Indian J. Genet., 74(2): 213-221 (2014)
10.5958/0975-6906.2014.00158.8

Marker detection and genetic analysis for rust resistance of recombinant and backcross inbred lines in groundnut (*Arachis hypogaea* L.)

Shridevi A. Jakkeral*, H. L. Nadaf, M. V. C. Gowda[1], R. S. Bhat[1], R. K. Patil[2], Babu Motagi, P. Kenchanagowda, Ganapati Mukri[3], B. Archana, Prakash Ganagshetty, K. Gangadhar and Lalitha Jaggal

Department of Genetics and Plant Breeding, University Agricultural Sciences, Dharwad, [1]GKVK Bangalore, Department of Biotechnology, [3]Directorate of Maize, New Dehli 110 012

(Received: February 2014; Revised: April 2014; Accepted: May 2014)

Abstract

The present work was conducted to study the genetic variation and identification of microsatellite markers linked to rust resistance in groundnut. An F_6 mapping population and three backcross populations (BC_1F_4, BC_2F_3 and BC_3F_2) were developed from a cross between the susceptible parent GPBD-5 and resistant parent GPBD-4. There were highly significant differences among recombinants for reaction to rust. A little difference was observed between PCV and GCV for reaction to rust. High heritability coupled with high genetic advance as per cent of mean was observed for reaction to rust in F_6, and backcross populations. Bulk segregant analysis in the segregating population of GPBD-5 x GPBD-4 indicated TC5A06 to be putatively linked to rust resistance i.e., single marker analysis (SMA). This marker can be used in marker assisted selection for rust resistance in groundnut improvement program.

Key words: Groundnut, rust resistance, genetic variation, bulk segregant analysis, SSR markers

Introduction

Groundnut (*Arachis hypogaea* L.) is a major oilseed crop of the semi-arid tropics and is unique in consumption pattern that can be consumed directly as an item of food and also utilized in diverse ways. India ranks second in groundnut production after China with an area of 4.93 million hectares and a production of 5.64 million tons during 2010 (FAOSTAT, 2012) [1]. But the average groundnut yield in the country is low (1.14 t/ha) compared to world average and that of China. The productivity is considered to be low because of number of constraints like abiotic (frequent droughts) and biotic stresses (attack by pests and diseases) [2].

Late leaf spot (LLS) and rust are the most destructive, widely distributed and economically important foliar diseases of groundnut causing severe damage to its production. These diseases are commonly present wherever groundnut is grown but their incidence and severity vary at locations and seasons depending on prevailing weather conditions. Rust (*Puccinia arachidis* Speg.) occurs in most of' the groundnut growing states in India but predominantly in South Indian states as conditions favour the development and spread of the disease. Pod yield losses in excess of 50% have been reported due to rust in groundnut [3]. The incidence of this disease also causes reduction in seed weight, total oil and protein content and alters fatty acid composition [4].

Several traits are known to influence resistance to rust and genetic variability for these components exists in germplasm [5]. Sporulation, lesion size, lesion number and latent period are important components that contribute to low field scores of the disease [6]. Resistance to rust in *Arachis hypogaea* L. is reported to be conferred either by a few recessive genes [7] or predominantly controlled by additive, dominance and additive x additive and additive x dominance genetic effects [8]. Digenic mode of inheritance [9], including the resistance conferred by duplicate complementary genes (9:7) has alo been reported [10]. Singh *et al*.,

*Corresponding author's e-mail: gowrishrigpb@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

[11] observed that rust resistance in diploid species was partially dominant as compared to the recessive nature in cultivated species of *Arachis hypogaea* Estimates of heritability from segregating populations are useful in understanding the genetic consequences of hybridization and inbreeding. They can help the breeder selecting and utilizing superior individuals in a population.

Due to simplicity, SSR markers were used in the present study to identify linked molecular markers for rust resistance in cultivated groundnut. Integration of molecular techniques into conventional plant breeding programme has facilitated marker assisted selection, as an attractive strategy for bringing in improvement complex traits. However, for rust resistance, recently few SSR [13] and RAPD markers [14] for yield and its attributing traits putatively linked to rust resistance loci have been identified. In view of above facts, an experiment was conducted to determine the extent of genetic variability generated through hybridization between two groundnut varieties resulting in recombinant inbred lines (RILs) and back cross population evaluated for assessing genetic variability for reaction to rust and same populations were used for identification of SSR marker for rust resistance

Materials and methods

Plant materials

A rust susceptible groundnut variety GPBD-5 was used as a female parent while GPBD-4 a resistant stock as pollen parent to develop the mapping popuations. The GPBD-5 derived from a cross, TG-49 x GPBD-4, is a Spanish bunch type high yielding and large seeded cultivar, and GPBD-4 (KRG-1 x ICGV 86855) is also an improved Spanish bunch groundnut variety with desirable combination of early maturity, high yield, high pod growth rate, desirable pod and kernel features, high oil content and better O/L ratio. Both GPBD-4 and GPBD-5 were developed at University of Agriculture Sciences, Dharwad [15].

