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QTL mapping for late leaf spot and rust resistance using an improved genetic map and extensive phenotypic data on a recombinant inbred line population in peanut (*Arachis hypogaea* L.)

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Abstract The linkage map for the recombinant inbred line (RIL) mapping population derived from late leaf spot (LLS) and rust disease susceptible (TAG 24) and resistant (GPBD 4) varieties of peanut was improved by adding 139 new SSR and transposable element (TE) markers. The improved map now has 289 mapped loci with a total map distance of 1730.8 cM and average inter-marker distance of 6.0 cM across 20 linkage groups. Quantitative trait loci (QTL) analysis using improved genetic map with 289 markers and comprehensive phenotypic data for LLS and rust from 11 seasons could identify a region on linkage group AhXV (B03 linkage group of B genome) which contributed significantly towards LLS and rust resistance. Of the five QTL mapped in this region, three showed high phenotypic variance explained (PVE) for both LLS and rust, and two QTL showed high PVE for only rust. The QTL flanked by GM2009-IPAHM103 had very high PVE of 44.5 % and 53.7 %, respectively for LLS and rust response. Another genomic region on AhXII (B10 linkage group of B genome) contained a QTL flanked by GM1839-GM1009 which had a PVE of 14.1–35.2 % for LLS resistance. A new QTL with marker interval GM1989-AhTE0839 on AhV (A05 linkage group of A genome) showed a PVE of 10.2 % for rust resistance. The new markers, AhTE0498 and AhTE0928 linked to rust resistance were validated using another RIL population of TG 26 × GPBD 4. The marker AhTE0498 showed 49.3–52.3 % PVE, indicating a strong marker validation in the new population. The improved map, QTL and markers for LLS and rust resistance reported in this study will be of immense utility in peanut molecular breeding.

Keywords Peanut · Recombinant inbred lines · SSR and AhTE markers · Late leaf spot and rust resistance · Improved molecular map · QTL analysis and validation

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Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed and food crop grown worldwide. Most commonly

grown Spanish bunch type varieties are highly susceptible to foliar fungal diseases like rust (*Puccinia arachidis* Speg,), early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (LLS) (*Phaeoisariopsis personata* [(Berk. and Curt) Deighton)]. In India, LLS and rust generally occur together and cause not only a yield loss up to 70 %, but also bring down the quality of the feed and fodder produce (Dwivedi et al. 2002). Breeding for resistant varieties is a preferred means of managing the foliar diseases over chemical control considering the additional cost and biological safety. The success of breeding for foliar disease resistance is influenced by the availability and identification of resistance sources, and combining resistance with high productivity and desirable pod features. Valencia landraces and wild species of peanut possess high level of resistance to foliar diseases, but the resistance is generally linked to low productivity, late maturity, poor adaptability and undesirable pod features (Wynne et al. 1991; Singh et al. 1997). Complex inheritance pattern of foliar disease resistance (Bromfield and Bailey 1972; Tiwari et al. 1984; Paramasivam et al. 1990) and interference among these diseases make phenotypic selection less effective.

Integration of genomic resources and tools like molecular markers, QTL and marker-assisted selection (MAS) with conventional breeding approaches would enhance the precision and speed of developing peanut cultivars with resistance to LLS and rust. In an attempt to map the genomic regions governing LLS and rust resistance, several recombinant inbred line (RIL) and introgression line (IL) mapping populations were developed at UAS, Dharwad, India (Bhat et al. 2012). Among them, two RIL populations TAG 24 × GPBD 4 and TG 26 × GPBD 4 involved LLS and rust susceptible (TAG 24 and TG 26) and resistant (GPBD 4) varieties. Initially, a linkage map of 462.24 cM was constructed based on the RILs of TAG 24 × GPBD 4 using 56 mapped SSR markers (Khedikar et al. 2010). This genetic map was further improved to cover 188 marker loci by adding 132 additional SSR loci which resulted in a total map distance of 1922.4 cM (Sujay et al. 2012). The QTL analysis using the extensive (eight seasons) phenotypic data could identify two major genomic regions governing resistance to LLS and rust (Khedikar et al. 2010; Sujay et al. 2012). One QTL region present on linkage group AhXV showed a maximum of 67.98 % and 82.96 % phenotypic variance explained (PVE) towards resistance to LLS and rust, respectively. The other QTL region on AhXII showed a maximum PVE of 62.34 % towards LLS resistance. Many of these QTL were also mapped (Varshakumari 2013) using introgression line population derived from a cultivated and foliar disease susceptible variety, ICGS 76 and a synthetic amphidiploid (ISATGR 278-18) obtained from *Arachis duranensis* (ICG 8138, A genome) × *Arachis batizocoi* (ICG 13160, K genome).

