



Research Paper

MARKER TRAIT ASSOCIATIONS AND PHENOTYPIC VARIATIONS FOR LATE LEAF SPOT AND RUST DISEASES IN ADVANCED BACKCROSS POPULATIONS IN GROUNDNUT (*Arachis hypogaea* L.)

Varsha Kumari¹, M.V.C.Gowda¹ and Vinod Tasiwal²

¹Department of Genetics and Plant Breeding,

²Department of Plant Pathology

University of Agricultural Sciences, Dharwad-580 005, Karnataka,
India.

Abstract

Late leaf spot (LLS) and rust are the two important foliar fungal diseases of groundnut (*Arachis hypogaea* L.) which cause significant economic losses globally. To introduce disease resistance from wild species into cultivated groundnut, synthetic amphidiploids, ISATGR 278-18 and ISATGR 5 were crossed to cultivated variety, DH 86 to develop BC₂F₄ introgression line population DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5 segregating for rust and LLS. Phenotypic data showed significant variation for the genotype, environment and genotype × environment interaction and high heritability for resistance to both the diseases. Single marker analysis (SMA) analysis in DH 86 × ISATGR 278-18 introgression population revealed a total of 21 markers associated with LLS and 25 markers for rust across seasons and the phenotypic contribution ranged from (4.067 to 76.571) and (5.056 to 68.337) per cent respectively. For DH 86 × ISATGR 5 introgression population, a total of 8 common markers were associated with LLS and rust across the seasons with phenotypic variations (4.249 to 63.730%). Molecular markers associated with the resistance to LLS and rust need to be validated before deployed for molecular breeding for improving disease resistance.

Key words: Groundnut, Synthetic amphidiploids, Single marker analysis (SMA), Rust, Late Leaf Spot.

INTRODUCTION

Peanut or groundnut (*Arachis hypogaea* L.) is the fourth-largest oilseed crop in the world and is cultivated in more than 100 countries, with the annual production of 38 million tonnes [1]. The largest producers of peanut are China and India, followed by the USA. Groundnut seeds contain 40-60% oil, 20-40% protein and 10-20% carbohydrate. Peanut has high nutritional value, possessing vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium [2]. Peanut is

mainly used for direct consumption, in the confectionary industry, for vegetable oil in cooking and also as a source for protein feed in the animal industry. These multiple uses of peanut make it an excellent cash crop for domestic as well as international trade. But the yield level of groundnut is severely affected by foliar diseases late leaf spot (*Phaeoisariopsis personata* [(Berk. and Curt.) Deighton] and rust (*Puccinia arachidis* Speng.). They often occur together and cause yield loss up to 50-70 per cent in groundnut [3]. Besides, they also affect the productivity and the quality of the seeds and fodder [4, 5]. Though, chemical control is possible, development of resistant cultivars is considered to be the best strategy to surmount the additional cost of production and hazardous effect of fungicides on the environment. Conventional resistance breeding alone would not give the expected results. Use of synthetic amphidiploid with desirable traits would enable breeders to effectively introgress the genes of some important traits in cultivated germplasm to develop introgression lines [6]. Development of genomic resources such as development of molecular markers, genetic maps, generation of functional genomics platforms that facilitate the identification of QTLs and discovery of genes associated with tolerance/resistance to abiotic, biotic stresses and agronomic traits will accelerate crop improvement programs through molecular breeding.

MATERIAL AND METHODS

The experimental material consisted of BC₂F₄ introgression line population (DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5) segregating for rust and late leaf spot (LLS) resistance. The populations were developed by crossing DH 86, a cultivated variety of groundnut which is highly susceptible to these foliar diseases and two synthetic amphidiploids (ISATGR 278-18 and ISATGR 5) that were highly resistant to rust and LLS. The two IL (Introgression Line) populations comprised of 51 introgression lines for DH 86 × ISATGR 278-18 and 32 lines for DH 86 × ISATGR 5 showing high resistance for LLS and rust. The two populations were evaluated at University of Agricultural Sciences, Dharwad. Phenotyping was carried for disease traits in three seasons viz., Kharif 2011, Summer 2012 and Kharif 2012 at UAS, Dharwad and genotyping was done at ICRISAT, Hyderabad.

