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Screening of Groundnut (*Arachis hypogaea* L.) Mini Core Collection against Late Leaf Spot Disease Caused by *Phaeoisariopsis personata* (Berk & M.A Curtis van Arx)

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Groundnut is considered as a "poor man's almond" possessing nutritional and medicinal properties constituting 44-50% edible oil, dietary protein (25-33%) and carbohydrates (20%). Diseases like early and late leaf spot caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* and rust (*Puccinia arachidis*) are causing yield loss upto 70% resulting in a lower productivity of the crop. Germplasm collection is a reservoir of genetic variability comprising of promising genes for various characters that can be incorporated in the breeding programme to develop and broaden the genetic base of cultivars. Thus the investigation was carried out to screen the mini core collection comprising of 188 accessions representing *fastigiata* (33), *vulgaris* (71), *peruviana* (2), *aequatoriana* (1), *hypogaea* runner (33) and *hypogaea* bunch (48) and identify the genotypes resistant to late leaf spot. The experiment was conducted in a Simple Lattice Design (15 × 15) in two replications during *kharif* 2011 at UAS, Bangalore. The cultivar TMV-2 highly susceptible to late leaf spot was used as a spreader row for natural disease incidence. Phenotypic screening was done using modified nine point scale. The genotypes such as ICG 5286, ICG 2773, ICG 111426, ICG 2857 and ICG 6022 recorded as resistant to late leaf spot can be used as a donor of disease resistance in breeding of disease resistant cultivars. SSR marker GM 1009 was validated through single marker analysis.

Key Words: Groundnut, mini core, late leaf spot, modified 9 point scale, germplasm

Introduction

Groundnut (*Arachis hypogaea* L.) is an important crop both in subsistence and commercial agriculture in arid and semi-arid regions of the world. Groundnut kernels are rich source of edible oil (44-50%), dietary protein (25-33%) and carbohydrates (20%). Diseases like early leaf spot and late leaf spot caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* and rust (*Puccinia arachidis*) are causing yield losses up to 70% (Grichar *et al.*, 1998; McDonald *et al.*, 1985; Miller *et al.*, 1990) resulting in a lower productivity of the crop. Among different approaches of disease management, growing resistant variety is the best environment friendly means of reducing yield losses from the diseases (Gibbons, 1980 and Subrahmanyam *et al.*, 1995). Therefore, it is important to identify sources of resistance that can be used in breeding programme to evolve resistant varieties.

Plant genetic resources are essential components to meet future food security needs of the world. Germplasm collection is a reservoir of genetic variability comprising of promising genes for various characters that can be incorporated in the breeding programme to develop and broaden the genetic base of cultivars. Screening for resistance to leaf spots and rust has been intensively carried out by many workers and a number of sources of resistance have been reported in both cultivated groundnut and wild *Arachis* species. The objective of the study was to screen the mini core collection of groundnut (*Arachis hypogaea* L.) for late leaf spot disease and identify the accessions resistant to late leaf spot disease under field conditions.

Materials and Methods

The field experiment was undertaken at University of Agricultural Sciences, Bengaluru during *kharif* 2011 favourable for natural spread of late leaf spot disease. The experimental site was located at an altitude of 899 m above Mean Sea Level and at 13.00^{o}N latitude and 77.35^{o} E longitude.

The experiment was conducted in *kharif* 2011 on 1st June 2011 in Simple Lattice (15 × 15) design with two replications. The cultivar TMV 2 was used as susceptible check and spreader row for natural late leaf spot disease incidence and its spread. The experimental material comprised of 225 genotypes which includes 188 accessions of groundnut mini core set procured from ICRISAT, Patancheru, Hyderabad, representing different

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*Author for Correspondence: E-mail:shivslucky@gmail.com species of *Arachis* such as *fastigiata* (33), *vulgaris* (71), *peruviana* (2), *aequatoriana* (1), *hypogaea* runner (33) and *hypogaea* bunch (48) and other advanced breeding lines. These genotypes are listed in Table 1.