The QTL region on AhXV was successfully validated using a wide range of resistant/susceptible breeding lines (Khedikar et al. 2010), Heteogeneous Inbred Family (HIF)-derived Near Isogenic Lines (NILs) (Yeri et al. 2014) and other mapping populations (Sukruth et al. 2015). QTL regions on AhXV and AhXII were introgressed to develop resistant backcross lines in the elite and popular varieties of peanut (Varshney et al. 2014a, 2014b). Since linkage mapping and QTL analysis for any trait is a long-term approach for map saturation and fine mapping, an effort was made in this study to improve the genetic map by mapping additional SSR and transposable element (*AhMITE1*)-specific markers, and to detect QTL for LLS and rust resistance using phenotypic data across 11 seasons (2004–2014). The QTL and their flanking markers identified here are of great utility in the molecular breeding for LLS and rust resistance in peanut.

Materials and methods

Genotyping of RILs

RIL population of TAG 24 × GPBD 4 was developed previously by crossing LLS and rust susceptible variety, TAG 24 (Patil et al. 1995) with a resistant variety, GPBD 4 (Gowda et al. 2002), and advancing the generations by single seed decent (SSD) (Khedikar et al. 2010; Sujay et al. 2012). Genomic DNA was isolated from the young leaves of RILs and their parents by following CTAB method with minor modifications (Cuc et al. 2008). DNA yield was quantified using Nano-Drop (UV technologies, USA). PCR for 1079 *A. hypogaea* genomic SSR (AHGS) (Shirasawa et al. 2012), 470 *A. hypogaea* EST-SSR (AHS) (Koilkonda et al. 2012) and 405 *A. hypogaea* transposable element (AhTE) markers (Shirasawa

et al. 2012a, 2012b) was carried out as described in Koilkonda et al. (2012) and Shirasawa et al. (2012a, 2012b). PCR products of AHGS and AHS markers were separated by polyacrylamide gel electrophoresis (PAGE) using Sequi-Gen (BIO RAD, Hercules, California, USA) followed by silver staining, while those of AhTE markers were separated on 10 % polyacrylamide gel. In addition, the data on 188 SSR markers were obtained from the previous studies (Khedikar et al. 2010; Sujay et al. 2012).

Phenotyping of RILs

Two hundred and sixty six RILs of TAG 24 × GPBD 4 were grown at IABT Garden of the Department of Biotechnology, UAS, Dharwad, India during the rainy season of 2011, 2013 and 2014 in randomized block design with two replications. Each replication consisted of 2 rows of 2.5 mt length with a spacing of 45 cm × 10 cm. The genotypes were subjected to field screening for rust and LLS reaction using spreader row technique (Subrahmanyam et al. 1995) in which the disease spreader plants [TMV 2 and Mutant 28-2 (Gowda et al. 2010)] were planted at regular interval of 10 rows. Disease scoring for both LLS and rust was done at 70, 80 and 90 days after sowing (DAS) according to modified 9-point scale (Subbarao et al. 1990). In addition to these data, LLS and rust scores collected (Khedikar et al. 2010; Sujay et al. 2012) during the previous eight seasons were employed.

