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VALIDATION AND DISCOVERY OF GENOMIC REGIONS LINKED TO LATE LEAF SPOT DISEASE RESISTANCE THROUGH QTL ANALYSIS IN AN $F_{2:3}$ MAPPING POPULATION OF PEANUT

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ABSTRACT

Peanut (*Arachis hypogaea* L.) yield is constrained due to foliar fungal diseases, late leaf spot (LLS) and rust, in most areas of the world; they reduce yield by more than 50% and adversely affect pod yield and fodder quality. In this context, an investigation was carried out with the experimental material consisting of 121 F_2 individuals developed from the cross TMV 2 × GPBD 4. It was forwarded to F_3 progeny rows and were evaluated during *kharif*-2021 to phenotype for late leaf spot disease on the 60th, 75th, 90th and 105th days after sowing at GKVK, Bengaluru. There were notable variations for LLS disease reaction in all $F_{2:3}$ progenies with greater genetic variability. Using the genotypic information of 94 F_2 individuals, a linkage map was constructed using 100 polymorphic SSR markers on 20 linkage groups. The linkage map was 3346.43 cM long, with an average of 33.46 cM inter-marker distance. A total of 15 QTLs were identified, accounting for 2.05–31.10% of the phenotypic variance. At LOD 2.5, seven major QTLs for LLS resistance were found. Following validation in RILs, the QTLs found in the study can be used for MAS (Marker-Assisted Selection).

Key words : Late leaf spot disease, $F_{2:3}$ populations, QTL mapping, Major QTL, Marker-Assisted Selection.

Introduction

Peanut (*Arachis hypogaea* L., AABB, 2n = 4x = 40) is one of the world's important crop, ranking fifth in oil production after soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), rapeseed (*Brassica napus* L.), and sunflower (*Helianthus annuus* L.). It produces over 45.95 million tons of pod yield on a global cultivated area of over 28.5 million hectares. Globally, India ranks first in area under cultivation and is the second largest producer in the world after China with 10.14 million tons with productivity of 1777 kg per hectare (FAO, 2020). A legume crop, peanut are primarily grown for their seed, which has 48–50% oil and 25–28% protein (Sukruth *et al*., 2015). The most common varieties grown in India are Spanish varieties, which are particularly vulnerable to foliar fungal diseases such as LLS (*Phaeoisariopsis personata* [(Berk. and Curt) Deighton]), early leaf spot (*Cercospora arachidicola* Hori), and rust (*Puccinia arachidis* Speg.).

However, the availability and identification of resistance sources, as well as the combination of resistance with high productivity and desirable pod traits, all affect the success of breeding for disease resistance. A significant degree of resistance to foliar diseases is seen in certain Valencia landraces and wild peanut species; nevertheless, this resistance is typically associated with low yield, late maturity, poor adaptation, and unattractive pod characteristics (Wynne *et al*., 1991; Singh *et al*., 1997). The accuracy and speed of developing peanut cultivars resistant to LLS may be improved by combining traditional breeding techniques with genomic tools such as markers, QTL analysis and MAS which

helps in shortening response to selection.

Two key genomic loci controlling resistance to LLS and rust have been identified as a result of QTL analysis in two RIL populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). Regarding resistance to LLS and rust, one QTL region on linkage group (LG) XV demonstrated 67.98% ($QTL_{R4\text{-}LLS}02$) and 82.96% ($QTL_{R4\text{-}Rust}03$) phenotypic variance explained (PVE), respectively. Regarding LLS resistance, the other QTL region on LG XII displayed a PVE of 62.34%. It was possible to identify and validate markers connected to LLS and rust resistance (Khedikar *et al.*, 2010 and Yeri *et al.*, 2014). To introduce resistant variations in the most elite and popular peanut varieties, the former QTL region ($QTL_{R4\text{-}LLS}02$) was introgressed (Varshney *et al.*, 2014). It is usually helpful to continuously validate the markers and QTL in new backdrops. In this regard, extremely LLS-susceptible Spanish varieties (TMV 2) and LLS-resistant Spanish varieties (GPBD 4) were used as parents to create $F_{2:3}$ mapping population at UAS, Bangalore, India. For the late leaf spot disease reaction, these F_3 families were phenotyped and genotypic data of F_2 individuals were utilised to detect and validate QTLs/markers using QTL analysis. Identified and validated QTLs/markers, would serve as an ample resource in selecting disease-resistant genotypes in groundnut crop improvement programs.