Each genotype was grown with a spacing of 40 cm between rows and 15 cm between plant to plant within the rows. After every six rows of different genotypes, a row of TMV 2 and two border rows of TMV 2 around the experimental plot was grown for uniform natural dispersal of pathogen spores. All the 225 genotypes were evaluated for late leaf spot disease incidence through visual screening method (Fig. 1) and modified nine point scale (Table 2) given by Subbarao *et al.* in 1990 (0: no disease severity and 9: 81-100% disease severity). The visual scores (1-9) and the extent of leaf area destroyed (0-100%) are linearly related. The field disease scores are mainly based on the extent of leaf area damage. The scores were converted into Percentage Disease Index (PDI) using the following formula:

$$\text{PDI (\%)} = \frac{\text{Sum of individual ratings}}{\text{Number of observations assessed}} \times \frac{100}{\text{Maximum disease rating}}$$

Phenotypic screening of all the 225 genotypes was done at five different intervals of time starting from 30% disease incidence to 100% *i.e.,* at 70 days after sowing (DAS), 80DAS, 90DAS, 100DAS and 110DAS. Five plants in each genotype were randomly selected for scoring and were tagged for easy identification during each scoring. Two replication data was pooled and the genotypes are categorised into different disease reaction groups based on the disease severity such as resistant, moderately resistant, susceptible, moderately susceptible and highly susceptible.

SSR marker GM 1009 identified to be linked to late leaf spot resistance (Sujay *et al.* 2011) was validated in different resistance and susceptible genotypic backgrounds. CTAB method of DNA extraction (Saghai-Maroof *et al.* 1984) was carried out during the study. DNA was quantified by loading the samples on 0.8% agarose gel containing 0.5 µl/10 ml Ethidium bromide (10mg/ml). The diluted DNA samples with the standard λ DNA molecular weight markers (5 ng/µl and 10 ng/µl) were loaded on 0.8% agarose gel and runned in 0.5X TBE (Tris borate EDTA) buffer at a constant voltage (80 V) for 20 min. The images of gels were documented under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England).

Polymerase Chain Reactions (PCR) were performed by using a Touch-Down PCR. DNA amplification was performed in 10 µl reaction mixture using Gene Amp® PCR system 9700. Before loading PCR products in the polyacrylamide gel electrophoresis (PAGE), amplification was checked on 1.2% agarose gel. For the separation of DNA fragments, non-denaturing PAGE was used.

Results and Discussion

All the 225 genotypes have shown different disease reaction to late leaf spot (Table 3). None of them are devoid of disease incidence. Among them few genotypes were resistant *viz.*, ICGV 91177 (2.3), ICGV 86699 (2.5), GBFD5272 (2.5), GPBD-4 (3.1), ICGV 86590 (3.1) and IGCV 99005 (3.6). The mini core comprised of accessions moderately resistant to late leaf spot such as ICG 5286 (4.1), ICG 2773 (4.3) ICG 11426 (4.3), M-282 (4.4), ICG 2857 (4.5), ICG 6022 (4.5), ICG 6766 (4.7), ICG 532 (4.8), ICG 2772 (4.8), ICG 5745 (4.9) and ICG 14475 (4.9). Several sources of resistance to late leaf spot and rust have been reported in *A. hypogaea* (Waliyar *et al.,* 1993; Anderson *et al.,* 1993; Mehan *et al.,* 1996; Singh *et al.,* 1997; Dwivedi *et al*., 2002).

The accessions identified as resistant to late leaf spot can be used as parents in hybridization programme to transfer the resistant gene to the agronomically superior cultivated genotypes to develop a variety resistant to late leaf spot. The core and mini core collections of various crops were evaluated to identify trait-specific diverse parents at ICRISAT. Due to the reduced size, the core and the mini core sets have been evaluated and characterized precisely and useful trait-specific accessions have been identified for use in breeding programme to develop varieties with a broad genetic base. Although there is an increase in the number of germplasm accessions in genebanks, there is no corresponding increase in their use by the crop improvement scientists, indicating that the collections were not being used to their full potential. Thus, a very large gap exists between availability and actual utilization of the material. Hence there is a need to utilize the germplasm in crop improvement programmes to till the genes for various traits including disease resistance.

Confirmation of Reaction to Late Leaf Spot and Validation of Markers

The SSR marker GM 1009 which was detected by Sujay *et al.* (2011) through comprehensive quantitative trait loci (QTL) analysis was tightly linked to late leaf spot resistance. The banding profile of selected genotypes were in confirmation of resistance *i.e.,* at 411bp length there

Table 1. List of mini core set of groundnut (*Arachis hypogaea* L.)