DNA was extracted from the fresh leaves of the parental genotypes and the introgression lines using the modified cetyltrimethylammonium bromide (CTAB) extraction method. Quality of the DNA was checked and quantified in 0.8 per cent agarose gel with known concentration of uncut lambda DNA of 50 ng/μl, 100 ng/μl and 200 ng/μl. Polymerase chain reaction (PCR) was carried out using a touchdown PCR profile and an amplification protocol appropriate for each pair of SSR primers. Amplification was checked by loading the PCR content on 1.2% agarose gel. However, the products were separated by capillary electrophoresis (ABI 3730XL DNA Analyzer, Applied Biosystems, USA). Initially the parents, DH 86, ISATGR 278-18 and ISATGR 5 were screened for polymorphism by using 430 SSR primers. The polymorphic markers were used genotype the ILs of the two populations. Analysis of variance (ANOVA), components of variation and correlation coefficient (r) were estimated using SAS software. Single marker analysis was performed to find out the association between the potential SSR markers and various agronomic and productivity traits including resistance to LLS and rust based on simple linear regression method.

RESULTS AND DISCUSSION

Analysis of variance across the seasons for disease scores of LLS and rust revealed significant variation among the genotypes, seasons and genotype \times season for the ILs of DH 86 \times ISATGR 278-18 and DH 86 \times ISATGR 5, indicating the need for screening in multiple environments. [7] and [8] found significant G \times E interaction for two diseases in a mapping population of 268 RILs obtained from the cross TAG 24 \times GPBD 4 and 146 RILs obtained from the cross TG 26 \times GPBD 4 respectively (Table 1). The genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) estimates for rust and LLS resistance in all the seasons were high, indicating higher magnitude of variation. In general, heritability was also high for these traits which led to high genetic advance. When the data was analyzed across the seasons, there was significant reduction in all the components of variation indicating predominant role for G \times E interaction for these traits (Table 2). Usually both LLS and rust occur together but the severity varies with the environment [3]. This would influence the precision of assessing the reaction of the genotypes.

Correlation among the rust and LLS resistance was studied using the data collected from (*kharif* 2012, *kharif* 2011) and (summer 2012). A negative association was found between rust and late leaf spot resistance. [8] also found the similar correlation using the RILs of TG 26 \times GPBD 4. However, [7] observed no association between the rust and LLS resistance among the RILs of TAG 24 \times GPBD 4 indicating material specific differences. Single marker analysis using linear regression was performed to find out association between phenotypic and genotypic data. The analysis revealed twenty markers associated with LLS resistance for DH 86 \times ISATGR 278-18 introgression population, and they were consistent across stages in different environments *viz.*, GM2009 (9.936-13.833%), GM2301 (5.977-10.616%), GM1536 (7.840-10.996%), GM1996 (9.576-19.900%), IPAHM103 (25.056-76.571%), TC3H07 (13.175-17.032%), PM137 (5.074-12.610%), GM1347 (8.409-11.212%), GM1290 (5.445-14.856%) and GM1494 (9.661-23.741%) (Table 3 a, b). Single marker analysis revealed twenty five markers associated with rust were consistent across stages in different environments *viz.*, TC7A02, TC9F04, GM2482, TC3A12, GM1988, GM1290, GM1012, TC9B07, GM1878, GM2024, GM1996, GM2009, GM2301, GM1536, IPAHM103, GM1954, GM1418, GM1494, IPAHM287, GM1836, TC3H07, PM137, TC5A06, GM1347 and GM1345 (Table 4 a, b).