Materials and Methods

Population development and Phenotypic screening of F_3

The mapping population was developed by crossing, TMV 2 as female (highly LLS susceptible Spanish variety) with GPBD 4 as male parent (LLS resistant Spanish variety), the resulting F_1 was advanced using the pedigree method without selection to create $F_{2:3}$ mapping populations at University of Agricultural Sciences (UAS), Bangalore, India (Fig. 1). For the late leaf spot disease reaction, F_3 families were assessed in augmented design with checks and parents (GKVK 5, TMV 2 and GPBD 4) in *kharif*-2021, while 121 F_2 individual plants were planted in compact family block design (CFBD) by following spacing of 30 × 20 cm for full genotypic expression along with checks and parents in the summer of 2021 at K-block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru, India (Fig. 2). Due to its high susceptibility towards creating natural epiphytotic conditions for the dissemination of LLS disease, the genotype TMV 2 was employed as a spreader row. After a 4-line pause, it was repeated. Using the spreader row technique (Subrahmanyam *et al.*, 1995),

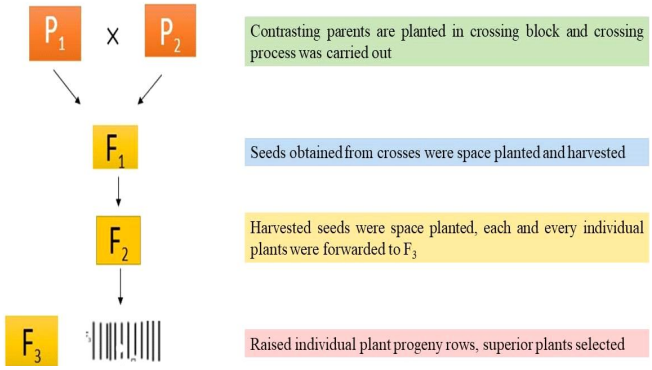


Fig. 1 : Schematic representation of handling and development of $F_{2:3}$ mapping population from the cross TMV 2 × GPBD 4 in peanut.



Fig. 2 : a & c: F_2 individual plants field view, b & d: F_3 Progeny rows for LLS disease screening at K- block, UAS, GKVK, Bengaluru.

which involves planting disease spreader plants (TMV 2) at regular intervals of four rows, progeny rows were subjected to field screening for LLS disease response. A modified 9-point scale was used to score the disease for LLS at 60, 75, 90 and 105 days after sowing (DAS) (Subbarao *et al.*, 1990) and disease score is converted to corresponding percent disease index (PDI) to use in further analysis (Fig. 3).

Genotyping of F_2 individuals

Genomic DNA was isolated from the young leaves (20-25 days old seedling) of F_2 and their parents by following the CTAB method with minor modifications (Cuc *et al.*, 2008). To check the polymorphism between the parents, 412 SSR markers were utilised and obtained 106 polymorphic markers between the parents (Fig. 4). PCR products were resolved using 4% (metaphor 3% + agarose 1%) Metaphor agarose gel electrophoresis (MAGE) with Sequi-Gen (BIO RAD, Hercules, California, USA) (Abdurakhmonov *et al.*, 2007). The alleles were represented by the generated marker data,

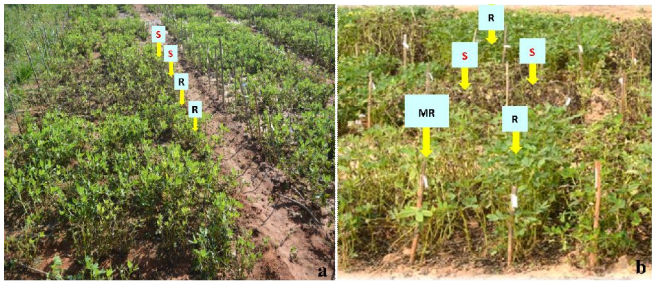


Fig. 3 : a & b: F_3 progeny rows segregating for late leaf spot disease field (*S* susceptible progeny row, *R* Resistant progeny row, *MR* Moderately resistant progeny row).