S.No	ICG No.	Origin	Species	Subspecies	Botanical type
1	36	India	*hypogaea*	*fastigiata*	VUL
2	76	India	*hypogaea*	*hypogaea*	HYR
3	81	Unknown	*hypogaea*	*fastigiata*	VUL
4	111	Unknown	*hypogaea*	*hypogaea*	HYB
5	115	India	*hypogaea*	*fastigiata*	FST
6	118	India	*hypogaea*	*vulgaris*	VUL
7	163	Unknown	*hypogaea*	*hypogaea*	HYR
8	188	India	*hypogaea*	*hypogaea*	HYB
9	297	UAS	*hypogaea*	*fastigiata*	FST
10	332	Brazil	*hypogaea*	*fastigiata*	FST
11	334	China	*hypogaea*	*fastigiata*	VUL
12	397	UAS	*hypogaea*	*fastigiata*	VUL
13	434	UAS	*hypogaea*	*fastigiata*	VUL
14	442	UAS	*hypogaea*	*fastigiata*	VUL
15	513	India	*hypogaea*	*hypogaea*	HYB
16	532	Unknown	*hypogaea*	*hypogaea*	HYB
17	721	UAS	*hypogaea*	*hypogaea*	HYB
18	862	India	*hypogaea*	*hypogaea*	HYR
19	875	India	*hypogaea*	*hypogaea*	HYR
20	928	Unknown	*hypogaea*	*hypogaea*	HYR
21	1137	India	*hypogaea*	*fastigiata*	VUL
22	1142	Benin	*hypogaea*	*fastigiata*	FST
23	1274	Indonesia	*hypogaea*	*fastigiata*	FST
24	1399	Malawi	*hypogaea*	*fastigiata*	FST
25	1415	Senegal	*hypogaea*	*fastigiata*	FST
26	1519	India	*hypogaea*	*fastigiata*	VUL
27	1668	UAS	*hypogaea*	*hypogaea*	HYB
28	1711	Bolivia	*hypogaea*	*fastigiata*	VUL
29	1973	India	*hypogaea*	*fastigiata*	VUL
30	2019	India	*hypogaea*	*fastigiata*	VUL
31	2106	India	*hypogaea*	*fastigiata*	VUL
32	2381	Brazil	*hypogaea*	*hypogaea*	HYR
33	2511	India	*hypogaea*	*hypogaea*	HYR
34	2772	Nigeria	*hypogaea*	*hypogaea*	HYB
35	2773	Tanzania	*hypogaea*	*hypogaea*	HYR
36	2777	India	*hypogaea*	*hypogaea*	HYR
37	2827	Argentina	*hypogaea*	*hypogaea*	HYR
38	2925	India	*hypogaea*	*hypogaea*	HYR
39	3027	India	*hypogaea*	*hypogaea*	HYB
40	3053	India	*hypogaea*	*hypogaea*	HYB
41	3102	India	*hypogaea*	*fastigiata*	
42	3240	Uganda	*hypogaea*	*fastigiata*	VUL
43	3343	India	*hypogaea*	*fastigiata*	VUL
44	3421	India	*hypogaea*	*fastigiata*	VUL
45	3584	India	*hypogaea*	*fastigiata*	VUL
46	3673	Korea	*hypogaea*	*fastigiata*	
47	3681	USA	*hypogaea*	*fastigiata*	FST
48	3746	Argentina	*hypogaea*	*fastigiata*	VUL
49	3775	Brazil	*hypogaea*	*fastigiata*	
50	3992	India	*hypogaea*	*hypogaea*	HYR
51	4156	Unknown	*hypogaea*	*hypogaea*	HYR
52	4343	India	*hypogaea*	*hypogaea*	HYR
53	4389	India	*hypogaea*	*hypogaea*	HYR
54	4412	USA	*hypogaea*	*hypogaea*	HYR
55	4527	Uganda	*hypogaea*	*hypogaea*	HYB
56	4538	India	*hypogaea*	*hypogaea*	HYB
57	4543	Unknown	*hypogaea*	*vulgaris*	VUL
58	4598	India	*hypogaea*	*hypogaea*	HYB
59	4670	Sudan	*hypogaea*	*fastigiata*	FST
60	4684	USA	*hypogaea*	*fastigiata*	VUL
61	4729	China	*hypogaea*	*fastigiata*	VUL
62	4746	Isreal	*hypogaea*		HYB
63	4750	Paraguay	*hypogaea*	*fastigiata*	VUL
64	4911	Malawi	*hypogaea*	*fastigiata*	VUL
65	4955	India	*hypogaea*	*fastigiata*	VUL
66	4998	China	*hypogaea*	*hypogaea*	HYR
67	5016	USA	*hypogaea*		
68	5051	USA	*hypogaea*		
69	5195	Sudan	*hypogaea*	*fastigiata*	VUL
70	5221	Argentina	*hypogaea*	*fastigiata*	FST
71	5236	Chile	*hypogaea*	*fastigiata*	VUL
72	5286	Zambia	*hypogaea*	*hypogaea*	HYB
73	5327	USA	*hypogaea*	*hypogaea*	HYB
74	5475	Kenya	*hypogaea*	*fastigiata*	FST
75	5494	Malaysia	*hypogaea*	*vulgaris*	VUL
76	5609	Sri Lanka	*hypogaea*	*fastigiata*	FST
77	5662	China	*hypogaea*	*hypogaea*	HYB
78	5663	China	*hypogaea*	*hypogaea*	HYB
79	5745	Puerto Rico	*hypogaea*	*hypogaea*	HYB
80	5779	India	*hypogaea*	*fastigiata*	VUL
81	5827	USA	*hypogaea*	*hypogaea*	HYR
82	5891	India	*hypogaea*	*hypogaea*	HYB
83	6022	Sudan	*hypogaea*	*fastigiata*	FST
84	6057	USA	*hypogaea*	*hypogaea*	HYB
85	6201	Cuba	*hypogaea*	*fastigiata*	FST
86	6263	Burkina Faso	*hypogaea*	*fastigiata*	VUL
87	6375	Unknown	*hypogaea*	*fastigiata*	VUL
88	6402	Unknown	*hypogaea*	*hypogaea*	HYB
89	6407	Zimbabwe	*hypogaea*	*fastigiata*	VUL
90	6646	Unknown	*hypogaea*	*fastigiata*	FST
91	6654	Unknown	*hypogaea*	*fastigiata*	VUL
92	6667	USA	*hypogaea*	*hypogaea*	HYB
93	6703	Paraguay	*hypogaea*	*hypogaea*	VUL
94	6766	USA	*hypogaea*	*hypogaea*	HYB
95	6813	Senegal	*hypogaea*	*hypogaea*	HYR
96	6888	Brazil	*hypogaea*	*fastigiata*	VUL