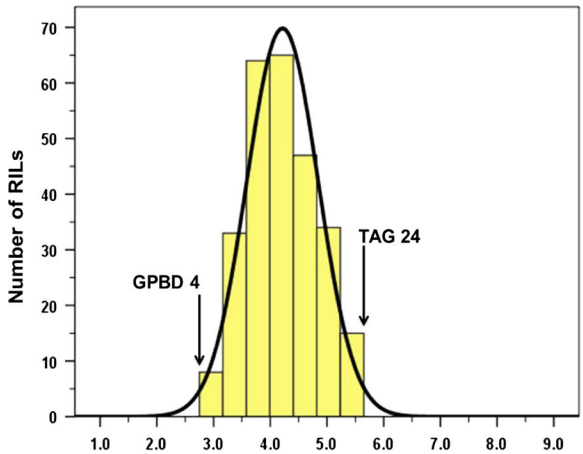
Statistical analysis

Analyses of variance (ANOVA), estimation of phenotypic and genotypic coefficients of variation (GCV and PCV), heritability (h^2_{bs}) and correlation among phenotypic traits were carried out for the pooled mean data of 11 seasons [rainy season of 2004, 2005, 2006, 2007 (early and late sown conditions), 2008, 2009, 2010, 2011, 2013 and 2014] using Windostat version 8 (developed by Indostat Service, Hyderabad, India). Markers showing 1:1 segregation ratio were identified. Linkage analysis was carried out for the markers to construct the linkage map using the Joinmap4 (Ooijen 2006). Association of the markers with LLS and rust resistance was tested by Single marker analysis (SMA) using Windows QTL Cartographer version 2.5 (Wang et al. 2007). QTL analysis was carried out for 37 traits of LLS and rust scores separately using the method of Composite Interval Mapping (CIM) with 1000 permutations using Windows QTL Cartographer version 2.5 (Wang et al. 2007). Significant QTL were identified by comparing the LOD scores with the threshold LOD. The new AhTE markers linked to rust resistance were validated using a new mapping population consisting of 148 RILs derived from TG 26 × GPBD 4. The phenotypic data on LLS and rust resistance across a maximum of 10 seasons were used for SMA.

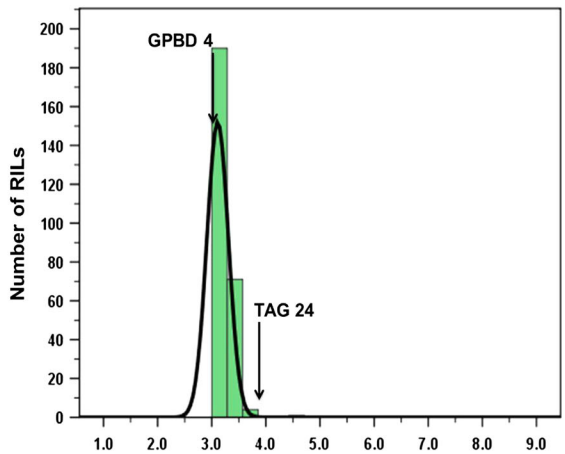
Results and discussion

Two hundred and sixty six RILs of TAG 24 × GPBD 4 were evaluated for reaction to LLS and rust during the rainy seasons of 2011, 2013 and 2014 under disease epiphytotic condition. In addition, the disease score collected during the previous eight seasons [rainy season of 2004, 2005, 2006, 2007 (early and late sown conditions), 2008, 2009 and 2010] were employed. ANOVA showed significant genotypic differences for resistance to LLS at 70, 80 and 90 days after sowing (DAS). TAG 24 showed scores of 9.0 and 7.0 for LLS and rust reaction, while GPBD 4 recorded 3.1 for LLS and 3.3 for rust response across the seasons. However, significant genotypic differences were observed only at 80 and 90 DAS for rust resistance. High phenotypic and genotypic coefficient variations were observed for all the traits except rust reaction at 80 DAS. Frequency distribution of the RILs followed a normal distribution for all the three stages of LLS development (Fig. 1). However, a bimodal distribution was observed for rust at 80 and 90 DAS as reported in the previous studies (Khedikar et al. 2010; Mondal et al. 2012; Sujay et al. 2012). The association analyses between the three stages across 11 seasons showed highly significant and positive correlation for LLS ($r = 0.747–0.902$; $P < 0.01$) and rust ($r = 0.419–0.955$; $P < 0.01$). However, LLS and rust showed significantly negative correlation ($r = -0.289$; $P < 0.01$), especially at later stages.

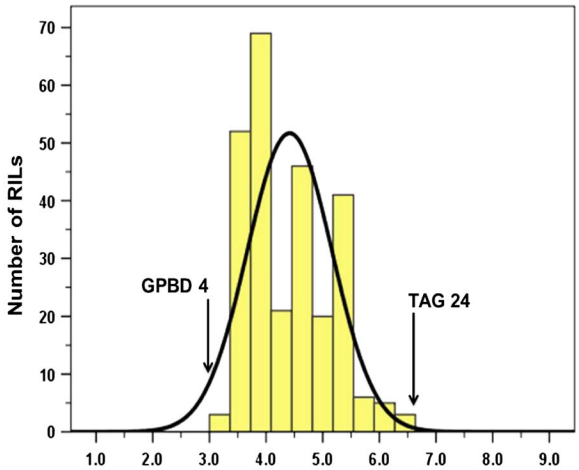
TAG 24 and GPBD 4 were screened with 1954 markers comprising of *A. hypogaea* genomic SSR (AHGS) (Shirasawa et al. 2012), *A. hypogaea* EST-SSR (AHS) (Koilkonda et al. 2012) and *A. hypogaea* transposable element (AhTE) markers (Shirasawa et al. 2012a, 2012b). Of them, 139 (7.1 %) were