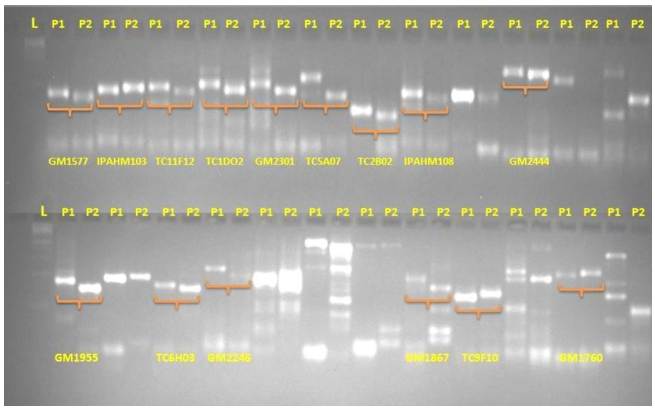


Fig. 4 : Gel image showing DNA bands from amplification products of SSR markers screened for parental polymorphism between parents (P1- TMV 2, P2-GPBD 4). The PCR products were resolved using 4% Metaphor agarose gel stained with ethidium bromide (L= 100 Base pair Ladder, P1- TMV 2, P2-GPBD 4).

which was scored as follows.

1. "A" or 2 - Homozygote at marker locus as that of recurrent parent (TMV 2)
2. "B" or 0 - Homozygote at marker locus as that of donor parent (GPBD 4)
3. "H" or 1 - Heterozygote at the marker locus
4. "U" or -1 - Missing data for the individual at the locus

Statistical analysis

A chi-square (χ^2) test was performed on the genotypic data obtained from polymorphic markers to test the null hypothesis of 1:2:1 expected Mendelian segregation for each marker. The program QTL IciMapping ver 4.2 (Wang *et al.*, 2019) software was used to construct a linkage map using genotyping data on 94 F_2 plants with their respective parents. Single marker analysis (SMA) was carried out by using QTL IciMapping software version 4.2 to identify SSR markers associated with QTLs at a LOD score threshold of 2.5 using the genotypic data of the F_2 population derived from TMV 2 × GPBD 4 and the phenotypic data on PDI of LLS disease of F_3. In order to identify, locate, and estimate size effects and to determine the mode of action of the QTLs controlling traits related to LLS resistance, the genotypic data of the F_2 population derived from TMV 2 × GPBD 4 of groundnut and the phenotypic data on PDI of LLS disease of F_3 were utilized. After implementing inclusive composite interval mapping (ICIM) using the "QTL IciMapping" software version 4.2, QTLs were identified at a LOD threshold of 2.5. Among the existing software tools, we found "QTL IciMapping" is the most user-friendly and facilitates the identification of QTLs based on various mapping population types. Each genotype was examined for co-segregation by examining the kind of allele and the phenotype in order to validate the marker in several $F_{2:3}$ populations. Genotypes with disease resistance (score <5.0) and resistant alleles at linked marker loci were deemed to be positive for co-segregation. In a population, a high percentage of individuals exhibiting co-segregation with both flanking markers was regarded as a good example of QTL/marker validation.

Results and Discussion

In the *Kharif* season of 2021, $F_{2:3}$ population (TMV 2 × GPBD 4) were assessed for reaction to LLS. Significant genotypic differences for resistance to LLS were observed in F_3 mapping population (Fig. 5). Productivity and quality attributes were significantly negatively correlated with LLS disease, although the two variables were generally positively and strongly correlated, among them pod per plant has shown higher magnitude of negative correlation with LLS disease (Fig. 6). Polymorphism between the parents of the $F_{2:3}$ population was found using SSR markers.106 SSR markers (29.12%) of the 412 markers used to screen the parents were found to be polymorphic (Supplementary Table 1). Additionally, the markers polymorphism information content (PIC) value was high. The segregation ratio for the markers was tested using the chi-square test. Six markers that showed segregation distortion among the markers were not included in the linkage map generation process. Of the 106 polymorphic markers, 100 genotyped polymorphic markers were employed after considering segregation

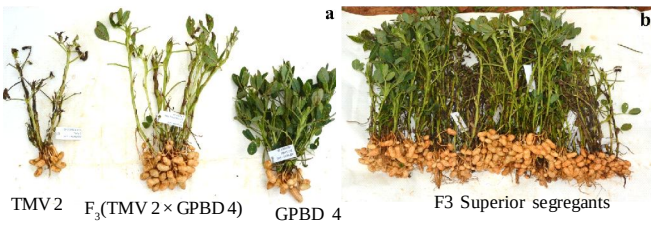


Fig. 5 : a: F_3 with its respective parents b: segregants of F_3 generation in the cross TMV 2 × GPBD 4 in peanut.

Table 1 : Distribution of SSR markers on the linkage map of $F_{2:3}$ population of the cross TMV 2 × GPBD 4 in peanut.