A cross between GPBD-5 and GPBF-4 was made during *kharif* 2009 at Main Agricultural Research Station [MARS] Dharwad and the F_1 hybrid was confirmed by appearance of some traits of male parents. The F_1 plants were selfed to produce F_2 and advanced through Single Seed Descent (SSD) till F_6 generation. Each F_6 line epitomizes the individual F_2 plant from which it is derived. Selected resistant F_2 plants were used to backcross to the recurrent parent GPBD-5 to produce BC_1, BC_2 and BC_3 populations. These populations were selfed in later generation *viz.,* BC_1F_4, BC_2F_3 and BC_3F_2 and evaluated in Randomized Block Design (RBD) with two replications except BC_3F_2. Ten seeds of each selected progeny (F_6, BC_1F_4, BC_2F_3 and BC_3F_2) populations were planted in 1 m rows with 30 cm and 10 cm inter and intra-row spacing, respectively. The two parents of respective populations were sown as controls after every 50 rows and 1st rows of each populations. All the necessary agronomic practices were followed to raise a healthy crop except for disease management.

Evaluation of rust resistance

Artificial disease epiphytotic conditions were created in experiments for the rust disease using Infector row technique. TMV2 was used as infector row for rust and planted at every 10th row as well as in the border rows around the field to maintain the effective inoculum load as suggested [16]. In order to encourage disease pressure, artificial inoculation with spraying of spore suspension was done from 40 days after sowing. Rust urediniospores were isolated by soaking and rubbing the infected leaves in water for 30 minutes. The filtered inoculum contained 20,000 urediniospore per ml suspension mixed with tween 8 (0.2 ml per 1.2 litres of water) as mild surfactant and atomized on the plants using Knapsack sprayer in the evening and high humid condition was created by frequent water spraying for three days following inoculation. Besides this infected leaf debris that were collected from the previous season were spread throughout the experimental area to serve as additional inoculum. Incidence of rust was recorded on 1-9 scale as suggested by Subbarao *et al.* [17]. Recombinants were evaluated for reaction to rust (90 DAS).

Statistical parameters

Genotypic coefficient of variation and phenotypic coefficient of variation [18] heritability [19], genetic advance [20], as a per cent over mean (GAM%) were computed by different statistical methods [21].

DNA extraction and Polymerase Chain Reactions (PCR)

DNA was extracted from F_6 and backcross (BC_1F_4, BC_2F_3 and BC_3F_2) populations and parents using CTAB method [22]. DNA quality was checked and quantified on 0.8% agarose gel with known concentration of uncut lambda DNA as standard. Polymerase Chain Reactions (PCR) were performed

using a Touch - Down PCR profile. DNA amplification was performed in 20 µl reaction mixture containing 20ng/µl template DNA, 10 pM/µl SSR primer pair (Forward and Reverse), 25 mM $MgCl_2$ (Qiagen), 2 mM dNTPs, 10X PCR buffer (Qiagen) and 5U/µl Taq DNA polymerase (Qiagen). Touch - Down PCR amplification using a programme in which the annealing temperature was lowered from 65 to 60 by 1°C every cycle, followed by 40 additional cycles at 59°C. After initial denaturation for 5 min at 95°C each cycle comprised 30 sec denaturation at 94°C, 45 sec anneaniling at 65°C and and 1 min extension at 72°C with a final extension for 10 min at 72°C. The PCR products were mixed with 2 µl of loading dye (0.25% bromophenol blue with 40% sucrose) and separated on 1.4% agarose gel using 1X TAE buffer of pH 8.0 containing ethidium bromide. The gel was documented using white/2UV Trans-illuminator of Ultra Violet products, London.The amplified products which showed less base pair size on agarose were separated on 4% metaphore agarose gel using the TAE buffer. The primere sequences earlier identified were used in the study [32, 33].