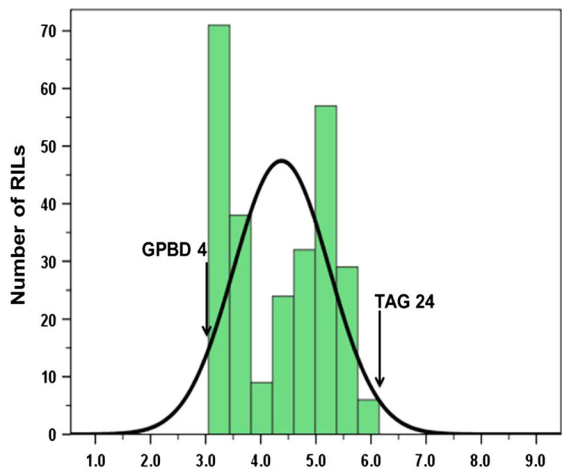
(A) LLS_70



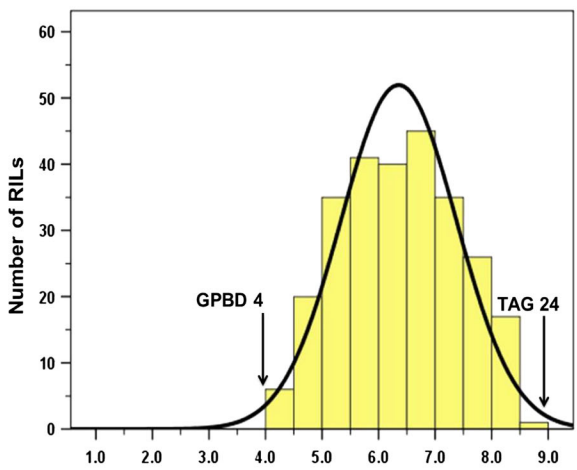
(D) Rust_70



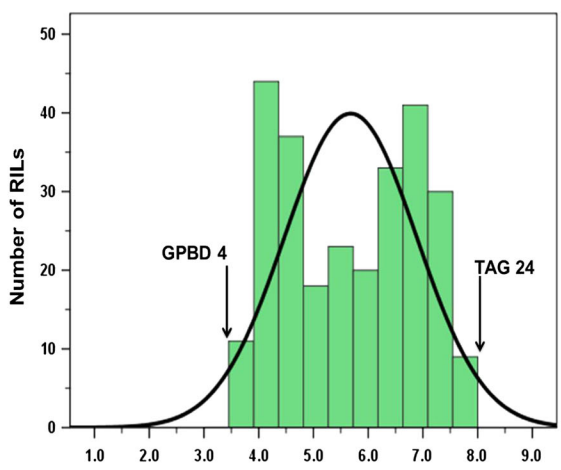
(B) LLS_80



(E) Rust_80



(C) LLS 90



(F) Rust 90

◀**Fig. 1** Frequency distribution of RILs of TAG 24 × GPBD 4 based on LLS and rust scores. LLS and rust scores at 70, 80 and 90 days after sowing across 11 seasons were pooled, and the means were used

found to be polymorphic between the two parents. Compared to SSR markers [28 out of 1079 (2.6 %)], AhTE markers [111 out of 405 (27.4 %)] showed a higher level of polymorphism. Previous studies have shown polymorphism of 22 % for transposon markers, which was higher than that of the SSR markers in peanut (Koilkonda et al. 2012; Pandey et al. 2012). Transposon markers, like SSR markers, represent potent, co-dominant, and PCR-based markers (Shirasawa et al. 2012). A clear advantage with transposon markers used in this study is that their PCR products, which differ by 205 bp (equal to the size of *AhMITE1*), can easily be resolved on 2.5 % agarose gel as well.

Two hundred and sixty six RILs were genotyped with 139 polymorphic markers. In addition, the genotypic data for 188 SSR markers (Khedikar et al. 2010; Sujay et al. 2012) were also used for analysis. Of the total 327 markers, 232 showed the expected (1:1) segregation. However, all the 327 markers were used for linkage map construction and an improved linkage map covering 1730.8 cM with an average inter-marker distance of 6.0 cM was constructed. The map consisted of 289 mapped marker loci in 20 linkage groups when compared to the map reported by Sujay et al. (2012) which carried 188 markers. The linkage groups (Supplementary Table 1) were named as described by Sujay et al. (2012) and Shirasawa et al. (2013). The size of the linkage groups ranged from ≤26.7 cM [AhXIX (A08 linkage group of A genome)] to 155.9 cM [AhIX (B09 linkage group of B genome)]. The genomic region on AhXV (B03) with high PVE was found to be mapped with four new AhTE markers (AhTE0498, AhTE0928, AhTE621 and AhTE0200). Also, the order of the markers in that region was different than that obtained earlier (Sujay et al. 2012; Varshakumari 2013). However, the current order of the markers was generated with very high LOD scores. A difference in marker order among genetic maps is not unexpected, as genetic mapping only gives an indication of the relative position of the markers to each other (Sourdille et al. 2003).