Single marker analysis revealed 24 (*kharif* 2012), 9 (*kharif* 2011) and 8 (summer 2012) markers associated with LLS across stages in different environments for DH 86 \times ISATGR 5 introgression population. Eight markers *viz.*, GM2638 (5.993-9.123%), Seq15C12 (4.567-6.011%), GM1954 (10.982-16.150%), TC1A02 (16.153-27.133%), GM1971 (44.254-63.730%), TC4G02 (24.361-26.192%), GM1988 (15.553-31.604%) and GM1996 (30.861-56.672%) were found to be common and consistent across stages and seasons (Table 5). Single marker analysis revealed 28 (*Kharif* 2012), 7 (*Kharif* 2011) and 8 (Summer 2012) markers associated with rust across stages in different environments for DH 86 \times ISATGR 5 introgression population were identified with the phenotypic variance ranging from 4.342% (GM2082) to 46.656% (GM1971) (Table 6). Out of these, seven markers *viz.*, TC1A02 (18.326-28.240%), GM1971 (17.812-46.656%), TC4G02 (12.399-32.428%), GM1988 (11.512-18.395%), GM1996 (9.695-46.687%), GM1954 (11.508-14.046%) and GM2638 (4.926-9.765%) were found to be consistent across stages and seasons. Further validation of these markers in advance introgression population would strengthen the reliable association of this marker with rust paving the way for application in MAS. The markers identified in the present study

are extremely important for integrating one or more desired traits in the superior cultivar. But before using them in the breeding program, they need to be validated in advance generation with advance markers viz., SNPs and DArT. However, few markers have already been detected in the other mapping population (TAG 24 × GPBD 4 and TG 26 × GPBD 4), [9] found significant for disease resistance (IPAHM103, GM2009, GM2301, GM1536, GM1996, TC6E01 and GM1954).

Table 1: Pooled ANOVA for disease resistance traits in DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5 introgression population

Source of variation	D.F.	F value DH 86 × ISATGR 278-18		F value DH 86 × ISATGR 5	
		Rust 90	LLS 90	Rust 90	LLS 90
Season	2	131.02**	239.11**	44.37**	112.84**
Replication × Season	3	35.08	35.81	34.18	20.57
Genotype	52	86.21**	123.07**	65.68**	127.21**
Season × Genotype	104	1.77**	3.51**	4.35**	3.82**
Pr > F		<.0001	<.0001	<.0001	<.0001
CV		7.16	7.69	8.58	7.94
Sed		0.43	0.45	0.45	0.41

*, **: Significant at 5% and 1% level of probability respectively

Rust 90- Rust score after 90 days of sowing

LLS90- late leaf spot score after 90 days of sowing

Table 2: Mean, range and genetic variability components for late leaf spot and rust in DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5 introgression population

Traits	MEAN	Range	PCV	GCV	h ² b.s %	GAM
DH 86 × ISATGR 278-18						
<i>Kharif 2012</i>						
Rust 90	6.64	2.0-8.5	46.62	43.25	86.24	52.43
LLS 90	6.71	3.0-9.0	63.70	50.18	83.09	55.37
<i>Kharif 2011</i>						
Rust 90	5.64	2.0-8.0	47.05	43.75	86.37	57.22
LLS 90	5.33	3.0-8.0	63.91	59.82	86.69	68.84
Summer 2012						
Rust 90	5.98	2.00-8.50	51.66	48.97	87.33	58.81
LLS 90	5.66	3.00-8.50	71.88	69.04	87.99	51.79
Across seasons						
Rust 90	6.08	2-8.33	44.46	43.93	84.89	42.72
LLS 90	5.9	3-8.5	50.02	49.41	83.18	56.00
DH 86 × ISATGR 5						
<i>Kharif 2012</i>						
Rust 90	5.47	2.0-8.5	53.93	50.18	86.40	52.23

LLS 90	5.81	3.0-9.0	66.59	53.30	94.27	42.39
<i>Kharif 2011</i>						
Rust 90	4.85	2.0-8.0	50.06	46.43	86.23	63.55
LLS 90	4.76	3.0-8.0	56.21	52.69	86.77	68.34
<i>Summer 2012</i>						
Rust 90	5.47	3.0-8.0	46.37	42.23	75.33	57.06
LLS 90	4.96	3.0-8.0	68.81	65.93	87.86	74.97
<i>Across seasons</i>						
Rust 90	5.26	2.33-8.16	40.34	39.65	81.74	41.74
LLS 90	5.17	3.0-8.33	57.57	57.00	84.15	54.15