Marker-phenotype association analysis

For analysis of molecular markers, recombinant inbred lines (F_6) and backcross populations were used and 150 specific SSR primers were used for screening parental genotypes *viz.,* GPBD-5 and GPBD-4. Single marker analysis (SMA) was performed to tag and confirm potential SSR markers linked to the trait based on phenotypic and genotypic data pertaining to the F_6 (RILs) direct cross and backcross populations.

Result and discussion

The genetic gain through selection depends on the quantum of variability and extent of heritability. In all the populations the analysis of mean sum of squares revealed the presence of significant differences among recombinants for reaction to rust indicating generation of genetic variability (Table 1). The decrease in the mean value of rust disease can be considered as desirable as it was evident from the rust reaction (5.66), (5.66) and (5.50) in F_6, BC_1F_4 and BC_2F_3 populations, respectively (Table 2). This indicated the presence of recombinants with reduced biotic stresses in segregating generations to make effective selection. The range of reaction to rust was wide (4-9) F_6, BC_1F_4 and BC_2F_3 generations (Table 2). This might be due to diversity among parental genotypes. The hybridization between diverse parents would enhance the variability and offers chances to isolate genotypes having desirable combination of characters aiming the recombinants. The recombination has led to the selection of genotypes with higher number of pods per plant, pod and kernel yield per plant, shelling per cent and 100 seed weight (g) (Tables 3 and 4).

The estimates PCV values were always higher than GCV suggesting the influence of environmental factors. Less difference observed between PCV and GCV in certain cases indicated greater role of genetic components and less influence by environment. The differences between PCV and GCV observed for

Table 1. Mean sum of squares for reaction to rust in of in the cross GPBD-5 x GPBD-4 in summer 2012

Generation/ population	F_6		BC_1F_4		BC_2F_3	
Source of variation	df	MSS	df	MSS	df	MSS
Replication	1	0.039	1	0.015	1	0.463
Recombinant lines	50	4.273**	39	1.342**	16	5.897**
Error	50	0.019	39	0.421	16	0.194

* - Significant at 5% probability level; ** - Significant at 1% probability level

Table 2. Mean, range and genetic variability for reaction to rust in different generations desired from GPBD-5 x GPBD-4 during summer 2012

Generation/population	Mean	Range	PCV (%)	GCV (%)	h^2 b.s. (%)	GA	GAM (%)
F_6	5.66	4-9	25.79	25.73	99.55	2.99	52.89
BC_1F_4	5.66	4-9	19.32	17.60	82.99	1.87	33.03
BC_2F_3	5.50	4-9	21.32	20.71	94.39	2.27	41.45

GCV – Genotypic coefficient of variation; PCV – Phenotypic coefficient of variation; GA – Genetic advance; GAM – Genetic advance as per cent of mean; h^2 b.s – Heritability in broad sense

Table 3. Superior recombinants identified with rust resistance in of the cross GPBD-5 x GPBD-4 during summer 2012 at MARS, Dharwad

Recombinant lines (RL)	No. of pods/ plant	Pod yield/ plant (g)	Kernel yield/ plant (g)	100-seed weight (g)	Reaction to rust at 90 DAS (1-9 scale)	Protein content (%)	Oil content (%)
			F_6 generation				
RL 37	43.5	43.1	30.5	41.5	4	27.38	48.92
RL 40	31.1	42.5	34.5	43	4	26.74	50.99
RL 29	46.5	42.25	34.5	49.5	4	27.85	48.36
RL 42	45.5	42.3	32.05	49.5	4	28.18	49.7
RL 41	43.2	40.5	29.05	47	4	28.98	48.68
Mean	19.09	24.83	15.93	36.21	5.66	-	-
TMV 2 (Check)	28.5	30.4	20.2	36	9	28.86	48.95
GPBD-5	48.8	42.9	32.4	43.2	8	29.41	49.48
GPBD-4	40.6	43.4	30.8	32	4	27.73	48.59
CD at 5%	3.18	8.68	5.03	2.74	0.27	-	-
CD at 1%	4.24	11.58	6.7	3.65	0.37	-	-
			BC_2F_3 population				
RL 27	43	40.0	32.0	40	4	26.93	49.37
RL 25	41	39.0	33.0	38	4	28.14	48.21
RL 2	40	38.50	31.0	42.0	4	27.95	48.05
RL 9	35	38.0	35.0	36	4	31.6	40.90
RL 5	42.75	37.0	33.75	46.0	4	28.65	48.75
Mean	17.8	29.5	17.4	39.5	5.5	-	-
TMV2(check)	31.3	23.5	21.3	22.3	9	26.6	40.4
GPBD-5	40.1	43.2	33.8	46.8	8	30.36	49.8
GPBD-4	43.1	40.2	31.2	32.9	4	30.16	48.0
CD at 5%	2.34	10.18	4.74	2.87	0.83	-	-
CD at 1%	3.22	14.02	6.52	3.96	1.14	-	-

