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Validation of SSR markers linked to late leaf spot and rust in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

The low productivity of Groundnut in India is ascribed to many biotic and abiotic stresses in the cultivation of the crop. Among the biotic stresses, the two major foliar diseases *viz.*, late leaf spot (*Phaeoisariopsispersonata* [(Berk. and Curt.) Deighton] and rust (*Puccinia arachidis* Speg.) causing yield loss up to 50-70 *per cent* (Subrahmaniyam *et al.*, 1984). The experiment was conducted at UAS, Bengaluru during 2011 and 2012 to validate the markers IPAHM 103, GM 2301 and GM 1009 identified to be linked to rust resistance and late leaf spot resistance respectively in different genetic backgrounds i.e., validating these markers over a set of resistant and susceptible genotypes for their utilization in marker assisted selection (MAS). During *kharif* 2011 minicore along with other advanced breeding lines were phenotypically screened for late leaf spot and rust disease using modified 9 point scale (Subbarao *et al.*, 1990). Few genotypes consisting of minicore accessions, advanced lines and local cultivars were selected for validation of the above mentioned markers. The DNA extraction was done as per CTAB method given by Saghai-Maroof*et al.* (1984). Polymerase Chain Reaction (PCR) was performed by using a Touch-Down PCR. Through single marker analysis it was found that these markers IPAHM 103, GM 2301 and GM 1009 were found to be associated with rust resistance and late leaf spot resistance respectively in this study. Hence these markers can be effectively used for MAS for selecting the resistant genotypes inearly generations of resistant breeding.

***Key words** : Groundnut, Late leaf spot, Rust, MAS, Validation.*

Introduction

Groundnut (*Arachis hypogaea* L.) also known as peanut, is an important oilseed crop in tropical and subtropical regions of the world. Late leaf spot caused by *Phaseoisariopsis personata* (Berk & curt) V. Arx. and rust by *Puccinia arachidis* Speg. are the most important foliar fungal diseases causing yield loss up to 50-70 per cent (Subrahmaniyam *et al.*, 1984). Though several effective fungicides are available to control the diseases but host-controlled resistance is considered as the best strategy to surmount additional cost of production and hazardous effect of fungicides on the soil and environment.

Recent advances in the area of crop genomics have offered molecular tools to assist breeding (Varshney *et al.* 2005a). Introgression of desired chromosomal segment in the progeny through precise monitoring using trait-linked marker, the process called marker-assisted selection (MAS), has been successfully applied in several cereal and some legume crops, resulting in the development of improved varieties/germplasm (Varshney *et al.*2006). Availability of molecular markers and genetic link-

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age maps is, however, the prerequisite for undertaking molecular breeding activities particularly identifying and localizing important genes, controlling qualitatively and quantitatively inherited traits (Varshney *et al.*, 2006). Among different marker systems analyzed in the groundnut, like other plant species, SSR markers have been found more informative and useful for genetic analysis and breeding applications (Gupta and Varshney, 2000). In the case of groundnut, several hundred SSR markers have been developed and characterized during last 5 years all over the world (Cuc *et al.*, 2008; Gautami *et al.* 2009) unpublished markers from University of California-Davis, USA and University of Georgia, USA). Several markers were linked to late leaf spot and rust resistance like IPAHM103, GM2009, GM1536, GM2301, GM2079 have been identified associated with a major QTL for rust resistance. In the case of LLS resistance GM1573, pPGPseq8D09 and GM 1009 have been found associated. Some of these markers are currently being deployed for molecular breeding for molecular breeding for rust. To test its association one has to go for its validation in different genetic backgrounds. Thus the present investigation was carried out to validate the markers IPAHM103, GM2301 and GM 1009 using different genetic backgrounds.