Table 3a: Single marker analysis for late leaf spot at different stages in DH 86 × ISATGR 278-18 introgression population

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
<i>Kharif 2012</i>							
LLS70K12	TC7A02	6.009	0.018 *	LLS80K12	GM1311	4.070	0.049 *
	TC9F04	6.349	0.015 *		TC7A02	6.381	0.015 *
	GM2482	7.711	0.008 **		TC9F04	7.252	0.010 **
	GM840	5.234	0.027 *		GM2482	14.040	0.000 ***
	GM2536	17.067	0.000 ***		GM2536	15.274	0.000 ***
	GM2142	5.703	0.021 *		TC3A12	25.576	0.00****
	TC3A12	18.196	0.00****		GM1988	65.457	0.00****
	GM1988	49.949	0.00****		GM1290	9.663	0.003 **
	GM1290	14.856	0.00 ***		GM1012	41.494	0.00****
	GM1012	27.972	0.00 ****		TC9B07	16.981	0.000 ***
	TC9B07	16.679	0.00 ***		GM1878	53.693	0.00****
	GM1878	41.354	0.00****		TC4G10	5.817	0.020 *
	GM2024	7.584	0.008 **		GM2024	16.643	0.000 ***
	GM2009	9.936	0.003 **		GM2009	12.157	0.001 **
	GM2301	6.919	0.011 *		GM2301	5.977	0.018 *
	GM1536	9.576	0.003 **		GM1536	7.840	0.007 **
	GM1996	9.576	0.003 **		GM1996	14.714	0.000 ***
	IPAHM103	25.056	0.00****		IPAHM103	34.366	0.00****
	GM1954	7.195	0.010 **		GM1954	11.181	0.002 **
	GM1494	9.661	0.003 **		GM1494	14.857	0.000 ***
	IPAHM287	5.264	0.026 *		IPAHM287	4.067	0.049 *
	GM1836	9.572	0.003 **		GM1836	9.910	0.003 **
	TC3H07	13.175	0.001 ***		TC3H07	17.032	0.000 ***
	PM137	5.074	0.029 *		TC1A02	6.331	0.015 *
TC5A06	8.083	0.006 **	PM137	10.893	0.002 **		
GM1347	11.212	0.002 **	TC5A06	6.676	0.013 *		
GM1345	29.402	0.00****	GM1347	10.425	0.002 **		
			GM1345	20.582	0.00****		
LLS90K12	GM1291	4.602	0.037 *	LLS90K12	GM2009	13.833	0.001 ***
	GM1311	4.589	0.037 *		GM2301	7.785	0.007 **

	TC7A02	7.003	0.011 *		GM1536	9.411	0.004 **
	TC9F04	6.937	0.011 *		GM1996	17.471	0.000 ***
	GM2482	15.387	0.000 ***		IPAHM103	39.863	0.000****
	GM2536	12.437	0.001***		GM1494	16.186	0.000 ***
	TC3A12	27.596	0.00****		IPAHM287	4.543	0.038 *
	GM1988	92.177	0.00****		GM1836	6.676	0.013 *
	GM1290	5.445	0.024 *		TC3H07	15.453	0.000 ***
	GM1012	43.170	0.00 ****		TC1A02	7.081	0.011 *
	TC9B07	18.511	0.00****		PM137	11.195	0.002 **
	GM1878	64.382	0.00****		TC5A06	7.557	0.008 **
	TC4G10	6.890	0.012 *		GM1347	9.897	0.003 **

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively.

