A QTL study on late leaf spot and rust revealed a major QTL and candidate marker for rust resistance to deploy in molecular breeding of groundnut (*Arachis hypogaea* L.)

Yogendra P Khedikar[1,2], M.V.C. Gowda[1], Kusuma V Patgar[1], Hari D Upadhyaya[2], Rajeev K Varshney[2,3,*]

[1]University of Agricultural Sciences (UAS), Dharwad-580 005, Karnataka, India;
[2]International Crops Research Institute for the Semi – Arid Tropics ICRISAT), Patancheru-502 324, A.P., India;
[3]Genomics towards Gene Discovery Sub Programme, Generation Challenge Programme (GCP), c/o CIMMYT, Mexico
*Address for correspondence: r.k.varshney@cgiar.org

Abstract

Late Leaf Spot (LLS) and rust are the major foliar diseases of groundnut that often occur together leading to 50-70% yield loss in the crop. A RIL population (TAG 24 × GPBD 4) was phenotyped for late leaf spot (LLS) for three seasons and rust for five seasons under artificial disease epiphytotics and genotyped with 67 SSR markers. A partial linkage map with 56 SSR markers on 14 linkage groups with 364.40 cM genome coverage was prepared. Composite interval mapping (CIM) showed 12 QTLs for LLS (1.2 to 5.6%) in three different environments. In case of rust, 14 QTLs were detected in five different environments with phenotypic variation ranged upto 54.4%. Furthermore, a major QTL associated with rust was identified by both CIM and single marker analysis (SMA) that contributed 17.6 to 54.4% phenotypic variation. One marker linked with this QTL was validated using a wide range of resistant / susceptible breeding lines as well as progeny lines of another mapping population (TG 26 × GPBD 4). This study provides the first set of molecular tools to the groundnut community to undertake molecular breeding for rust resistance.

Trait phenotyping

- Phenotyping of mapping population along with the parental genotypes for LLS was carried out in three environments *viz*., Rainy 2004 (EL-I), 2005 (EL-II) and 2006 (EL-III) at Dharwad, India.
- A total of 268 RILs derived from TAG 24 (S) and GPBD 4 (R) parents were used
- Rust was phenotyped in five environments *viz*., Rainy 2004 (ER-I), Rainy 2005 (ER-II), Post rainy 2007 (ER-III), Rainy 2007 (ER-IV) and Rainy 2008 (ER-V).
- Artificial disease epiphytotics were created in separate screening experiments for LLS and rust using "spreader row technique"(Fig. 1).
- Disease scoring was done at 70 days (LLS-I) and 90 days (LLS-II) for LLS.
- Rust was scored at 70 days (LR-I), 80 days (LR-II) and 90 days (LR-III), 105 days (LR-IV), 113 days (LR-V) and 120 days (LR-VI) in different seasons by using modified 9 point scale.
- Components of resistance to rust *viz*., incubation period (IP), latent period (LP) and Infection Type (IT) were also studied.

Fig.1: RILs segregating for a) rust and b) LLS

Marker genotyping

- Initially the parents, TAG 24 and GPBD 4 were screened for polymorphism by using 1089 available SSR markers from different sources.
- Of these, 67 markers (6.15%) were found polymorphic which were then employed for genotyping the mapping population.

Construction of genetic linkage map

- Linkage analysis was performed using MAPMAKER/ EXP V 3.0.
- A total of 67 polymorphic markers were used to construct linkage map.
- 56 markers were mapped on 14 LGs spanning 364.40 cM (Fig. 2).
- Average marker distance in the map is 8.7cM.
- Number of markers mapped per linkage group ranged from two (LG 3, LG 4, LG 13 and LG 14) to ten (LG 8).
- Linkage groups were ranged from 5.50 cM (LG 9) to 94.00 cM (LG 6).

