results had exhibited the higher PCV and GCV estimates were recorded for plant height, primary branches per plant, secondary branches per plant, hundred seed weight. Higher heritability and genetic advance as a per cent of mean was observed for all traits included in this study except kernel width and oil content that suggested for effective selection for genetic improvement of such traits. Assessment of phenotypic variation quantified that the inter cluster between VI and VII

was maximum the maximum, followed by the clusters III and VII. The crosses generated between parents from these clusters could deliver a greater level of heterosis in F_1 Similarly, the germplasm were subjected for screening against late leaf spot both under in vitro and in vivo conditions and as such, 5 genotypes were found to be resistant, 45 genotypes were moderately resistant, 126 genotypes were susceptible and 48 genotypes were highly susceptible under natural conditions. Further, upon artificial screening, two genotypes found to be resistant, 14 were moderately resistant, 118 were susceptible and 90 were highly susceptible. Molecular analysis for LLS resistance among the groundnut germplasm also had shown the exact band size as the resistant check attributing resistance to late leaf spot disease among ICG 15233, ICG 8760, ICG 76, ICG 111, ICG 297, ICG 405, ICG 238, ICG 4598, ICG 5051, ICG 6057, ICG 8253, ICG 8285, ICG 10701, ICG 15234. Hence, use of such resistant genotypes as donor can help to tailor LLS resistance in locally adopted and superior groundnut varieties.

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Conflict of interest

The all authors declared that they have no conflict of interest.

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