K12- Kharif 2012, K11- Kharif 2011, S12-summer 2012

Table 3b: Single marker analysis for late leaf spot at different stages in DH 86 × ISATGR 278-18 introgression population

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
<i>Kharif 2011</i>				Summer 2012			
LLS90K11	GM1291	5.471	0.023 *	LLS90S12	GM1291	5.221	0.027 *
	GM1311	5.504	0.023 *		GM1311	5.276	0.026 *
	TC7A02	9.489	0.003 **		TC7A02	9.074	0.004 **
	TC9F04	9.467	0.003 **		TC9F04	9.072	0.004 **
	GM2482	24.816	0.000****		GM2482	25.119	0.000****
	GM2536	11.110	0.002 **		GM2536	11.996	0.001 **
	GM1290	7.467	0.009 **		GM2142	4.196	0.046 *
	GM1012	65.832	0.000****		TC3A12	27.785	0.000****
	TC9B07	15.001	0.000 ***		GM1988	86.707	0.000****
	GM1878	51.431	0.000****		GM1290	6.669	0.013 *
	GM2024	22.512	0.000****		GM1012	61.629	0.000 ****
	GM2009	13.217	0.001 ***		TC9B07	15.528	0.000 ***
	GM2301	9.925	0.003 **		GM1878	63.824	0.000 ****
	GM1536	9.645	0.003 **		TC4G10	6.083	0.017 *
	GM1996	18.406	0.000****		GM2670	4.589	0.037 *
	IPAHM103	76.571	0.000****		GM2024	23.412	0.000****
	GM1954	16.593	0.000 ***		GM2009	13.723	0.001 ***
	GM1418	6.833	0.012 *		GM2301	10.616	0.002 **
	GM1494	21.397	0.000****		GM1536	10.996	0.002 **
	IPAHM287	7.169	0.010 *		GM1996	19.900	0.000 ****
GM1836	8.516	0.005 **	IPAHM103	66.847	0.000 ****		
TC3H07	13.375	0.001 ***	GM1954	15.280	0.000 ***		
TC1A02	6.773	0.012 *	GM1418	4.673	0.036 *		

	PM137	10.449	0.002 **		GM1494	23.741	0.000 ****
	TC5A06	7.041	0.011 *		IPAHM287	6.894	0.012 *
	GM1347	8.409	0.006 **		GM1836	7.715	0.008 **
	GM1345	16.366	0.000 ***		TC3H07	15.653	0.000 ***
					TC1A02	8.666	0.005 **
					PM137	12.610	0.001 ***
					TC5A06	6.786	0.012 *
					GM1347	9.401	0.004 **
					GM1345	19.460	0.000 ****

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively.

K12- Kharif 2012, K11- Kharif 2011, S12-summer 2012

Table 4a: Single marker analysis for rust at different stages for DH 86 × ISATGR 278-18 introgression population in kharif 2012

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
Kharif 2012							
RUST70K1 2	TC7A02	6.262	0.016 *	RUST80K1 2	TC7A02	5.056	0.029 *
	TC9F04	5.949	0.018 *		TC9F04	5.147	0.028 *
	GM2482	6.621	0.013 *		GM2482	5.240	0.026 *
	GM840	6.090	0.017 *		GM840	5.667	0.021 *
	GM2536	15.468	0.000 ***		GM2536	14.688	0.000 ***
	TC3A12	17.686	0.000 ***		GM2142	4.051	0.050 *
	GM1988	44.754	0.000*** *		TC3A12	16.008	0.000 ***
	GM1290	17.277	0.000***		GM1988	36.317	0.000*** *
	GM1012	33.397	0.000*** *		GM1290	20.956	0.000*** *
	TC9B07	20.144	0.000*** *		GM1012	28.386	0.000*** *
	GM1878	48.328	0.000*** *		TC9B07	17.728	0.000 ***
	GM2024	9.492	0.003 **		GM1878	39.037	0.000*** *
	GM2009	8.399	0.006 **		GM2024	7.904	0.007 **
	GM2301	6.638	0.013 *		GM2009	7.291	0.009 **
	GM1536	7.212	0.010 **		GM2301	6.129	0.017 *
	GM1996	13.931	0.000 ***		GM1536	7.215	0.010 **
IPAHM10	26.678	0.000*** *	GM1996	12.959	0.001 ***		
GM1954	7.244	0.010 **	IPAHM10	24.58	0.000***		