Linkage group (LG)	No. of markers	Length (cM)	Average inter-marker distance (cM)
1	5	178.76	35.75
2	16	587.83	36.74
3	4	141.57	35.39
4	2	66.43	33.22
5	6	211.25	35.21
6	5	108.91	21.78
7	6	205.69	34.28
8	3	84.26	28.09
9	7	203.68	29.09
10	13	439.52	33.81
11	8	261.10	32.64
12	3	94.19	31.39
13	3	132.92	44.30
14	4	148.32	37.08
15	2	62.31	31.16
16	2	50.79	25.39
17	2	67.05	33.52
18	5	194.97	38.99
19	2	51.67	25.83
20	2	55.19	27.59
Total	**100**	**3346.43**	**33.46**

cM : Centimorgan

distortion. The linkage map was constructed using QTL IciMapping ver 4.2 (Wang *et al*., 2019) software with marker segregation data.

A minimum LOD of 2.5 and a maximum distance of 50.0 cM were used to create linkage groups, and 20 linkage groups were mapped with 100 markers. With an average marker distance of 33.46 cM, the linkage map spanning 3346.43 cM was constructed (Fig. 7). Each linkage group has consisted of two (LG-4, LG-15, and LG-19) to sixteen (LG-2) markers. The total length (cM), average marker density (cM), number of markers mapped, and number of marker loci mapped on each linkage group has been enlisted (Table 1). Recently, a few genetic maps based on RIL populations have been developed in cultivated groundnut (Varshney *et al*., 2009; Hong *et al*., 2010; Khedikar *et al*., 2010; Ravi *et al*., 2011; Sarvamangala *et al*., 2011).

Single marker analysis was used to identify significant marker-trait associations. Although SMA confounds size effects and QTL location, it provides preliminary results

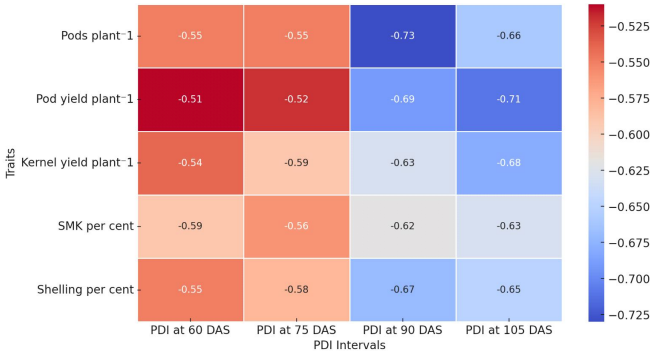


Fig. 6 : Heatmap representing phenotypic correlation for PDI of LLS disease at four intervals with pod yield and its attributing traits in F_3 populations in peanut.

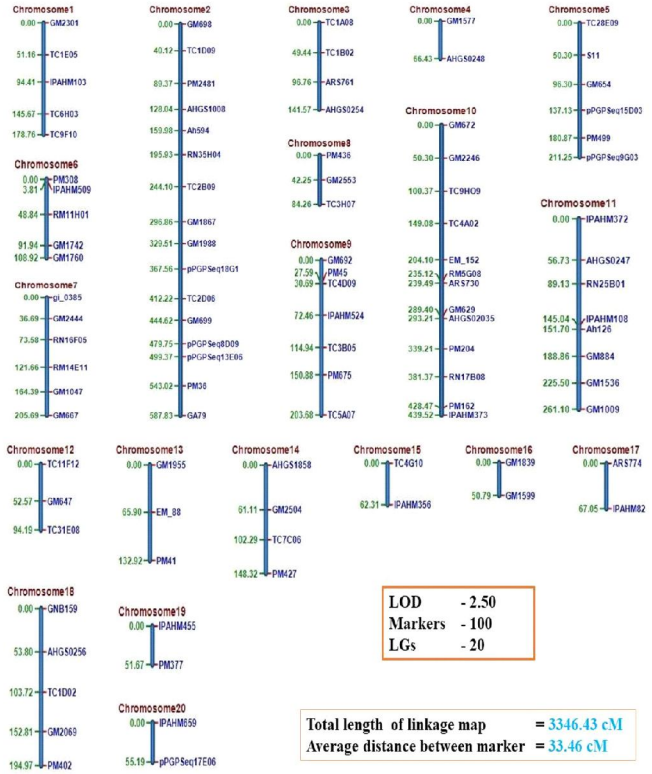


Fig. 7 : Genetic linkage map for $F_{2:3}$ population of the cross TMV 2 × GPBD 4 in peanut.