Association of markers with the phenotypic variation in 37 traits (LLS at 70 DAS from 8 seasons, LLS at 80 DAS from 2 seasons and LLS at 90 DAS from 9 seasons, rust at 70 DAS from 2 seasons, rust at 80 and 90 DAS from 8 seasons each) was worked out using single marker analysis (SMA) in QTL Cartographer. Overall, 22–40 markers showed significant association with LLS and rust resistance. AhTE0446 was the major marker with highest PVE values (16.5 %, 7.7 % and 12.1 %, respectively) for LLS resistance at all the three stages (70, 80 and 90 DAS) (Table 1). However, it was observed that AhTE0446 was an unmapped marker. IPAHM103 followed by GM2301, GM2079, GM1536, GM2009 and AhTE0498 showed high PVE for rust response at all the three stages. AhTE0928 recorded high PVE only for rust reaction at 80 and 90 DAS. IPAHM103 exhibited PVE of 9.0 %, 50.7 % and 49.0 % for rust resistance at 70, 80 and 90 DAS, respectively.

QTL analysis was carried out for 37 phenotypic traits using Composite Interval Mapping (CIM) with 1000 permutations in QTL Cartographer to identify the genomic regions linked to quantitative variation at LLS and rust resistance. Remarkable QTL peaks were observed for LLS at 70 DAS during 2008, LLS at 90 DAS during 2008 and 2009, rust at 80 DAS during 2008 and 2009 and rust at 90 DAS during 2008 and 2009. Four and six major QTL were detected for LLS and rust resistance, respectively with LOD scores exceeding the threshold level (Table 2). Of the four QTL for LLS resistance, three were mapped on a single genomic region (17.8–31.7 cM) on linkage group AhXV, and one QTL was present in a 24.2 cM region of linkage group AhXII (B10 linkage group of B genome) (Fig. 2). The QTL flanked by GM2009:IPAHM103 on AhXV recorded the highest PVE of 44.5 % followed by the QTL flanked by GM1839:GM1009 on AhXII with a maximum PVE of 35.2 %. Additive effect for alleles at LLS resistance-linked QTL ranged from −1.3 to 1.1. Interestingly, TAG 24 contributed the LLS resistance allele at three QTL regions on AhXV. But the LLS resistance allele at QTL on AhXII was contributed by GPBD 4.

For rust resistance, five of the total six QTL were mapped on a single genomic region (0–43.3 cM) on AhXV. The QTL flanked by AhTE0498-GM2009 recorded the highest PVE of 70.4 % followed by the QTL flanked by GM2079-AhTE0928 with a maximum PVE of 64.6 %. It was observed that the QTL flanked by GM2009-IPAHM103 on AhXV showed a high PVE of 53.7 %. A QTL flanked by GM1989-AhTE0839 on AhV (A05 linkage group of A genome) had a PVE of 10.2 %. Additive effect for alleles at rust

Table 1 Single marker analysis for top 10 markers among the RILs of TAG 24 × GPBD 4