* - Significant at 5% probability level; **- Significant at 1% at probability level

Table 4. Superior recombinants (Single plant observation) identified with rust resistance in BC_3F_2 population of cross GPBD-5 x GPBD-4 during summer 2012 at MARS, Dharwad

Recom-binant lines (RL)	Plant height (cm)	No. of primary branches/ plant	Leaf length (cm)	Leaf width (cm)	No. of pods/ plant	Pod yield/ plant (g)	Kernel yield/ plant (g)	Shelling per cent	No. of thrips/ leaf let (30 DAS)	Reaction rust at 90 DAS (1-9 scale)	Protein content (%)	Oil content (%)
RL 30	20.2	6	5.3	2.5	42.2	47.4	37.2	78.5	3.1	4.0	27.78	48.20
RL 31	24	9	4.8	2.1	49.8	47.2	34.4	72.9	2.9	4.0	27.06	46.02
RL 32	25.3	10	4.6	2.5	48.3	45.8	32.3	70.5	3.0	4.0	27.04	48.72
RL 33	24	8	5.6	2.5	44.4	45.1	34.4	76.3	3.0	4.0	26.76	49.46
RL 34	23.6	6	5.3	2.6	42.2	44.7	30.1	67.3	3.0	4.0	27.28	47.15
RL 35	23.2	8	5.3	2.2	46.1	43.8	32.3	73.7	3.5	4.0	28.11	48.17
RL 36	20.5	5	5.0	2.0	40.5	43.2	31.4	72.7	3.0	4.0	29.95	48.05
RL 37	24.2	7	4.3	2.6	44.2	42.6	33.2	77.9	1.8	4.0	29.98	49.08
RL 38	22.3	11	5.7	2.5	47.3	41.3	32.2	78.0	2.0	4.0	29.40	47.93
Mean	14.0	6.9	4.3	2.7	22.5	26.8	19.6	73.2	2.4	5.6	-	-
TMV2(check)	29.6	7.0	6.0	3.0	32.3	34.5	19.8	61.0	2.3	9	27.20	40.10
GPBD-5	24.8	8	5.6	2.1	42.6	44.6	33.4	71.1	6.0	8	23.01	48.80
GPBD-4	23.6	5	5.1	2.3	42.1	42.2	20.7	73.7	1.6	4	30.06	48.60

reaction to rust among $F_{6,}$ BC_1F_4 BC_2F_3 were less. High heritability coupled with high genetic advance as per cent of mean was observed for reaction to rust. The results indicate that, this trait is under the control of additive gene action. These results are similar with the findings of Khedikar [23] who reported LLS disease score at 70 and 90 DAS. Similarly genetic advance can however, help to predict the extent of improvement that can be achieved for this trait. A high genetic gain along with high heritability would suggest suitable conditions for making effective selection.

Identification of high productive rust resistant recombinants

Generally, lines which are potential for one or more characters, such as resistance to disease may be useful for breeding. The potential top 5 lines with disease score 4 at 90 DAS , with >30 total number of pods/plant, >20g kernel yield per plant (g), > 25 g of 100- seed weight, > 60 per cent of shelling and a pod yield/plant of >10 g with values of protein and oil content are given in the Tables 3 and 4. Several lines recorded improvement in at least one of these characters indicating scope for breaking the undesirable association. Thus, potential variability was evident in the cross high frequency for resistance, however the cross was less potential in generating resistant segregants in combination with other desirable traits. It is thus suffested that there is a need for raising large segregating population or intermating among the selected segregants to get desirable recombinations. Krishnakanth *et al*. [24] reported similar results in F_5 genotypes for resistance to stem and pod rot in groundnut.

Multiple backcrossing, in general, recorded higher percentage of superior lines for all productivity parameters studied and disease resistance. BC_3F_2 population showed higher number of recombinant lines (30-34) which were found superior and at par number of pods per plant, kernel and pod yield per plant and shelling per cent as compared to recurrent parent GPBD-5. In BC_1F_4 population, recombinants (12, 29, 18 and 20) lines were found to be superior with pod yield per plant. The recombinant line (12) was at par with resistant number of pods per plant, pod and kernel yield, shelling per cent and medium 100 seed weight as compared to GPBD-5.