that aid in the detection and characterization of QTL using more advanced analytical approaches. Markers were shown to have a significant correlation for resistance to late leaf spot disease in SMA analysis (Table 2). 15 QTLs were identified for late leaf spot disease resistance at different intervals of disease incidence through inclusive composite interval mapping (ICIM) method using the QTL IciMapping ver 4.2 (Wang *et al*., 2019) software. Seven of the 15 QTLs are M-QTLs for resistance to late leaf spot disease at various crop stages between 60 and 105 DAS. Two M-QTLs for late leaf spot disease resistance at 60 DAS were found to explain phenotypic variance of 14.69 per cent (GM2246–TC9H09) to 16.61 per cent (GM654–pPGPSeq15D03). Of these, one major QTL

Table 2 : Single marker analysis, linkage group, identity, position and size effects of markers linked to late leaf spot resistance in $F_{2:3}$ population of the cross TMV 2 × GPBD 4 in peanut.

Trait Name	Marker Name	Linkage Group	Position (cM)	LOD	PVE (%)	Additive effects
90D LLS Resistance	GM2301	1	0	2.57	8.62	-1.89
90D LLS Resistance	IPAHM103	1	94.41	2.79	9.31	-2.09
90D LLS Resistance	TC11F12	12	0	3.03	10.04	-1.95
90D LLS Resistance	IPAHM82	17	67.05	7.42	22.16	-3.42
105D LLS Resistance	GM2301	1	0	2.67	8.91	-1.94
105D LLS Resistance	IPAHM103	1	94.41	2.86	9.49	-2.15
105D LLS Resistance	TC11F12	12	0	3.26	10.71	-2.06
105D LLS Resistance	IPAHM82	17	67.05	7.27	21.69	-3.40

90D LLS Resistance 90 Days late leaf spot resistance, *105D LLS Resistance* 105 Days late leaf spot resistance, *cM* centimorgan *LOD* Logarithm of the odds, *PVE* Phenotypic variation explained

(M-QTL) was found on linkage group 5 (LG-5), flanked by markers GM654 and pPGPSeq15D03, explaining a greater phenotypic variance of 16.65 percent at a LOD score of 4.26 with negative additive effects (-0.24), indicating favorable alleles contributed by the resistant parent GPBD 4 in the study (Fig. 7). Another major QTL was found on LG-10, explaining phenotypic variance of 14.69 per cent with negative additive effects (-0.08) at a LOD score of 4.18.

Similarly, at 75 DAS, two novel QTLs for resistance to late leaf spot disease were identified. With negative additive effects (-0.66) and a LOD score of 2.62, one major QTL (M-QTL) found on linkage group 4 (LG-4) and flanked by markers GM1577 and AHGS0248 explained a higher proportion of the phenotypic variance (10.84%). With negative additive effects (-0.81) and a LOD score of 2.91, the second minor QTL on linkage group 9 (LG-9) flanked by markers IPAHM524 and TC3B05 explained 8.5% of the phenotypic variance. Therefore, it is necessary to validate minor QTLs across various genetic backgrounds and mapping population grown under different geographical regions (Table 3).

At 90 DAS, five QTLs for late leaf spot disease resistance were found in the $F_{2:3}$ mapping population. Phenotypic variance for these five QTLs ranges from 3.01 percent (TC1E05–IPAHM103) to 31.10 percent (ARS774–IPAHM82). The resistant parent GPBD 4 of the study is the source of favorable alleles, as evidenced by the detection of M-QTLs on linkage groups 17 and 18 flanked by markers (ARS774–IPAHM82) and (GNB159–AHGS0256). These M-QTLs explained a greater phenotypic variance of 10.47 and 31.10 percent at LOD scores of 2.84 and 12.34 with negative additive effects (-4.16) and (-0.75), respectively Additionally, the other three are minor QTLs flanked by markers (GM2301–TC1E05), (TC1E05–IPAHM103) and (TC7C06–PM427), which explain 3.32, 3.01, and 5.47 phenotypic variances with LOD scores of 2.86, 2.51 and 2.60, respectively (Table 3). Khedikar *et al*. (2010), Sujay *et al*. (2012), Mondal *et al*. (2012), and Kolekar *et al*. (2016) also reported similar findings, demonstrating a strong correlation between markers GM2301 and IPAHM103 and genomic regions/QTLs governing resistance to late leaf spot.