Sl. no.	Marker	LLS_70		LLS_80		LLS_90	
		F	PVE (%)	F	PVE (%)	F	PVE (%)
1	AhTE0446	54.0 (0.000)**	16.5	23.2 (0.000)**	7.7	41.5 (0.000)**	12.1
2	TC2G05	9.2 (0.003)**	3.8	7.3 (0.007)**	2.8	7.2 (0.008)**	3.4
3	GM1577	10.0 (0.002)**	3.7	–	–	–	–
4	GM1076	5.5 (0.020)*	3.7	–	–	–	–
5	Lec1	12.1 (0.001)**	3.4	7.6 (0.006)**	2.2	11.1 (0.001)**	3.2
6	PM436	11.4 (0.001)**	3.4	–	–	10.5 (0.001)**	3.0
7	AhTE0839	8.9 (0.003)**	3.3	–	–	10.8 (0.001)**	3.9
8	Seq 17C09	8.3 (0.004)**	3.2	5.6 (0.019)*	2.1	–	–
9	Seq 7G02	8.1 (0.005)**	3.0	–	–	–	–
10	gi1107	12.1 (0.001)**	3.0	7.5 (0.006)**	2.1	11.1 (0.001)**	3.2
11	Seq 17E3	–	–	4.7 (0.032)*	2.6	–	–
12	TC3H02	–	–	6.1 (0.013)*	2.2	–	–
13	TC9F10	–	–	4.7 (0.032)*	2.0	–	–
14	TC6H03	–	–	4.7 (0.032)*	2.0	–	–
15	PM3	–	–	5.3 (0.022)*	2.0	–	–
16	IPAHM103	–	–	–	–	9.5 (0.002)**	3.2
17	AhTE0498	–	–	–	–	10.1 (0.002)**	3.2
18	GM2009	–	–	–	–	11.8 (0.001)**	3.0
19	GM2301	–	–	–	–	10.1 (0.002)**	2.8
		Rust_70		Rust_80		Rust_90	
1	IPAHM103	26.7 (0.000)**	8.97	287.2 (0.000)**	50.71	273.3 (0.000)**	49.0
2	GM2301	28.3 (0.000)**	7.53	301.2 (0.000)**	40.81	306.5 (0.000)**	42.6
3	GM2079	24.5 (0.000)**	6.33	200.4 (0.000)**	30.45	207.1 (0.000)**	32.1
4	Seq 13E06	11.2 (0.001)**	4.08				
5	GM1536	16.6 (0.000)**	3.48	206.8 (0.000)**	29.93	206.0 (0.000)**	31.0
6	GM2009	16.2 (0.000)**	3.39	166.2 (0.000)**	24.96	173.3 (0.000)**	26.7
7	AhTE0164	7.0 (0.009)**	3.27	–	–	–	–
8	Seq 19D06	7.4 (0.007)**	3.17	–	–	–	–
9	Seq 18E07	6.9 (0.009)**	3.15	–	–	–	–
10	AHGS2254	8.55 (0.004)**	2.89	–	–	–	–
11	AhTE0498	–	–	106.5 (0.000)**	21.02	105.4 (0.000)**	20.9
12	AhTE0621	–	–	48.3 (0.000)**	15.46	43.7 (0.000)**	14.5
13	GM1954	–	–	45.9 (0.000)**	10.39	43.2 (0.000)**	9.7
14	AhTE0928	–	–	28.9 (0.000)**	9.85	25.4 (0.000)**	8.8
15	AhTE0200	–	–	30.6 (0.000)**	8.48	27.7 (0.000)**	8.1

LLS_70: late leaf spot (LLS) score at 70 days after sowing (DAS); LLS_80: LLS score at 80 DAS; LLS_90: LLS score at 90 DAS; Rust_70: rust score at 70 DAS; Rust_80: rust score at 80 DAS; Rust_90: rust score at 90 DAS

*, ** Significance at 5 % and 1 %, respectively

resistance-linked QTL ranged from 0.1 to 2.1. GPBD 4 contributed rust resistance alleles at all the QTL regions.

A detailed comparison of the current QTL map with the previous map (Sujay et al. 2012) obtained using the same RIL population of TAG 24 × GPBD 4 revealed

Table 2 Details of QTL detected for late leaf spot and rust resistance among the RILs of TAG 24 × GPBD 4

QTL in the marker interval	Linkage group	cMs	Trait, stage and season	LOD	Additive effect	Phenotypic variance explained (%)
			LLS			
GM2009-IPAHM103	AhXV (B03)	17.8–26.8	LLS at 90 DAS of 2008	22.2	−1.3	44.5
IPAHM103-GM2301	AhXV (B03)	26.8–29.3	LLS at 70 DAS of 2008	12.6	−0.3	17.5
GM2301-GM1536	AhXV (B03)	29.3–31.7	LLS at 90 DAS of 2009	8.0	−0.5	17.0
GM1839-GM1009	AhXII (B10)	101.5–125.7	LLS at 70 DAS of 2004, 2005, 2006, 2008, 2009, LLS at 90 DAS of 2005, 2006, 2009	6.5–14.9	0.2–1.1	14.1–35.2
			Rust			
AhTE0498-GM2009	AhXV (B03)	0.0–17.8	Rust at 80 DAS of 2009, Rust at 90 DAS of 2007E	21.4–30.9	0.6–0.9	62.7–70.4
GM2079-AhTE0928	AhXV (B03)	34.0–40.3	Rust at 80 DAS of 2006, Rust at 90 DAS of 2006, 2007E, 2008, 2009	14.3–56.0	0.6–2.1	19.42–64.6
GM2009-IPAHM103	AhXV (B03)	17.8–26.8	Rust at 80 DAS of 2006, 2007E, 2007L, 2008, Rust at 90 DAS of 2006, 2007E, 2007L	31.3–54.5	0.5–2.1	30.0–53.7
IPAHM103-GM2301	AhXV (B03)	26.8–29.3	Rust at 80, 90 DAS of 2007L	40.9–42.3	1.5–1.6	34.7–35.9
GM2301-GM1536	AhXV (B03)	29.3–31.7	Rust at 80 DAS of 2006	35.3	1.1	34.0
GM1989-AhTE0839	AhV (A05)	65.8–80.5	Rust at 70 DAS of 2014	2.3	0.1	10.2