Material and Methods

The present investigation was carried out during *kharif* 2011 and summer 2012 at UAS, Bangalore, India. During *kharif* 2011. The experimental material comprised of 225 genotypes which includes 188 accessions of groundnut mini core set representing fastigiata (33), vulgaris (71), peruviana (2), aequatoriana (1), hypogaea runner (33) and hypogaea bunch (48) and other advanced breeding lines. The experiment was taken in Simple Lattice Design (15 × 15) with two replications. The cultivar TMV 2 was used as susceptible check or spreader row for natural late leaf spot and rust disease incidence and its spread. Each genotype was grown with a spacing of 40 cm between rows and 15 cm between plants within the rows. All these set of different genotypes were phenotypically screened for late leaf spot and rust using modified 9 point scale (Subbarao *et al.*1990). A set of twenty two genotypes with differential reaction to late leaf spot and rust resistance (Table 1) were selected for validating the markers IPAHM 103, GM 2301 and GM 1009 linked to rust resistance and late leaf spot resistance respectively during summer 2012 in randomised block design with two replication.DNA samples from each 22 genotypes were extracted using CTAB method of Saghai-Maroof *et al.* (1984). DNA samples were amplified using Touch-Down PCR. The quality and quantity was checked by using 0.8 % (w/v) agarose gel electrophoresis. Three SSR primer pairs specific to cultivated groundnut selected from the previous study (Khedikar *et al.* 2010 and Sujay, *et al.*, 2011) were used. The results were obtained on the Non-Denaturing Polyacrylamide Gel Electrophoresis (PAGE). The markers were validated using single marker analysis (SMA).

Results and Discussion

Validation of marker: The marker GM 1009 detected by Sujay *et al.* (2011) through comprehensive Quantitative Trait Locus (QTL) analysis was tightly linked to late leaf spot resistance and the markers IPAHM 103 and GM 2301 identified as a tightly

Table 1. List of genotypes selected for validation based on their disease score during *kharif* 2011

Sl. No	Genotypes	Disease score in *kharif* 2011		Sl. No	Genotypes	Disease score in *kharif* 2011	
		Late leaf Spot	Rust			Late leaf Spot	Rust
1	ICGV 87165	4.40	1.70	12	TMV 2	8.30	6.50
2	ICGV 86590	3.10	1.00	13	ICG 2857	4.50	3.00
3	ICGV 99003	4.00	1.60	14	ICG 2773	4.80	4.40
4	ICGV 99004	5.10	5.00	15	ICG 3027	4.40	4.00
5	ICGV 99005	3.60	4.00	16	ICG 5286	4.10	4.00
6	ICGV 86699	2.50	3.00	17	ICG 11426	4.30	4.30
7	ICGV 93021	5.00	1.00	18	ICG 13942	6.00	5.60
8	ICGV 91177	2.30	1.50	19	ICG 10036	6.50	4.50
9	GPBD 4	3.10	2.00	20	ICG 13099	5.60	5.80
10	GBFDS 272	2.50	2.50	21	ICG 5745	4.90	4.30
11	M 282	4.80	2.00	22	GKVK 13	4.30	4.70

linked SSR markers for rust resistance by both CIM and single marker analysis (SMA) were validated under different genetic backgrounds.

Data scoring and data analysis (Single marker analysis)

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 (Fig 1) for GM 1009 and at 160bp, 137bp for IPHM 103 and GM 2301 (Fig. 2 and Fig. 3) respectively. 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 2) using one way regression analysis (Sax, 1923) using 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 all themarkers in this study and hence these markers were found to be associated with diseases. The results were in confirmation with Khedikar *et al.* (2010) and Sujay *et al.* (2011).

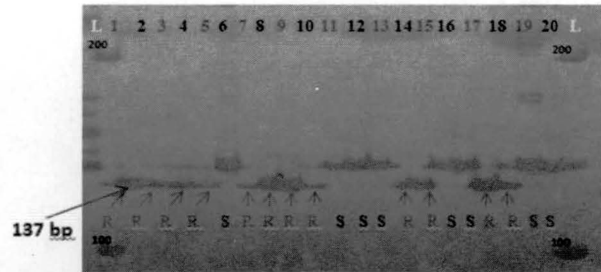

Fig. 2. Profile of 20genotypes for SSR marker GM 2301.