HYB: *Hypogaea* bunch; HYR: *Hypogaea* runner; VUL: *Vulgaris;* FST: *Fastigiata*

HYB: *Hypogaea* bunch; HYR: *Hypogaea* runner; VUL: *Vulgaris;* FST: *Fastigiata*

Table 1. Contd.

S.No	ICG No.	Origin	Species	Subspecies	Botanical type
97	6892	USA	*hypogaea*	*hypogaea*	HYB
98	6913	USA	*hypogaea*	*hypogaea*	HYB
99	6993	Brazil	*hypogaea*	*hypogaea*	HYR
100	7000	USA	*hypogaea*	*hypogaea*	HYR
101	7153	India	*hypogaea*	*hypogaea*	HYR
102	7181	India	*hypogaea*	*fastigiata*	FST
103	7190	Brazil	*hypogaea*	*vulgaris*	VUL
104	7243	USA	*hypogaea*	*hypogaea*	HYR
105	7906	Zimbabwe	*hypogaea*	*vulgaris*	VUL
106	7963	USA	*hypogaea*	*hypogaea*	VUL
107	7969	Zimbabwe	*hypogaea*	*vulgaris*	VUL
108	8083	Russia & CISs	*hypogaea*	*vulgaris*	VUL
109	8106	Peru	*hypogaea*	*fastigiata*	FST
110	8285	USA	*hypogaea*	*hypogaea*	HYB
111	8490	Somalia	*hypogaea*	*hypogaea*	HYR
112	8517	Bolivia	*hypogaea*	*fastigiata*	FST
113	8567	Uruguay	*hypogaea*	*vulgaris*	VUL
114	8760	Zambia	*hypogaea*	*hypogaea*	HYR
115	9037	Cote d'Ivoire	*hypogaea*	*hypogaea*	HYR
116	9157	Puerto Rico	*hypogaea*	*vulgaris*	VUL
117	9249	Mauritius	*hypogaea*	*vulgaris*	
118	9315	USA	*hypogaea*	*fastigiata*	FST
119	9418	Martinique	*hypogaea*	*vulgaris*	VUL
120	9507	Philippines	*hypogaea*	*vulgaris*	VUL
121	9666	India	*hypogaea*	*hypogaea*	HYB
122	9777	Mozambique	*hypogaea*	*hypogaea*	HYB
123	9809	Mozambique	*hypogaea*	*vulgaris*	VUL
124	9842	Tanzania	*hypogaea*	*hypogaea*	HYB
125	9905	Zambia	*hypogaea*	*hypogaea*	HYR
126	9961	Unknown	*hypogaea*	*hypogaea*	HYB
127	10036	Peru	*hypogaea*	*peruviana*	FST
128	10092	Zimbabwe	*hypogaea*	*fastigiata*	
129	10185	USA	*hypogaea*	*hypogaea*	HYB
130	10384	Nigeria	*hypogaea*	*vulgaris*	VUL
131	10474	Cuba	*hypogaea*	*fastigiata*	FST
132	10479	Uruguay	*hypogaea*	*hypogaea*	
133	10554	Argentina	*hypogaea*	*fastigiata*	FST
134	10566	Congo	*hypogaea*	*fastigiata*	FST
135	10890	Peru	*hypogaea*	*fastigiata*	FST
136	11088	Peru	*hypogaea*	*peruviana*	FST
137	11109	Taiwan	*hypogaea*	*hypogaea*	HYR
138	11144	Argentina	*hypogaea*	*fastigiata*	FST
139	11219	Mexico	*hypogaea*	*hypogaea*	HYR
140	11249	Tanzania	*hypogaea*	*vulgaris*	VUL
141	11322	India	*hypogaea*	*hypogaea*	HYB