2007E: early condition of 2007 and 2007L: late condition of 2007

a few new outcomes. A region of 20.6 cM on AhXV of the previous map carried three QTL (GM2009-GM1536, GM1536-GM2301 and IPAHM103-GM1954) governing both LLS and rust resistance. In the present study, this region was reduced to 13.9 cM, which was further resolved to identify five QTL; two (AhTE0498-GM2009 and GM2079-AhTE0928) contributing towards only rust resistance and three (GM2009-IPAHM103, IPAHM103-GM2301 and GM2301-GM1536) contributing for both LLS and rust resistance. In addition, this study identified a new QTL (GM1989-AhTE0839) for rust resistance on AhV. A 29.3 cM region on AhXII carried a major QTL (GM1573/GM1009-pPGPseq 8D09) for LLS resistance in the previous map (Sujay et al. 2012). This QTL was detected only among the RILs of TAG 24 × GPBD 4, but not among the RILs of TG 26 × GPBD 4. In the present study, the length of this region was reduced to 24.2 cM by the markers (GM1839-GM1009) flanking the major QTL for LLS resistance.

The present map was also compared with the map constructed using introgression line (IL) mapping population differing for LLS and rust resistance (Varshakumari 2013). In this map, the region on AhXV was 49.5 cM, and it carried 3–4 major QTL for LLS and rust resistance. The one mapped between GM2301 and GM1536 contributing to rust resistance was common between the current map and the previous map (Varshakumari 2013). Thus, the results of QTL analysis in this study not only confirmed the previously reported QTL but also identified new QTL for LLS and rust resistance.

In general, rust resistance-linked markers showed strong co-segregation with the phenotype, while LLS resistance-linked markers displayed weak co-segregation with the phenotype in our previous validation studies (Yeri et al. 2014; Sukruth et al. 2015; Yeri 2015). The new LLS resistance-linked marker, GM1839 is being validated in a new mapping population of TG 26 × GPBD 4 as well as several backcross and introgression lines. However, considering the advantages of AhTE markers over SSR markers in genotyping, the two rust resistance-linked AhTE markers, AhTE0498 and AhTE0928 (with very high PVE %) were validated using a new RIL mapping population (TG 26 × GPBD 4). The parents TG 26 and GPBD 4 showed polymorphism for both the markers i.e., AhTE0498 and AhTE0928. One hundred and forty eight RILs of TG 26 × GPBD 4