Six QTLs with phenotypic variances ranging from 2.05 percent (TC1E05–IPAHM103) to 20.15 percent (ARS774–IPAHM82) were found to be resistant to late leaf spot disease at 105 DAS. ARS774–IPAHM82 and ARS774–IPAHM82 were the markers flanking two of the major QTLs found on the same linkage group 17. These QTLs explained a greater phenotypic variance of 17.44% and 20.15% at LOD scores of 10.74 and 10.89 with negative additive effects (-4.37) and (-0.78), respectively, indicating favorable alleles originating from the resistant parent GPBD 4. Additionally, four minor QTLs flanked by markers (GM2301–TC1E05), (TC1E05–IPAHM103) and (GNB159–AHGS0256) explained 2.29, 2.05, 3.85 and 6.96 percent of the phenotypic variance at LOD scores of 2.98, 2.59, 2.51, and 2.77, respectively (Table 3).

Validation of QTLs/Markers

Validating the QTLs and their effects is a necessary step before MAS can be used effectively to improve any trait. With the aim of validating the QTLs/markers associated with LLS resistance in the groundnut $F_{2:3}$ population of the cross TMV 2 × GPBD 4, were phenotyped for LLS disease reaction at multiple points, specifically 60 DAS, 75 DAS, 90 DAS, and 105 DAS in *Kharif*-2021. The progenies showed notable differences for disease, and the F_3 lines showed a fairly broad spectrum of distinct disease sensitivities to LLS disease. As QTL analysis is completed, it has enabled validation

Table 3 : Linkage group, identity, position and size effects of QTLs controlling LLS resistance, pod yield and yield related traits in $F_{2:3}$ population of the cross TMV 2 × GPBD 4 in peanut.

Trait Name	QTL Name	Linkage Group (LG)	Position (cM)	Flanking Marker (FM)		LOD	Distance between FM (cM)	PVE (%)	Additive effects
60D LLS Resistance (2 QTLs)	q60DLLS Resistance-5-1	5	112	GM654	pPGPSeq15D03	4.26	16	16.61	-0.24
	q60DLLS Resistance-10-1	10	73	GM2246	TC9HO9	4.18	28	14.69	-0.08
75D LLS Resistance (2 QTLs)	q75DLLS Resistance-4-1	4	30	GM1577	AHGS0248	2.62	32	10.84	-0.66
	q75DLLS Resistance-9-1	9	91	IPAHM524	TC3B05	2.91	28	8.5	-0.81
90D LLS Resistance (5 QTLs)	q90DLLS Resistance-1-1	1	0	GM2301	TC1E05	2.86	14.5	3.32	-1.56
	q90DLLS Resistance-1-2	1	94	TC1E05	IPAHM103	2.51	39	3.01	-2.13
	q90DLLS Resistance-14-1	14	114	TC7C06	PM427	2.60	48	5.47	-1.73
	q90DLLS Resistance-17-1	17	41	ARS774	IPAHM82	12.34	12	31.10	-0.75
	q90DLLS Resistance-18-1	18	26	GNB159	AHGS0256	2.84	42	10.47	-4.16
105D LLS Resistance (6 QTLs)	q105DLLS Resistance-1-1	1	0	GM2301	TC1E05	2.98	14.5	2.29	-1.60
	q105DLLS Resistance-1-2	1	94	TC1E05	IPAHM103	2.59	38	2.05	-2.18
	q105DLLS Resistance-14-1	14	116	TC7C06	PM427	2.51	51	3.85	-1.79
	q105DLLS Resistance-17-1	17	39	ARS774	IPAHM82	10.89	12	20.15	-0.78
	q105DLLS Resistance-17-2	17	52	ARS774	IPAHM82	10.74	19	17.44	-4.37
	q105DLLS Resistance-18-1	18	26	GNB159	AHGS0256	2.77	48	6.96	-4.11

60D LLS Resistance 60 Days late leaf spot resistance, *75D LLS Resistance* 75 Days late leaf spot resistance, *90D LLS Resistance* 90 Days late leaf spot resistance, *105D LLS Resistance* 105 Days late leaf spot resistance, *LOD* Logarithm of the odds, *PVE* Phenotypic variation explained.



Fig. 8 : QTLs for traits related to LLS resistance and yield related traits identified on QTL map of peanut in the cross TMV 2 × GPBD 4.

in addition to the identification of potential QTLs for LLS resistance.