However all the recombinant linew were superior in resistance in the BC_3F_2 involving GPBD-5 and GPBD-4 in the generated more number of desirable recombinant lines showing improvement in individual and combination of complexly inherited characters. Cultivated groundnut being an allotetraploid with similar sets of genome [25] contains more genes controlling the same character would be possible. Epistatic gene interactions are most likely to be involved in the inheritance of quantitative traits. Hence, in backcrosses, due to repeated recombination provide an opportunity for shuffling of genes, the possibility of realizing desirable recombinants would increase. The existence of linkage and/or pleiotrophy of undesirable characters with resistance and higher yields may get broken which leads to greater reshuffling of genes to recover desirable recombinants. Multiple back crosses are much superior in producing desirable lines [26].

Iroume and Knauft [27] suggested that an index combining yield and disease severity traits may be useful in selecting for disease resistant productive crosses in early generation. Based on available information and the results of present study, a breeding strategy (backcross) can be proposed for incorporating rust resistance to the adapted cultivars of groundnut. The lines in the segregating population could be used to backcross the recurrent parent (GPBD-5) selection for realizing greater reshuffling of genes. However, in groundnut, the conventional recurrent selection cannot be resorted to as the hybridization itself is difficult. Therefore, repeated backcrossing of the few selected group of superior lines provides an opportunity for multiparental gene recombination. The selection of lines superior for resistance, productivity and desirable pod features can be done in early (BC_3F_2) generations. Backcrossing of the selected lines should be repeated till desirable recombinations are recovered in higher frequencies.

Frequency distribution

The variation existed for yield and its component traits and reaction to rust in direct and backcross populations are presented graphically using frequency distribution of mean from recombinants in all populations. The yield and its components traits and rust disease scores were plotted on X-axis against genotype frequency on Y-axis with equal class intervals. The resulting histogram showed near normal curves for all agronomic traits and reaction to rust in all the populations. In general the distribution was normal and within the parental limit for all yield component traits and reaction to rust. Transgressive segregants were also observed in both the directions (Figs. 1, 2a&b).

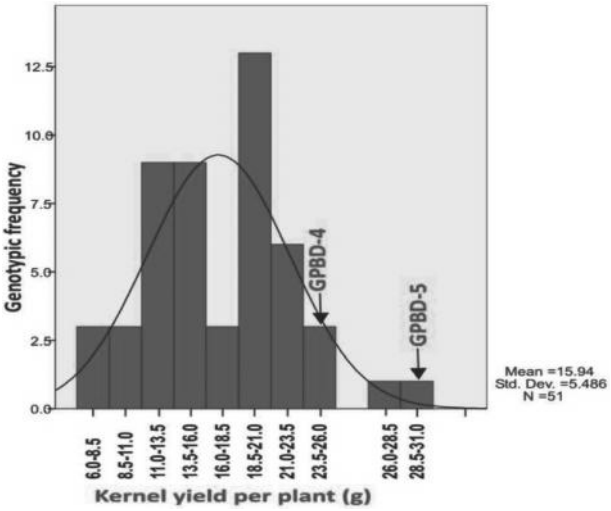

Fig. 1. Frequency distribution of F6 population for kernel yield/plot in the cross of GPBD-5xGPBD-4

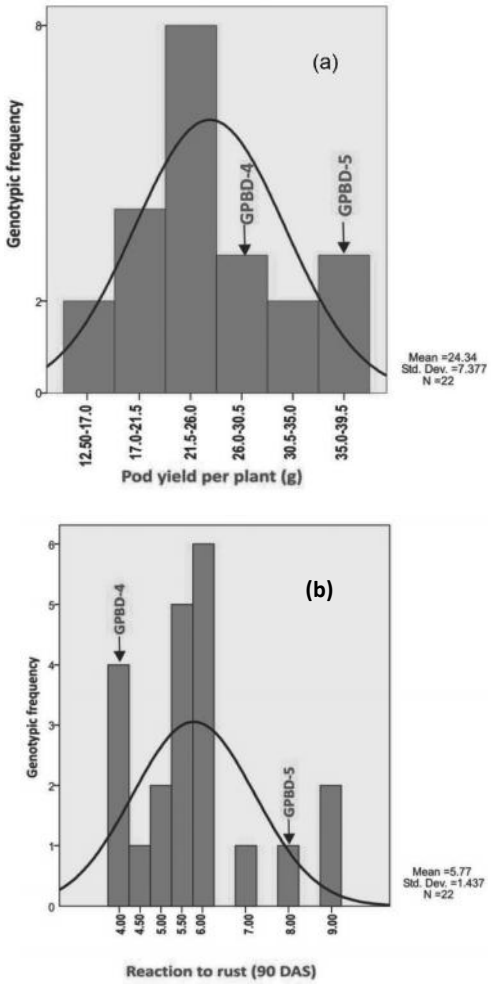

Fig. 2. Frequency distribution of BC_2F_3 ppopulation for (a) pod yield/plant and (b) reaction to rust in the cross of GPBD-5 x GPBD-4