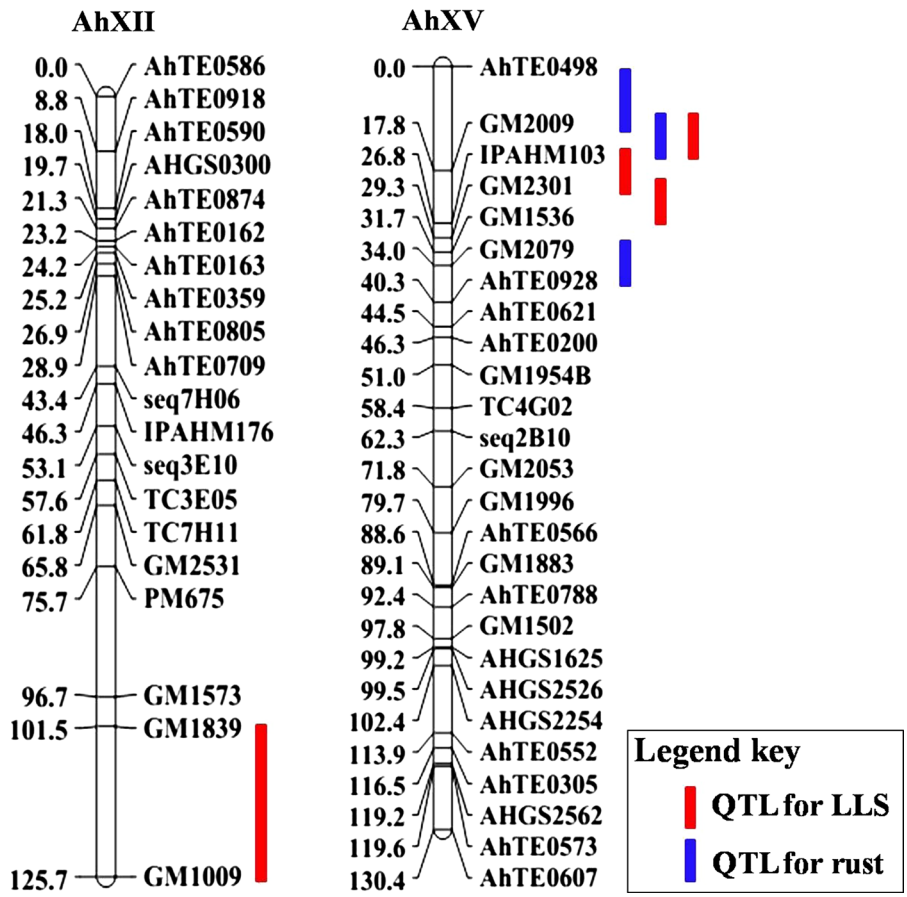


Fig. 2 Linkage groups AhXII (B10) and AhXV (B03) with QTL detected for late leaf spot and rust resistance

were genotyped with AhTE0498 and AhTE0928. Using the phenotypic data of 10 seasons, marker-trait association was studied using SMA. The RILs differed significantly for LLS at 70 and 90 DAS and for rust resistance at 80 and 90 DAS. These traits also showed high variability. LLS ($r = 0.578–0.885$; $P < 0.01$) and rust ($r = 0.304–0.936$; $P < 0.01$) resistance at different stages were significantly and positively correlated. However, LLS and rust resistance were negatively correlated. AhTE0498 and AhTE0928 showed significant association (Table 3) with rust scores at 80 and 90 DAS, indicating a strong marker validation. However, only AhTE0498 showed significant association with rust score at 70 DAS. The R^2 values were higher for AhTE0498 when compared to those of AhTE0928. Highest R^2 of 52.3 % at 90 DAS followed by 49.4 % at 80 DAS was recorded for AhTE0498. Mondal et al. (2013) also observed a strong linkage between AhTE0498 and rust resistance in another RIL population of VG 9514 and TAG 24, indicating its usefulness in marker assisted selection (MAS).

Zhou et al. (2014) reported the first high density SNP-based linkage map using 1621 SNP and 64 SSR marker loci from a RIL population derived from parents differing for LLS resistance. In future, SNP markers and genotyping by sequencing (GBS) can be used to enrich the map reported in this study for detecting QTL for LLS and rust resistance. Further, the fact that AhTE markers were developed for *AhMITE1* insertion sites in peanut genome (Gowda et al. 2010; Shirasawa et al. 2012a, 2012b), and *AhMITE1* has transposition preference for genic regions (Wessler 1998; Zhang et al. 2000; Wessler 2001), gives a hint that the QTL region can be dissected with ease for gene(s) governing LLS and rust resistance. Currently, these LLS and rust resistance-linked markers are being employed for marker assisted backcrossing (MABC) in two elite, but disease susceptible varieties (JL 24 and TMV 2) of peanut.

Table 3 Single marker analysis for AhTE0498 and AhTE0928 among the RILs of TG 26 × GPBD 4

Sl. no.	Marker	Rust_70		Rust_80		Rust_90	
		F	PVE (%)	F	PVE (%)	F	PVE (%)
1	AhTE0498	12.1 (0.000)**	7.8	140.5 (0.000)**	49.4	158.1 (0.000)**	52.3
2	AhTE0928	0.8 (0.360)	0.6	19.0 (0.000)**	11.7	18.2 (0.000)**	11.2

PVE: phenotypic variance explained; Rust_70: rust score at 70 DAS; Rust_80: rust score at 80 DAS, Rust_90; rust score at 90 DAS

*, ** Significance at 5 % and 1 %, respectively

Overall, such an improved map and detailed QTL analysis detecting new markers would increase the level of genomic resources for use in peanut breeding to improve LLS and rust resistance.

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