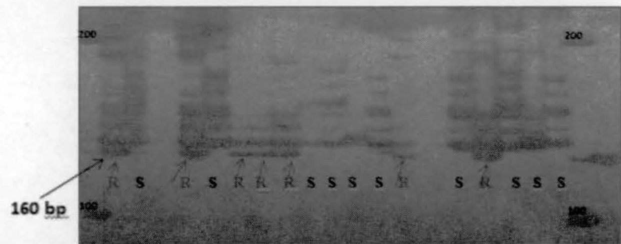

Fig 3. Profile of 17 genotypes for SSR marker IPHM 103.

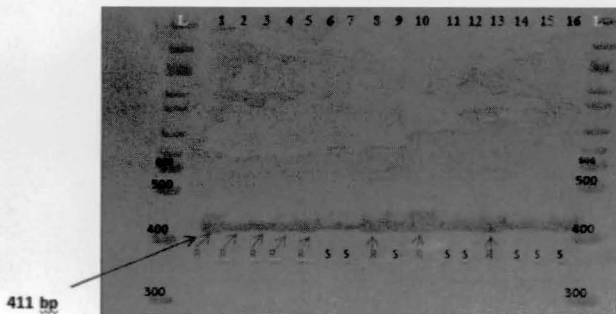

Fig. 1. Profile of 16 genotypes for SSR marker GM 1009.

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 2. Single marker analysis for validation of SSR markers

SSR Marker	Disease	Source of variation	Degrees of freedom	Mean sum of square	Pr> F	R Square
GM 1009	Late leaf spot	Model	1	6.503*	0.0483	0.250506
		Error	14	1.389		
		Corrected total	15			
GM 2301	Rust	Model	1	14.981*	0.0096	
		Error	18	1.787		
		Corrected total	19			
IPHM 103	Rust	Model	1	6.508*	0.0490	
		Error	15	2.386		
		Corrected total	16			

DNA fragments were separated on 6% non-denaturing Poly-Acrylamide Gel Electrophoresis (PAGE). The left side arrow shows the resistant band at 137 bp. Resistance genotypes are marked as (R) and susceptible genotypes are marked as (S). Lane L, 100bp DNA ladder, Lane 1- 4, 6-9,13,14,17,18 , ICGV 93021, GPBD 4, ICG 87165, GBFD 5272, ICGV 86699, ICG 5745, ICGV 99005, ICG 11426, GKVK 13 and ICG 2857 respectively. Lane 5, 10-12, 16, 19, 20, M 282, ICG 5286, ICG 2773, ICG 10036, ICGV 13099 and TMV 2 respectively.

DNA fragments were separated on 6% non-denaturing Poly-Acrylamide Gel Electrophoresis (PAGE). The left side arrow shows the resistant band at 160bp. Resistance genotypes are marked as (R) and susceptible genotypes are marked as (S). Lane L, 100bp DNA ladder, LANE 1, 4, 6-8, 13, 16, ICGV 86590, GPBD 4, ICGV 87165, GBFD 5272, ICGV 86699, ICG 11426 and GKVK 13 respectively. Lane 2, 5, 9-12, 15, 17-19, ICGV 93021, M 282, ICG 5745, ICG 3027, ICG 5286, ICG 2773, ICGV 99004, ICG 2857, ICG 13099 and TMV 2 respectively

While validating the these marker over a set of resistant and susceptible genotypes, the markers IPAHM 103, GM 2301 and GM 1009 had found to be association with rust and late leaf spot resistance respectively through single marker analysis. Hence these tightly linked markers could be utilized in the marker assisted breeding programme over a wide range of genetic background.

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