A common marker found to be linked in several earlier late leaf spot (LLS) disease studies is GM2301 (Khedikar *et al*., 2010; Sujay *et al*., 2012; Kolekar *et al*., 2016), which is strongly associated with genomic region/QTLs ($QTL_{R4\text{-}LLS}09$, $QTL_{R4\text{-}LLS}03$) governing resistance to late leaf spot in linkage group AhXV (B3) that explained phenotypic variance ranging from 4.0 to 17.37 per cent in the crosses TAG 24 × GPBD 4 and TG 26 × GPBD 4. The same marker GM2301 linked with LLS disease resistance at the peak stage of disease at 90DAS (q90DLLS Resistance-1-1) and at 105DAS (q105DLLS Resistance-1-1) in the linkage group LG-1, which explained phenotypic variance ranging from 2.29-3.32 per cent in the present study. Similarly, IPAHM103 (Khedikar *et al*., 2010; Sujay *et al*., 2012; Kolekar *et al*., 2016) was tightly linked with genomic region/QTLs ($QTL_{LLS}10$, $QTL_{R4\text{-}LLS}04$, $QTL_{R5\text{-}LLS}03$) governing resistance to late leaf spot disease in linkage groups LG6 (B5) and AhXV (B3) with phenotypic variance ranging from 2.6 – 63.17 per cent in the crosses TAG 24 × GPBD 4 and TG 26 × GPBD 4. The same IPAHM103 marker showed linkage with LLS disease resistance at the first peak stage of disease at 90DAS (q90DLLSResistance-1-2) and at 105DAS (q105DLLSResistance-1-2) in the linkage group LG-1, with phenotypic variance ranging from 2.05-3.01 per cent in the current experiment, results were in accordance with Sukruth *et al*. (2015). Markers GM2301 and IPAHM103 were successfully validated in two backcross (DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5) and RIL (TG 19 × GPBD 4) populations with phenotypic variance of 12.10 and 11.68 per cent, respectively (Table 3 and 4).

Marker TC9H09 (Khedikar *et al*., 2010) was one of the flanked marker to *$QTL_{LLS}03$* in linkage group LG9(B7) with phenotypic variance of 3.80-4.80 per cent in the cross TAG 24 × GPBD 4. The same marker TC9H09 linked to LLS disease resistance at 60DAS (q60DLLSResistance-10-1) in linkage group LG-10 in the current study. GM1577 (Khedikar *et al*., 2010; Sujay *et al*., 2012) was one of the flanked marker to $QTL_{R4\text{-}LLS}05$ in linkage group AhV with phenotypic variance of 10.81-15.34 per cent in the crosses TAG 24 × GPBD 4 and TG 26 × GPBD 4. The same marker GM1577 was linked to LLS disease resistance at 75 DAS (q75DLLS Resistance-4-1) in linkage group LG-4 in our experiment. LLS disease resistance QTL (qF2LS12) was associated with SSR marker GNB159 (Wang *et al*., 2013) in linkage group AhXVIII(A9) with phenotypic variance of 11.59 per cent in the cross Tifrunner × GT-C20. The same marker GNB159 was found to be linked with LLS disease resistance at 90DAS (q90DLLSResistance-18-1) and 105DAS (q105DLLSResistance-18-1), which explained phenotypic variance ranging from 6.96 to 10.47 percent that was noticed in present study. Another marker, TC7C06 (Wang *et al*., 2013) was one of the flanked marker to *qF5LS5* in linkage group LGT6 (A6) in the

Table 4 : List of SSR markers linked to LLS resistance validated with linkage groups in comparison with reported linkage groups in peanut.

Validated Marker	Linkage group (LG) in present study	Linkage group (LG) reported	References
GM2301	LG-1	AhXV (B3)	Khedikar *et al.* (2010); Sujay *et al.* (2012) and Kolekar *et al.* (2016)
IPAHM103	LG-1	LG6(B5) and AhXV(B3)	Khedikar *et al.* (2010); Sujay *et al*. (2012) and Kolekar *et al.* (2016)
TC9H09	LG-10	AhXV(B3)	Khedikar *et al.* (2010)
GM1577	LG-4	AhV	Khedikar *et al*. (2010) and Sujay *et al*. (2012)
GNB159	LG-18	AhXVIII(A9)	Wang *et al.* (2013)
TC7C06	LG-14	LGT6 (A6)	Wang *et al.* (2013)

LLS Late leaf spot disease.

cross Tifrunner × GT-C20. The same marker, TC7C06, found linked to LLS disease resistance at 90DAS (q90DLLS Resistance-14-1) and 105DAS (q105DLLS Resistance-14-1) in linkage group LG-14, which explained phenotypic variance of 3.85-5.47 per cent in the cross TMV 2 × GPBD 4 of the $F_{2:3}$ population in the present study (Table 3). From the results of the study, it suggests that for LLS disease, phenotyping at 90 (Ramakrishnan *et al*., 2020) and 105 DAS would give reliable data; also, a possible reason may be that generally disease severity will be at its peak during these stages, ensuring a uniform spread of pathogen load, ensuring reliable disease score data.