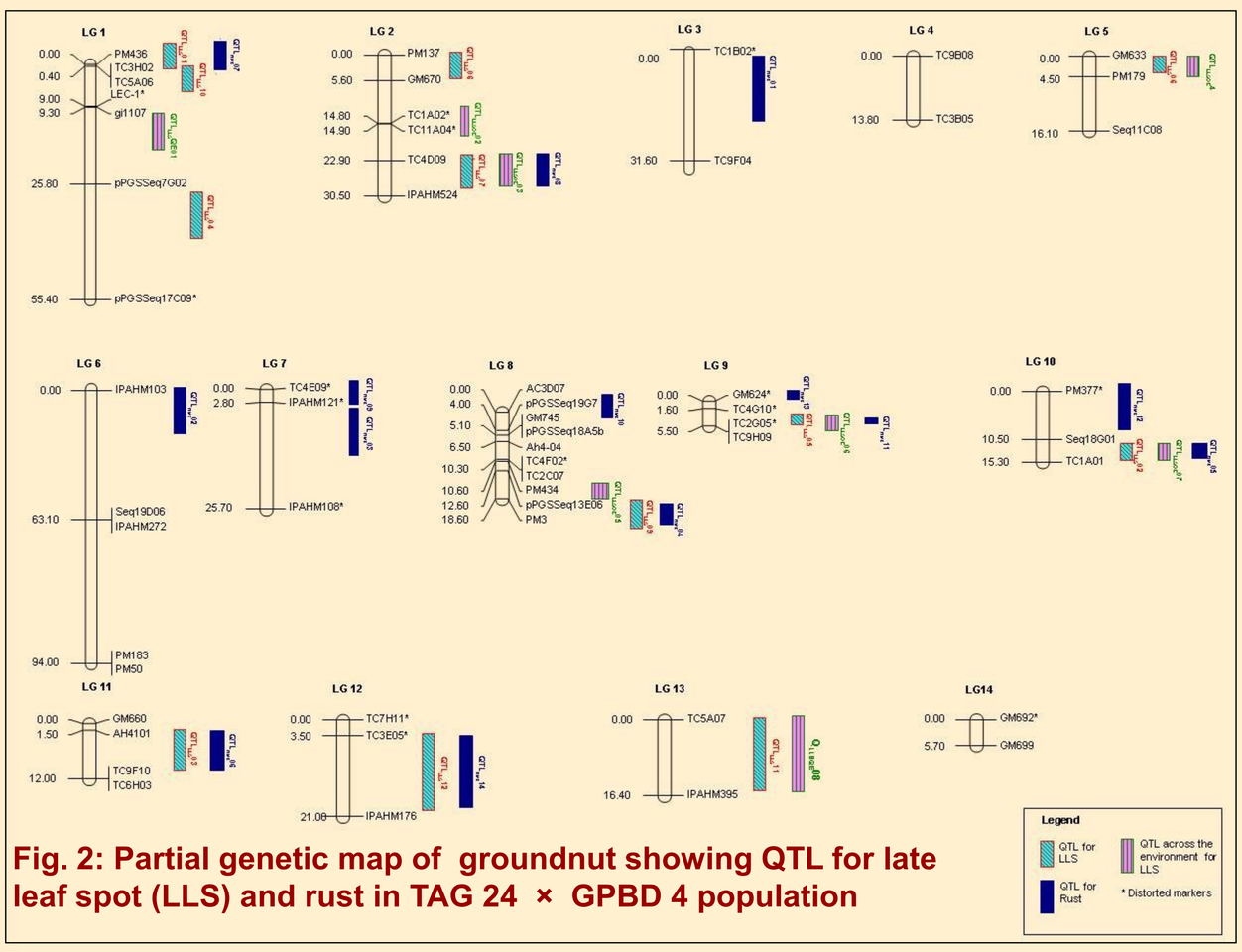

Fig. 2: Partial genetic map of groundnut showing QTL for late leaf spot (LLS) and rust in TAG 24 × GPBD 4 population

QTLs for LLS

- Positive correlation between different stages of LLS (*r* = 0.37 to 0.87) and rust (*r* = 0.37 to 0.98) and independent nature of inheritance between both diseases was observed.
- High to very high heritability (40.87 to 82.81%) estimates in LLS and rust (34.0 to 89.0 %) was observed.
- QTL analysis for LLS revealed 12 QTLs (1.2 to 5.6%) and 8 QTLs (0.7 to 4.3%) across seasons (Table 1).