					3	7	*
	GM1959	4.528	0.038 *		GM1954	6.638	0.013 *
	GM1418	5.513	0.023 *		GM1959	5.913	0.019 *
	GM1494	10.36	0.002 **		GM1418	5.858	0.019 *
	1						
	IPAHM28	5.944	0.018 *		GM1494	8.637	0.005 **
	7						
	GM1836	11.37	0.001 **		IPAHM28	5.374	0.025 *
	6				7		
	TC3H07	11.38	0.001 **		GM1836	12.97	0.001 ***
	5				3		
	TC1A02	4.092	0.049 *		TC3H07	10.54	0.002 **
					6		
	PM137	5.945	0.018 *		PM137	4.611	0.037 *
	TC5A06	5.717	0.021 *		TC5A06	5.234	0.026 *
	GM1347	9.641	0.003 **		GM1347	10.35	0.002 **
					6		
	GM1345	22.25	0.000***		GM1345	23.76	0.000***
	0		*		9		*
	IPAHM17	4.827	0.033 *				
	6						
RUST90K1	TC7A02	5.956	0.018 *	RUST90K1	GM1536	8.270	0.006 **
2	TC9F04	6.090	0.017 *	2	GM1996	14.09	0.00 ***
					5		
	GM2482	8.098	0.006 **		IPAHM10	34.62	0.000***
					3	6	*
	GM840	5.721	0.021 *		GM1954	8.692	0.005 **
	GM2142	15.74	0.000***		GM1418	5.098	0.028 *
	8						
	TC3A12	21.27	0.000***		GM1494	10.96	0.002 **
	5		*		1		
	GM1988	55.55	0.000***		IPAHM28	5.522	0.023 *
	1		*		7		
	GM1290	17.84	0.000***		GM1836	11.56	0.001 **
	9				0		
	GM1012	35.80	0.000***		TC3H07	13.82	0.001 ***
	8		*		1		
	TC9B07	20.60	0.000***		TC1A02	5.264	0.026 *
	9		*				
	GM1878	48.03	0.000***		PM137	5.970	0.018 *
	1		*				
	GM2024	10.50	0.002 **		TC5A06	6.701	0.013 *
	2						
	GM2009	8.614	0.005 **		GM1347	11.81	0.001 **
					8		
	GM2301	7.604	0.008 **		GM1345	27.50	0.000
					2		****

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively; K12- *kharif* 2012, K11- *kharif* 2011, S12-summer 2012, R²(%)-percentage of phenotypic variance, pr(F)- probability value

Table 4b: Single marker analysis for rust at different stages for DH 86 × ISATGR 278-18 introgression population in *kharif* 2011 and summer 2012

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
<i>Kharif</i> 2011				Summer 2012			
RUST90K1 1	TC7A02	8.144	0.006 **	RUST90S1 2	GM1291	4.321	0.043 *
	TC9F04	8.009	0.007 **		GM1311	4.310	0.043 *
	GM2482	13.809	0.001 ***		TC7A02	8.398	0.006 **
	GM2536	13.865	0.001 ***		TC9F04	8.555	0.005 **
	GM2142	5.819	0.020 *		GM2482	16.969	0.000 ***
	TC3A12	18.088	0.000*** *		GM840	4.414	0.041 *
	GM1988	47.833	0.000*** *		GM2536	14.248	0.000 ***
	GM1290	17.250	0.000***		GM2142	5.171	0.027 *
	GM1012	47.377	0.000*** *		TC3A12	24.963	0.000*** *
	TC9B07	14.824	0.000 ***		GM1988	68.337	0.000*** *
	GM1878	38.601	0.000*** *		GM1290	14.788	0.000 ***
	GM2024	12.407	0.001 ***		GM1012	56.149	0.000*** *
	GM2009	10.781	0.002 **		TC9B07	17.977	0.000*** *
	GM2301	11.013	0.002 **		GM1878	50.861	0.000*** *
	GM1536	8.931	0.004 **		GM2024	17.799	0.000 ***
	GM1996	19.603	0.000*** *		GM2009	12.240	0.001 **
	IPAHM103	57.307	0.000*** *		GM2301	10.677	0.002 **
GM1954	9.206	0.004 **	GM1536	9.432	0.003 **		
GM1418	8.105	0.006 **	GM1996	18.914	0.000*** *		
GM1494	17.344	0.000 ***	IPAHM103	66.070	0.000*** *		
IPAHM287	8.851	0.005 **	GM1954	11.331	0.001 **		