142	11426	India	*hypogaea*	*hypogaea*	HYB
143	11457	India	*hypogaea*	*hypogaea*	HYR
144	11515	China	*hypogaea*	*vulgaris*	VUL
145	11651	China	*hypogaea*	*vulgaris*	VUL
146	11687	India	*hypogaea*	*vulgaris*	VUL
147	11855	Korea	*hypogaea*	*hypogaea*	HYB
148	11862	Korea	*hypogaea*		HYB
149	12000	Mali	*hypogaea*	*hypogaea*	HYB
150	12189	Isreal	*hypogaea*	*vulgaris*	VUL
151	12276	Bolivia	*hypogaea*	*hypogaea*	HYB
152	12370	India	*hypogaea*	*hypogaea*	HYR
153	12625	Ecuador	*hypogaea*	*aeguatoriana*	FST
154	12672	Bolivia	*hypogaea*	*hypogaea*	HYB
155	12682	India	*hypogaea*	*vulgaris*	VUL
156	12697	India	*hypogaea*	*vulgaris*	VUL
157	12879	Myanmar	*hypogaea*	*vulgaris*	VUL
158	12921	Zimbabwe	*hypogaea*	*vulgaris*	VUL
159	12988	India	*hypogaea*	*vulgaris*	VUL
160	13099	Unknown	*hypogaea*	*hypogaea*	HYR
161	13491	Central Afr. Rep	*hypogaea*	*vulgaris*	VUL
162	13603	Indonasia	*hypogaea*	*vulgaris*	VUL
163	13723	Niger	*hypogaea*	*hypogaea*	HYR
164	13787	Niger	*hypogaea*	*hypogaea*	HYB
165	13856	Uganda	*hypogaea*	*fastigiata*	FST
166	13858	Uganda	*hypogaea*	*fastigiata*	FST
167	13982	USA	*hypogaea*	*hypogaea*	VUL
168	14008	Central Afr. Rep	*hypogaea*	*hypogaea*	HYB
169	14106	Zaire	*hypogaea*	*fastigiata*	FST
170	14118	Zaire	*hypogaea*	*vulgaris*	VUL
171	14127	Zaire	*hypogaea*	*fastigiata*	VUL
172	14466	Nigeria	*hypogaea*	*hypogaea*	HYB
173	14475	Nigeria	*hypogaea*	*hypogaea*	HYB
174	14482	Nigeria	*hypogaea*	*hypogaea*	HYB
175	14523	Unknown	*hypogaea*		
176	14630	Brazil	*hypogaea*	*fastigiata*	FST
177	14705	Cameroon	*hypogaea*	*hypogaea*	HYB
178	14710	Cameroon	*hypogaea*	*fastigiata*	FST
179	14985	Unknown	*hypogaea*	*vulgaris*	VUL
180	15042	Unknown	*hypogaea*	*fastigiata*	FST
181	15190	Costa Rica	*hypogaea*	*hypogaea*	HYB
182	15287	Brazil	*hypogaea*	*fastigiata*	HYB
183	15309	Brazil	*hypogaea*	*fastigiata*	FST
184	15419	Ecuador	*hypogaea*	*fastigiata*	FST
	Controls				
1	156	India			
2	2738	India			
3	13941	ICRISAT			
4	13942	ICRISAT			

HYB: *Hypogaea* bunch; HYR: *Hypogaea* runner; VUL: *Vulgaris;* FST: *Fastigiata*

HYB: *Hypogaea* bunch; **HYR**: *Hypogaea* runner; **VUL**: *Vulgaris;* **FST**: *Fastigiata*