Table 1: QTLs associated with LLS in TAG 24 × GPBD 4 population in different stages and seasons

QTL	Marker Interval	LG	Position (cM)	LOD	Phenotypic variation (R^2_P, %)	Additive effect
$QTL_{LLS}01$	PM436-TC3H02[a]	1	0.0	2.73	3.3	-0.149
$QTL_{LLS}02$	Seq18G01-TC1A01[ab]	10	14.0	3.06 – 5.22	1.5–1.7	-0.116 – -0.121
$QTL_{LLS}03$	Ah4-101-TC9F10[ab]	11	10.0 –12.0	2.68 – 2.71	3.8–4.0	-0.159 – -0.163
$QTL_{LLS}04$	Seq7G02-Seq17C09[b]	1	36.0	3.67	3.7	-0.197
$QTL_{LLS}05$	TC4G10-TC2G05[bde]	9	4.0	2.86 – 5.27	1.8–3.5	-0.154 – -0.158
$QTL_{LLS}06$	PM137-GM670[c]	2	0.0	3.33	1.2	0.151
$QTL_{LLS}07$	TC4D09-IPAHM524[cd]	2	30.0	3.75 – 4.20	3.7 – 4.3	-0.264 – -0.292
$QTL_{LLS}08$	GM633-PM179[cd]	5	2.0	4.19 – 4.26	3.0–5.6	-0.213 – -0.273
$QTL_{LLS}09$	Seq13E06-PM3[c]	8	14.0	2.77	4.00	-0.283
$QTL_{LLS}10$	TC3H02-Lec1[d]	1	6.0	2.75	2.9	-0.261
$QTL_{LLS}11$	TC5A07-IPAHM395[d]	13	0.0	4.59	2.4	-0.181
$QTL_{LLS}12$	TC3E05-IPAHM176[e]	12	20.0	2.54	2.7	-0.178

[a] LLS-I EL-I; [b] LLS-II EL-I; [c] LLS-I EL-II; [d] LLS-II EL-II; [e] LLS-II EL-III

QTLs for Rust

- In the case of rust, 14 QTLs (0.1 to 54.4%) were detected (Table 2).
- A SSR locus (IPAHM103) amplified by IPAHM marker (developed by Cuc et al. 2008) was found to be promising marker for rust in groundnut.

Table 2: QTLs associated with rust in TAG 24 × GPBD 4 population in different stages and seasons