Considerable efforts have been made to identify sources of resistance to rust in groundnut because of its importance worldwide. Rust resistant sources are available in wild species, interspecifc derivatives and cultivated species of groundnut. Mere identification of markers based on resistant or susceptible germplasm lines will not have any practical utility in the breeding programme but tagging of the markers with the traits of interest and assessing their contribution towards phenotypic variation will substantiate the utility of markers. Varma *et al.* [28] screened 25 SSR markers in two mapping populations (ICGV 99003 × TMV 2 and ICGV 99005 × TMV 2) and identified five markers associated with rust resistance. Mace *et al.* [29] used 23 SSR markers in 22 genotypes and identified 12 markers associated with resistance to LLS and rust. In the present investigation, all these markers were employed but none were found to be associated with rust indicating genotype specific association.

Since resistance to rust and late leaf spot were complex with several components of resistance (30), the power of QTL detection could be increased by phenotyping the mapping population for the components of resistance like incubation period, latent period, lesion size, lesion on main stem for LLS and rust and also number of pustule, pustule diameter for rust. Classical genetic analyses indicated multiple recessive genes governing resistance to LLS, but few recessive genes for rust resistance. Recently, five SSR loci were identified to be associated with rust resistance through comparison of results obtained from locus by-locus AMOVA and Kruskal-Wallis one-way ANOVA on the diverse set of 22 cultivated groundnut genotypes [31]. In the present investigation, 150 specific SSR primers from RIL population of cross TAG-24 x GPBD-4 and primers screened and used in earlier reports [32, 33] were utilized for screening parental genotypes *viz.,* GPBD-5 and GPBD-4.

Analysis of molecular marker

One hundred and fifty SSR primers were used for screening parental genotypes *viz.,* GPBD-5 and GPBD-4. A total of 36 polymorphic primers only 13 were polymorphic between the parents of the cross GPBD-5 x GPBD-4 indicating low polymorphism in the parental genotypes. The tagging population of the cross, GPBD-5 x GPBD-4 was subjected to bulk segregant analysis to identify putatively linked markers for rust resistance. Out of 13 SSR markers, only two were found polymorphic between resistant and susceptible bulks. These two markers were analyzed again on individual

eight resistant and susceptible plants. Of the two markers one SSR primer (TC5A06) (Fig. 3) was found polymorphic indicating that this marker is putatively linked to rust resistance gene. Rust resistant lines were identified based on this markers in direct (F_6) population and backcross (BC_1F_4, BC_2F_3 and BC_3F_2) populations.

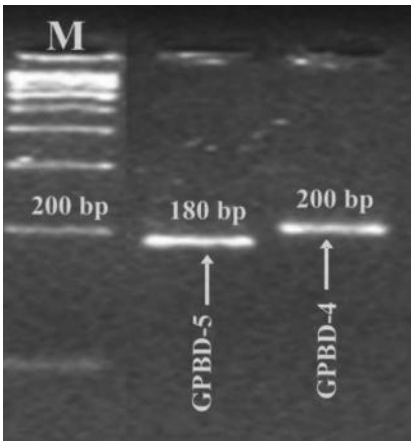

Fig. 3. Tc5A06 primer showing polymorphism between parents GPBD-5 and GPBD-4

SSR markers and rust disease association analysis

The polymorphic marker identified based on analysis of bulked extremes were used to screen direct (F_6) population and backcross (BC_1F_4, BC_2F_3, BC_3F_2) populations to establish the association between the respective marker and phenotype. Single marker analysis (SMA) was used for simple linear regression method [34] to find out the significant marker trait. The analysis revealed that TC5A06 marker accounted for 31.20 per cent variation in F_6 population, 68.90 per cent in BC_1F_4 population, 81.40 per cent in BC_2F_3 population and in BC_3F_2 population; 72.50 per cent of the total variation for rust resistance in the cross of GPBD-5 x GPBD-4 (Table 5).