	GM1836	10.58 9	0.002 **		GM1418	7.365	0.009 **
	TC3H07	12.74 7	0.001 ***		GM1494	19.12 4	0.000*** *
	TC1A02	4.365	0.042 *		IPAHM28 7	7.679	0.008 **
	PM137	5.106	0.028 *		GM1836	10.75 2	0.002 **
	TC5A06	5.814	0.020 *		TC3H07	14.90 8	0.000 ***
	GM1347	9.300	0.004 **		TC1A02	6.405	0.015 *
	GM1345	24.86 4	0.000*** *		PM137	7.677	0.008 **
					TC5A06	6.503	0.014 *
					GM1347	10.21 3	0.002 **
					GM1345	24.59 1	0.000*** *

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively; K12- *kharif* 2012, K11- *kharif* 2011, S12-summer 2012, R²(%)-percentage of phenotypic variance, pr(F)- probability value

Table 5: Single marker analysis for late leaf spot at different stages in DH 86 × ISATGR 5 introgression population

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
<i>Kharif</i> 2012							
LLS70K1 2	GM2638	7.912	0.009 **	LLS80K1 2	GM2638	7.504	0.010 *
	Seq15C1 2	5.357	0.028 *		Seq15C1 2	5.939	0.021 *
	GM1954 2	12.38	0.001 **		GM1954 4	13.11	0.001 **
	TC1A02 3	27.13	0.000*** *		TC1A02 1	26.10	0.000*** *
	GM1971 7	44.80	0.000*** *		GM1971 4	44.25	0.000*** *
	TC4G02 8	24.44	0.000*** *		TC4G02 1	24.99	0.000*** *
	GM1988 2	19.72	0.000 ***		GM1988 4	31.60	0.000*** *
	GM1996 1	30.86	0.000*** *				
LLS90K1 2	GM2082	4.249	0.048 *	LLS90K1 2	GM1971	63.73 0	0.000*** *
	GM2638	9.123	0.005 **		TC4G02	39.85 8	0.000*** *
	Seq15C1 2	6.011	0.020 *		GM1988	15.55 3	0.000 ***

	GM1954	12.03 2	0.002 **		GM1996	53.04 2	0.000*** *
	TC1A02	21.96 2	0.000*** *				
<i>Kharif 2011</i>				Summer 2012			
LLS90K1 1	GM2082	4.511	0.042 *	LLS90S12	GM2638	5.993	0.020 *
	GM2638	7.442	0.011 *		Seq15C1 2	4.567	0.041 *
	Seq15C1 2	5.652	0.024 *		GM1954	10.98 2	0.002 **
	GM1954	16.15 0	0.000 ***		TC1A02	16.15 3	0.000 ***
	TC1A02	19.40 3	0.000 ***		GM1971	54.89 9	0.000*** *
	GM1971	62.33 4	0.000*** *		TC4G02	26.19 2	0.000*** *
	TC4G02	24.36 1	0.000*** *		GM1988	20.80 0	0.000 ****
	GM1988	23.58 6	0.000*** *		GM1996	54.12 0	0.000 ****
GM1996	56.67 2	0.000*** *					

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively.

K12- *Kharif 2012*, K11- *Kharif 2011*, S12-summer 2012

Table 6: Single marker analysis for rust at different stages in DH 86 × ISATGR 5 introgression population

Traits	Marker	R ² (%)	pr(F)	Traits	Marker	R ² (%)	pr(F)
<i>Kharif 2012</i>							
RUST70K 12	GM2082	4.400	0.044 *	RUST80K 12	GM208 2	4.342	0.046 *
	GM2638	8.932	0.006 **		GM263 8	7.848	0.009 **
	Seq15C1 2	7.075	0.012 *		Seq15C 12	5.265	0.029 *
	GM1954	11.70 3	0.002 **		GM195 4	13.139	0.001 **
	TC1A02	22.84 6	0.000** **		TC1A02	25.699	0.000*** *
	GM1971	46.65 6	0.000** **		GM197 1	23.657	0.000 ****
	TC4G02	32.42 8	0.000** **		TC4G02	15.759	0.000 ***
	GM1988	16.66	0.000		GM198	18.395	0.000