QTL	Marker Interval	LG	Position (cM)	LOD	Phenotypic variation (R^2_P, %)	Additive effect
$QTL_{rust}01$	TC1B02-TC9F04[acefghijkl]	3	0.0-24.0	3.03-4.06	0.3-5.9	0.094 – 0.360
$QTL_{rust}02$	IPAHM103-Seq19D06[acdefghijklmn]	6	0.0-10.0	3.94-45.49	17.6-54.4	-0.179 – 2.27
$QTL_{rust}03$	IPAHM121-IPAHM108[af]	7	26.0-28.0	2.60-2.77	0.1-2.0	-0.015– - 0.210
$QTL_{rust}04$	Seq13E06-PM3[ab]	8	14.0-18.0	3.18-3.34	0.8-0.1	0.057 – 0.142
$QTL_{rust}05$	Seq18G01-TC1A01[cghk]	10	12.0-14.0	2.83-4.35	0.1-1.1	0.048 – -0.375
$QTL_{rust}06$	Ah4-101-GM660[a]	11	12.0	2.64	2.3	0.205
$QTL_{rust}07$	PM436-TC3H02[bck]	1	0.0-8.0	2.73-2.90	0.2-5.4	0.065 – 0.291
$QTL_{rust}08$	TC4D09-IPAHM524[bl]	2	30.0	3.11-3.29	0.1-1.7	-0.057 – 0.155
$QTL_{rust}09$	TC4E09-IPAHM121[bc]	7	2.0	3.33-3.94	0.1-0.7	-0.039 – -0.097
$QTL_{rust}10$	AC3D07-Seq19G07[b]	8	2.0	4.83	2.0	-0.253
$QTL_{rust}11$	TC4G10-TC2G05[fg]	9	2.0	2.68-4.88	1.5-6.6	-0.087 – -0164
$QTL_{rust}12$	PM377-Seq18G01[f k]	10	2.0-10.0	3.19-4.44	1.2-1.7	0.079 – 0.379
$QTL_{rust}13$	GM624-TC4G10[hk]	9	0.0	3.42-3.47	0.8-6.5	-0.129 – 0.206
$QTL_{rust}14$	TC3E05-IPAHM103[l]	12	4.00	2.61	0.6	-0.109

[a] LR-III ER-I; [b] LR-I ER-II; [c] LR-III ER-II; [d] LR-II ER-IV EI; [e] LR-III ER-IV EI; [f] LR-I ER-IV EII; [g]LR-II ER-IV EII; [h] LR-III ER-IV EII; [i] LR-IV ER-III; [j] LR-V ER-III; [k] LR-VI ER-III; [l] IP ER-V; [m] LP ER-V; [n] IT ER-V

Validation of the putative marker – IPAHM 103

- In order to confirm association of IPAHM 103 with rust , Single Marker Analysis (SMA) and Composite Interval Mapping (CIM) was conducted; these analysis showed the phenotypic variation upto 54% contributed by the QTL associated with IPAHM 103.
- For marker validation, a total of 157 elite resistant and susceptible lines and a new mapping population (TG 26 x GPBD 4) segregating for rust were used (Table 3).
- A chi-square test of independence revealed highly significant differences at 0.01 level between resistant and susceptible for this marker.
- Phenotypic variation was 17.6 to 54.4% in TAG 24 × GPBD 4 and 24.1 to 51.96% in TG 26 × GPBD 4.

Table 3: Estimates of phenotypic variation for IPAHM 103 using SMA and CIM analysis in TAG 24 × GPBD 4 and TG 26 GPBD 4 mapping populations

Environment	Stages of Scoring	TAG 24 × GPBD 4		TG 26 × GPBD 4	
		SMA (R^2_P, %)	CIM (R^2_P, %)	SMA (R^2_P, %)	CIM (R^2_P, %)
ER-I	LR-III	19.11	17.6	-	-
ER-II	LR-I	28.07	29.4	32.79	32.3
	LR-III	28.35	29.7	27.98	28.9
ER-III	LR-I	-	-	33.01	32.7
	LR-III	-	-	37.49	36.7
	LR-IV	39.46	39.9	-	-
	LR-V	39.45	38.8	-	-
	LR-VI	34.04	33.6	-	-
ER-IV EI	LR-I	-	-	24.86	24.1
	LR-II	53.95	51.9		
	LR-III	54.42	54.4	38.5	35.8
	LR-IV	-	-	36.86	35.1
ER-IV EII	LR-I	36.59	37.5	49.39	46.1
	LR-II	40.58	40.0	-	-
	LR-III	33.80	34.8	51.96	48.9
ER-V	IP	7.91	6.7	-	-
	LP	34.88	34.2	-	-
	IT	48.91	46.6	-	-

*EI – Experiment 1; EII – Experiment 2

Acknowledgements

This work was supported by grants from the Department of Biotechnology (DBT) and National Fund of Indian Council of Agricultural Research (ICAR), Government of India and Generation Challenge Programme (GCP).