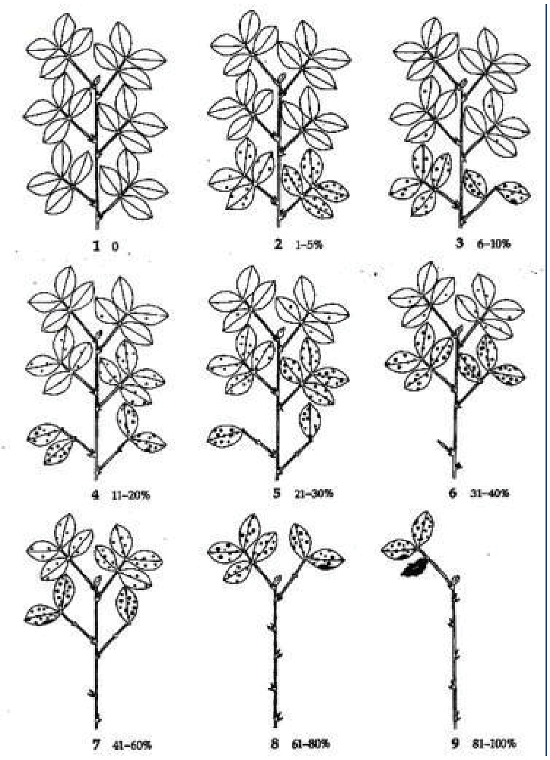

Fig. 1. Diagram showing leaf symptoms used for scoring late leaf spot disease resistance (Subbarao *et al.*, 1990)

was presence of band in case of resistant genotypes and absent in case of susceptible genotypes (Fig. 2).

Clear and unambiguous bands were scored for their presence or absence with the score 1 indicating their presence and 0 indicating their absence of band on the gel at 411bp. The data matrix of binary codes thus obtained was subjected to further analysis. The genotypic and phenotypic data obtained from set of individuals were subjected to single marker analysis (Table 4) using one

Table 2. Modified 9-point scale used for field-screening of groundnut genotypes for late leaf spot disease resistance (Subbarao *et al.*, 1990)

Disease score	Description	Disease severity (%)
1	No disease	0
2	Lesions present largely on lower leaves; no defoliation	1-5
3	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaf lets evident on lower leaves	6-10
4	Lesions present on lower and middle leaves but severe on lower leaves; defoliation of some leaf lets evident on lower leaves	11-20
5	Lesions present on lower and middle leaves, over 50 % of defoliation of lower leaves	21-30
6	Severe; eosins on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; some defoliation on middle leaves	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and middle leaves	41-60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves evident.	61-80
9	Almost all leaves defoliated, leaving bare stem; some leaflets may remain, but show severe leaf spot	81-100