		4	***		8		***
	GM1996	45.919	0.000**		GM1996	13.833	0.001***
RUST90K 12	GM2082	5.828	0.022 *	RUST90K 12	GM1971	17.812	0.000***
	GM2638	9.765	0.004 **		TC4G02	12.399	0.001 **
	Seq15C12	4.349	0.046 *		GM1988	14.476	0.001***
	GM19548	11.508	0.002 **		GM1996	9.695	0.004 **
	TC1A020	28.240	0.000**		TC5A06	4.466	0.043 *
Kharif 2011				Summer 2012			
RUST90K 11	GM2638	4.926	0.034 *	RUST90S 12	GM2638	6.742	0.014 *
	GM19541	12.301	0.001 **		Seq15C12	5.658	0.024 *
	TC1A026	18.326	0.000***		GM1954	14.046	0.001***
	GM19710	35.720	0.000**		TC1A02	21.880	0.000****
	TC4G028	15.168	0.001***		GM1971	46.643	0.000****
	GM19882	11.512	0.002 **		TC4G02	21.859	0.000****
	GM19969	33.289	0.000**		GM1988	11.885	0.002 **
					GM1996	46.687	0.000****

Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively; K12- kharif 2012, K11- kharif 2011, S12-summer 2012, R²(%)-percentage of phenotypic variance, pr(F)- probability value

ACKNOWLEDGEMEN

The research presented in this article is a contribution from Ph. D research work to UAS-Dharwad Karnataka.

REFERENCES

1. FAO. Food and Agricultural Organization of the United Nation, FAO Statistical Database. <http://faostat.fao.org/faostat/collections?subset=agriculture> 2012.
2. Savage, G. P. and Keenan, J. I., 1994, The composition and nutritive value of groundnut kernels. In: The Groundnut Crop: a scientific basis for improvement. (Eds.) Smart, J., Chapmon and Hall London. pp. 173-213.
3. Subramanyam, P., Williams, J. H., McDonald, D. and Gibbons, R. W., 1984, The influence of foliar diseases and their control by selective fungicides on a range of groundnut (*Arachis hypogaea* L.) genotypes. *Ann. Appl. Biol.*, **104**: 467-476.
4. Wynne, J. C., Beute, M. K. and Nigam, S. N., 1991, Breeding for disease resistance in peanut (*Arachis hypogaea* L.). *Annual Review of Phytopathology.*, **29**: 279-303.

5. Singh, A. K., Mehan, V. K. and Nigam, S. N., 1997, Sources of resistance to groundnut fungal and bacterial diseases: An update and appraisal. *Information Bulletin No. 50*. International Crops Research Institute for the Semi Arid Tropics, Patancheru, Andhra Pradesh, India, p. 48.
6. Crouch, J. H., 2001, Molecular marker-assisted breeding: a perspective for small to medium-sized plant breeding companies. *Asia and Pacific Seed Association Technical Report No.*, **30**: 1-14.
7. Khedikar, Y. P., 2008, Molecular tagging and Mapping of resistance to late leaf spot and rust in Groundnut (*Arachis hypogaea* L.). *Ph.D. Thesis*, Uni. Agric. Sci. Dharwad (India).
8. Sarvamangala, C., 2009, Construction of genetic linkage map and qtl analysis for foliar disease resistance, nutritional quality and productivity traits in groundnut (*Arachis hypogaea* L.). *Ph.D. Thesis*, Uni. Agric. Sci. Dharwad (India).
9. Sujay, V., Gowda, M. V. C., Pandey, M. K., Bhat, R. S., Khedikar, Y. P., Nadaf, H. L., Gautami, B., Sarvamangala, C., Lingaraju, S., Radhakrishan, T., Knapp, S. J. and Varshney R. K., 2012., Quantitative trait locus analysis and construction of consensus genetic map for foliar disease resistance based on two recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). *Mol Breeding.*, **30**: 773–788.