Table 5. Single marker analysis in the cross of GPBD-5 x GPBD-4

Trait	Marker	Populations	R^2 adjusted
Rust	TC5A06	F_6	31.20
		BC_1F_4	68.90
		BC_2F_3	81.40
		BC_3F_2	72.50

Peanut is one of the most important crops in the world, both for oil and as a protein source. DNA markers have significant advantages over protein or phenotypic markers. Marker assisted selection (MAS) can improve the efficiency of conventional breeding especially in the case of low heritable and recessive traits, where phenotypic selection is difficult, expensive, lack accuracy or precision. Development of disease is mostly erratic and it varies according to season, location and year. Moreover creation of artificial disease epiphytotics is costly and time consuming and also availability of hot spot for this particular disease is one of the paramount factors for screening and MAS can act as an elixir in such circumstances. Identification of resistant lines at seedling stage is possible, when MAS is employed. Linkage drag is also one of the serious problems while transferring resistance from unadapted wild and weedy germplasm into elite lines and it can be dissected out through tightly linked markers. It can help in the introgression of resistance from wild relatives and fastest recovery of the recurrent parent genome can be achieved by using foreground and background selection approach. Since resistance to rust reported to be governed by recessive genes, MAS can save one generation of selfing to select recessive genes using linked markers.

Validation of this identified markers in germplasm lines of different genetic background and mapping population indicated that it could be directly used for marker-assisted breeding for rust resistance in groundnut.

Genetic analysis revealed that the parents involved in the study differed for many genes which resulted in creating large amount of genetic variability for the yield and yield components and reaction to rust in recombinant inbred lines (F_6) and backcross population. This suggested the scope of this material and parents in the future breeding programme. These superior recombinants obtained should be maintained and forwarded to further generation to stabilize them.

Reference

1. **FAOSTAT. 2012.** Statistical data on crops, groundnut, area, production and productivity of Indi &world,2012.http://faostat.fao.org/site/567/default.aspx.#ancor.

2. **Padmaja D., Brahmeswara Rao, Eswari K. B. and S. Madhusudhan Reddy.** 2013. Genetic Variability, Heritability for Late leaf Spot tolerance and Productivity Traits in a Recombinant Inbred Line Population of Groundnut (*Arachis hypogaea* L.)

Journal of Agriculture andVeterinary Science (IOSR-JAVS Volume **5**, Issue 1 (Sep.- Oct. 2013), PP 36-41.