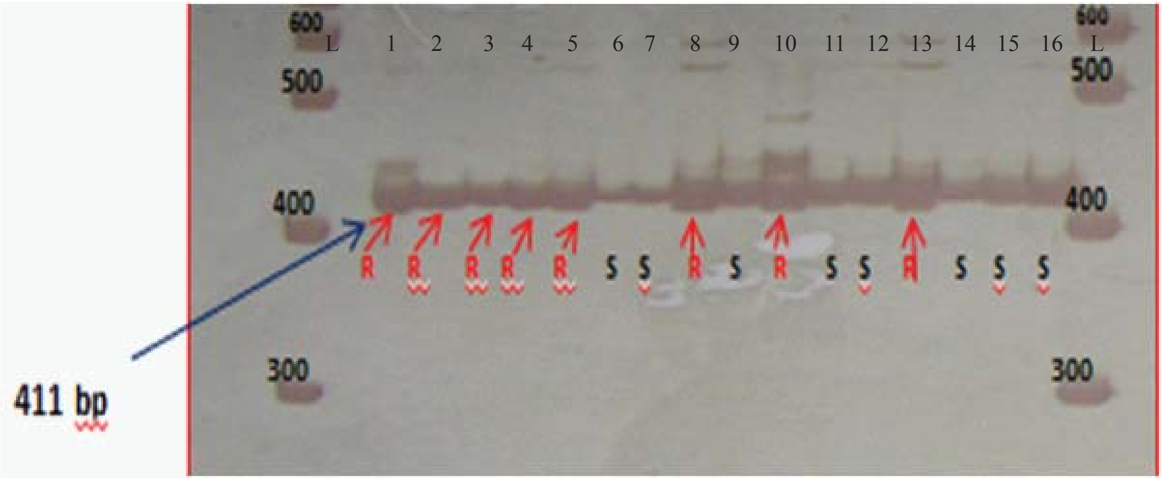

Fig. 2. Banding profile of 16 genotypes for marker GM 1009. DNA fragments were separated on 6% non-denaturing poly acrylamide gel electrophoresis (PAGE). The left side arrow shows the resistant band at 411bp. Resistance genotypes are marked as (R) and susceptible genotypes are marked as (S). Lane L, 100bp DNA ladder, lane 1-5, 8, 10 and 13, ICGV 86699, GPBD 4, ICG 2857, ICG 2773, ICG 5286, ICG 5745, M-282 and ICG 11426 respectively. Lane 6, 7, 9, 11, 12, 14 and 15, ICGV 99003, ICG 3027, GKVK 13, ICGV 99004, ICGV 93021, ICG 13099 and ICG 10036, respectively.

Table 3. Reactions of genotypes to late leaf spot during *kharif* 2011

Disease reaction	Resistant (0-3.5)*		Moderately Resistant (3.6-4.9)*	
No. of genotypes	7		18	
	Genotypes/Lines	Score	Genotypes/Lines	Score
	ICGV 91177	2.3	GPBD 5	4.0
	ICGV 86699	2.5	ICGV 99003	4.0
	GBFD5272	2.5	ICG 5286	4.1
	GPBD-4	3.1	GKVK-13	4.2
	ICGV 86590	3.1	ICG 2773	4.3
	IGCV 99005	3.6	ICG 11426	4.3
			M-282	4.4
			ICGV 87165	4.4
			ICG 2857	4.5
			ICG 6022	4.5
			ICG 6766	4.7
			ICG 532	4.8
			ICG 2772	4.8
			ICG 5745	4.9
			ICG 14475	4.9

*Disease score

Disease reaction	Moderately Susceptible (5.0-6.5)*							
No. of genotypes	88							
	Genotypes/Lines	Score	Genotypes/Lines	Score	Genotypes/Lines	Score	Genotypes/Lines	Score
	1	2	1	2	1	2	1	2
	ICGV 99004	5.0	ICG 1973	5.5	ICG 7000	5.9	ICG 6813	6.2
	ICG 4412	5.0	ICG 4389	5.5	ICG 9961	5.9	ICG 11109	6.2
	ICG 3027	5.0	ICG 6913	5.5	JL-24	5.9	GKVK17	6.2
	ICG 2925	5.0	ICG 11088	5.5	ICG 163	6.0	ICG 14710	6.3
	1	2	1	2	1	2	1	2
	ICG 5663	5.0	ICG 9666	5.5	ICG 5016	6.0	ICG 188	6.3
	ICG 9037	5.0	ICG 12276	5.5	ICG 4343	6.0	ICG 6057	6.3
	ICG 14466	5.0	ICG 156	5.6	ICG 4156	6.0	ICG 11862	6.3
	ICGV 93021	5.0	ICG 2511	5.6	ICG 7963	6.0	ICG 6892	6.3
	ICG 2381	5.1	ICG 5662	5.6	ICG 8490	6.0	ICG 9777	6.3
	ICG 4527	5.1	ICG 11457	5.6	ICG 8285	6.0	ICG 8106	6.3
	ICG 14008	5.1	ICG 11515	5.6	ICG 13942	6.0	ICG 334	6.4
	ICG 4538	5.2	ICG 12000	5.6	GKVK 8b	6.0	ICG 12625	6.4
	ICG 14482	5.2	ICG 13099	5.6	NCAC 17090	6.0	ICG 13941	6.4
	ICG 4746	5.2	ICG B 37C	5.6	ICG 15190	6.0	GKVK 12	6.4
	ICG 5827	5.2	ICG 6993	5.7	ICG 721	6.1	ICG 76	6.5
	ICG 12672	5.2	ICGV 87264	5.7	ICG 2777	6.1	ICG 10036	6.5
	VL-1	5.2	ICG 875	5.8	ICG 10479	6.1		
	ICG 5051	5.3	ICG 5327	5.8	ICG 13787	6.1		
	ICG 5891	5.3	ICG 8760	5.8	GKVK 4	6.1		
	ICG 9842	5.3	ICG 14705	5.8	ICG 9905	6.2		
	ICG 513	5.4	R 8801	5.8	ICG 14630	6.2		
	ICG 6667	5.4	ICG 4955	5.9	ICG 15419	6.2		
	ICG 14523	5.4	ICG 4598	5.9	ICG 862	6.2		
	ICG 928	5.5	ICG 3053	5.9	ICG 5475	6.2		

*Disease score

Contd.