As one of the flanking markers for genomic region/ QTLs governing resistance to LLS disease, namely q60DLLS Resistance-10-1, q75DLLS Resistance-4-1, q90DLLS Resistance-14-1, q90DLLS Resistance-18-1, q90DLLS Resistance-1-1, q90DLLS Resistance-1-2, q105DLLS Resistance-1-1, q105DLLS Resistance-14-1, q105DLLS Resistance-1-2 and q105DLLS Resistance-18-1, SSR markers, namely GM 2301, TC9H09, GM1577, GNB159, TC7C06, and IPAHM 103, were validated as markers linked to LLS resistance in the study (Khedikar *et al*., 2010; Sujay *et al*., 2012; Wang *et al*., 2013; Sukruth *et al*., 2015; Kolekar *et al*., 2016). Hence, these validated markers to LLS resistance can be effectively utilized to develop LLS resistant cultivars using marker-assisted foreground and background selection of MABC (marker-assisted backcross) breeding and other MAS (marker-assisted selection) breeding programs.

However, despite being reported as linked markers to QTLs/genomic regions controlling late leaf spot disease resistance in various biparental populations in previous studies (Khedikar *et al*., 2010; Sujay *et al*., 2012; Wang *et al*., 2013; Kolekar *et al*., 2016), the SSR markers namely TC9F10, pPGPSeq18G1, TC11F12, GM1839, GM1988, GM1867, and TC4A02 did not validate in the present study (Table 3 and 4).

In the present study, despite being validated, the markers GM2301, TC9H09, GM1577, GNB159, TC7C06, and IPAHM 103 were found on linkage groups that differed from those reported in the majority of instances. Some possible explanations might be the number of markers utilized in the study, the size of the population, the sort of mapping population used in previous studies, and the LOD score used in mapping. Therefore, we could have verified these SSR markers in the same appropriate linkage groups if everything had been taken care of, perhaps with a good saturated map. Nevertheless, in the majority of the cases, these markers were successful in differentiating the resistant and susceptible progenies/ genotypes, indicating their potential to be used in a marker-assisted breeding program for LLS disease resistance in groundnut.

Conclusion

Molecular markers linked with QTLs and major genes for traits of interest are being routinely developed in several crops using materials derived from planned crosses such as F_2, RIL, DH populations, etc. Many times, the markers identified QTLs in specific populations may not be useful in other populations. Hence, validation in other mapping population/s is highly important before using these markers in MAS. Validation of the identified markers/QTLs in a set of genotype/s is one of the best methods because the results can be generalized more than the results obtained from biparental populations. Hence, it is believed that the markers that have been validated have a potential use in the MAS program for introgression in breeding to late leaf spot resistance in groundnut. The study noted the inability to validate QTLs

using the same flanking marker and linkage group. However, we could validate markers associated with resistance to late leaf spot disease. The current investigation has made a substantial contribution to the discovery of new key QTLs/markers that contribute to LLS resistance, in addition to the previously published tightly linked markers GM2301 and IPAHM 103 (Khedikar *et al*., 2010; Sujay *et al*., 2012; Kolekar *et al*., 2016). A significant portion of phenotypic diversity is also contributed by validated markers linked to LLS disease resistance, such as GM 2301, TC9H09, GM1577, GNB159, TC7C06, and IPAHM 103. There are two late leaf spot-resistant QTLs, namely q90DLLSResistance-1-1 and q105DLLSResistance-1-1, validated between markers GM2301 and IPAHM103 (Kolekar *et al*., 2016), but due to less saturation of the map, one more marker, TC1E05, is noticed between these flanking markers. We could have confirmed other QTLs with the same flanking markers and explaining a substantial phenotypic variation, if the map had been more saturated. Further work is in progress for re-confirmation of validated/identified genomic regions in recombinant inbred lines (RILs) of same cross. These markers may serve as tools for introgression/screening for LLS resistance QTLs/ genomic regions and other MAS breeding programs, thus enhancing the speed and accuracy of crop improvement programs.

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