3. **Sandhikar R. N., Bulbule S. V. and Mayee C. D.** 1989. Prediction models for rust epidemic in groundnut. Indian J. Mycol. Plant Pathol., **19**: 60-67.
4. **Dwivedi S. L., Nigam S. N., Subrhamanyam P., Jambunathan R., Nagabhushanam V. S., Reddy P. M., Rahunath K. and McDonald D**. 1993. Effect of foliar disease control by chlorothalonil an pod yield and quality characteristics of confectionary groundnuts (*Arachis hypogaea* L.). J. Scient. Food Agri., **63** : 265-271.
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. **Aquino V. M., Shokes F. M., Gorbet D. W. and Nutter F. W.** 1995. Late leaf spot progression on peanut as affected by components of partial resistance. Plant Disease, **79**: 74-78.
7. **Paramasivam K., Jayasekhar M., Rajsekharan R. and Veerbadhiran P.** 1990. Inheritance of rust resistance in groundnut (*A. hypogaea* L). Madras Agril. J., **77**: 50-52.
8. **Varman P., Ravendran T. S. and Ganapathy T.** 1991. Genetic analysis of rust resistance in groundnut *Arachis hypogaea* L., J. Oilseed Res., **8**: 35-39.
9. **Bromfield K. R. and Bailey W. K.** 1972. Inheritance of resistance to *Puccinia arachidis* in peanut. Phytopathol., **62**: 748.
10. **Motagi.** 2001. Genetic analysis of resistance to late leaf spot and rust vis-à-vis productivity in groundnut (*Arachis hypogaea* L.). Ph. D. Thesis, Univ. Agril. Sci., Dharwad, India, pp. 1- 165.
11. **Singh A. K., Subrahmanyam P. and Moss J. P.** 1984. The dominant nature of resistant to *Puccinia arachidis* in certain wild *Arachis* species. Oleagineux, **39**: 535-538.
12. **Dwivedi S. L., Pande S., Rao J. N. and Nigam S. N.** 2002. Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut (*Arachis hypogaea* L.). Euphytica, **125**: 81-88.
13. **Khedikar Y. P., Gowda M. V. C. and Sarvamangala C.** 2010. A QTL study on leaf spot and rust revealed on major QTL for molecular breeding for rust resistance in groundnut. TAG., **7**: 1-14.
14. **Suvendu Mondal, Anand M. Badigannavar and G. S. S. Murty.** 2007. RAPD markers linked to a rust resistance gene in cultivatedgroundnut (*Arachis hypogaea* L.) Euphytica 159:233-239.
15. **Gowda M. V. C., Motagi B. N., Naidu G. K., Diddimani S. B. and Sheshagiri R.** 2002. GPBD 4 : A Spanish bunch groundnut genotype resistant to rust and late leaf spot. Intr. Arachis Newsletter, **22**: 29-32.
16. **Subrahmanyam P., McDonald D., Waliyar F., Reddy L. J., Nigam S. N., Gibbons R. W., Rao V. R., Singh A. K., Pande S., Reddy P. M., Subba Rao P. V.** 1995. Screening methods and sources of resistance to rust and late leaf spot of groundnut. In: Information bulletin no. 47. ICRISAT, Patancheru India
17. **Subbarao P. V., Subrahmaniyam P. and Reddy P. M.** 1990. A modified nine point disease scale for assessment of rust and late leaf spot of groundnut. *In*: Second International Congress of French Phytopathological Society, 28-30 November, 1990, Montpellier, France, p. 25.
18. **Burton G. W. and Devane E. M.** 1953. Estimating heritability in tall Fescue (*Festuca aruniodinacea*) from replicated clonal material. Agron. J., **45**: 478-481.
19. **Hanson G. H., Robinson H. F. and Comstock R. E.** 1956. Biometrical studies of yield in segregating population of Korean lespedeza. Agron. J., **48**: 267-282.
20. **Robinson H. F.** 1949. Quantitative genetics in relation to breeding on the centennial of Mendelism. Indian J. Genet., **26** : 171-187.
21. **Johnson H. W., Robinson H. F. and Comstock R. F.** 1955. Estimates of genetic and environmental variability in soybean. Agronomy Journal, **47**: 314-318.
22. **Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W.** 1984. Ribosomal DNA spacer length polymorphism in barley. Mendelian inheritance, chromosomal location and population dynamics. Proc. Nat. Acad. Sci., USA, **81**: 8014-8018.
23. **Khedikar Y. P.** 2008. Molecular tagging and Mapping of resistance to late leaf spot and rust in groundnut (*Arachis hypogaea* L.). Ph.D. Thesis. University of Agricultural Science, Dharwad, India.
24. **Krishnakanth A., Naidu G. K. and Gowda M. V. C.** 2005. selection for resistance to stem and pod rot in groundnut (*Arachis hypogaea* L.). Journal of Oilseeds Research, **22**: 226-227.
25. **Kochert G., Halward T., Branch W. D. and Simpson C. E.** 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet., **81**: 565-570.
26. **Hanson W. D.** 1959. The breakup of initial linkage blocks under selected mating systems. Genetc., **44** : 857-868.

27. **Iroume R. N. and Knauft D. F.** 1987. Heritability and correlations for pod yield and leaf spot resistance in peanut (*Arachis hypogaea* L.) implications for early generation selection. Peanut Sci., **14**: 46-50.

28. **Varman P., Ravendran T. S. and Ganapathy T.** 1991. Genetic analysis of rust resistance in groundnut *Arachis hypogaea* L. J. Oilseed Res., **8**: 35-39.

29. **Mace E. S., Phong D.T., Upadhyaya H. D., Chandra S. and Crouch J. H.** 2006. SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. Euphytica, **152**: 317-330.

30. **Dwivedi S. L., Pande S., Rao J. N. and Nigam S. N.** 2002. Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut (*Arachis hypogaea* L.). Euphytica, **125**: 81-88.

31. **Mace E. S., Varshney R. K., Mahalakshmi V., Seetha K., Gafoor A., Leeladevi Y. and Crouch J. H.** 2007. *In silico* development of simple sequence repeat markers within the aeshynomenoid / dalbergoid and genistoid clades of the Leguminosae family and their transferability to *Arachis hypogaea*, groundnut. Plant Sci., **174**: 51-60.

32. **Ferguson M. E., Burow M. D., Schulze S. R., Bramel P. J., Paterson A. H., Kresovich S. and Mitchell S.** 2004. Microsatellite identification and characterization in peanut (*A. hypogaea* L.). Theor. Appl. Genet., **108**: 1064-1070.

33. **Moretzsohn M. C., Hopkins M. S., Mitchell S. E., Kresovich S., Valls J. F. M. and Ferreira M. E.** 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol., **4**: 11.

34. **Haley C. A. and Knott S. A.** 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity, **69**: 315-324.