Disease reaction Susceptible (6.6-7.5)*					Highly susceptible (7.6- 9.0)*					
No. of genotypes 88					81					
	Genotypes	Score	Genotypes	Score	Genotypes	Score	Genotypes	Score	Genotypes	Score
	ICG 1668	6.6	ICG 11651	7.0	ICG 4911	7.6	ICG 3102	8.0	ICG 8567	8.3
	ICG 6646	6.6	ICG 8517	7.0	ICG 11322	7.6	ICG 2019	8.0	TMV 2	8.3
	ICG 11855	6.6	ICG 13713	7.1	ICG 11144	7.6	ICG 5609	8.0	ICG 36	8.4
	ICG 13982	6.6	GKVK 2	7.1	ICG 10474	7.6	ICG 9809	8.0	ICG 5221	8.4
	ICG 115	6.7	ICG 81	7.2	ICG 9507	7.6	ICG 9315	8.0	ICG 7190	8.4
	ICG 6402	6.7	ICG 1415	7.2	ICG 9157	7.6	ICG 14127	8.0	ICG 4750	8.4
	ICG 7969	6.7	ICG 2738	7.2	ICG 3775	7.7	ICG 4684	8.1	ICG 6407	8.4
	ICG 10185	6.7	ICG 6263	7.2	ICG 3673	7.7	ICG 6375	8.1	ICG 7181	8.4
	ICGV86155	6.7	ICG 13603	7.2	ICG 6703	7.7	ICG 10556	8.1	ICG 10890	8.4
	ICG 297	6.8	ICG 13856	7.2	ICG 397	7.8	ICG 12879	8.1	ICG 10384	8.5
	ICG 3992	6.8	ICG 1274	7.3	ICG 6888	7.8	ICG 6654	8.2	ICG 10092	8.5
	NCAC 343	6.8	ICG 9418	7.3	ICG 12189	7.8	ICG 7906	8.2	ICG 5195	8.6
	ICG 7153	6.8	ICG 13491	7.3	ICG 3746	7.8	ICG 11219	8.2	ICG 4729	8.6
	ICG 10554	6.8	GKVK 1	7.3	ICG 5494	7.8	ICG 12988	8.2	ICG 12921	8.6
	GKVK 8a	6.8	ICG 13858	7.3	ICG 9249	7.9	ICG 1519	8.3	ICG 1137	8.7
	ICG 4998	6.9	ICG 15309	7.3	ICG 332	7.9	ICG 2106	8.3	ICG 1711	8.7
	ICG 434	6.9	ICG 3681	7.4	ICG 4670	7.9	ICG 4543	8.3	ICG 3421	8.7
	ICG 14118	6.9	ICG 11687	7.5	ICG 3584	7.9	ICG 3343	8.3	ICG 8083	8.8
	ICG14985	6.9	ICG 12682	7.5	ICG 1142	7.9	ICG 11249	8.3		
	ICG 118	7.0	ICG 106	7.5	ICG 12697	7.9				
	ICG 6201	7.0			ICG 442	8.0				
	ICG 7243	7.0			ICG 1399	8.0				

*Disease score

Table 4. Single marker analysis for validation of SSR marker GM 1009

SSR Marker	Disease	Source of variation	Degrees of freedom	Mean sum of square	Pr > F	R^2
GM 1009	Late leaf spot	Model	1	6.503*	0.0483	0.250506
		Error	14	389		
		corrected total	15			

way regression analysis (Sax, 1923) using Statistical Package for the Social Sciences (SPSS) software. All the marker data and the mean phenotypic traits value of genotypes were used for calculating two marker classes and their variances. The significant threshold for association of marker to the trait was set at P B 0.05 for single marker analysis. The adjusted R2 (phenotypic variance) value was used as per cent of variance explained by the marker on the particular trait of test and used as a measure of the magnitude of association. The MSS was significant for the marker in this study and hence the marker was found to be associated with diseases. The result was in confirmation with Sujay *et al*. (2011).

Thus markers in Marker Assisted Selection (MAS) can offer an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes.

Plant genetic resources conserved in the form of germplasm mini core collection preserved the variation in the entire collection of groundnut and the mini core accessions, which only includes 1.29% of the entire collection, represents the total diversity contained in the core collection. This mini core subset drastically reduces the number of entries to be evaluated and provides a working collection of groundnut germplasm that can be extensively examined for all economically important traits. In this study evaluation of mini core collection helped in identifying the sources of resistance to late leaf spot disease which can be used as a donor of disease resistance in breeding for disease resistant cultivars.

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