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IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) FOR LATE LEAF SPOT DISEASE RESISTANCE IN GROUNDNUT (*ARACHIS HYPOGAEA L.)*

D. Shoba, N. Manivannan*, P. Vindhiyavarman and S.N. Nigam[1]

Department of Oilseeds,
Tamil Nadu Agricultural University, Coimbatore- 641 003, INDIA

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ABSTRACT

TMV 2, a LLS susceptible genotype and the COG 0437, a LLS resistant genotype were crossed and their F_2 population was used for marker analysis in the present investigation. The phenotypic mean data on F_2:3 progenies were used as phenotype. Seventy seven SSR markers were used for the parental polymorphism. Among SSR markers, nine markers were found polymorphic between the parents TMV 2 and COG 0437. Eight markers formed a linkage group and covered a distance of 37.2 cM with an average 4.65 cM at LOD 3.0. The composite interval mapping analysis resulted in two QTLs *viz.,* each one for hundred kernel weight and LLS severity score with 6.1 and 37.9 R^2 respectively. The nearest marker for QTLs of hundred kernel weight and LLS severity score were Ah 4-26 and PM 384 respectively. The markers PMc 588 (3.9 cM) and Ah 4-26 (4.3cM) are the flanking markers for PM 384 and hence these flanking markers can be used for marker assisted breeding for LLS resistance. The parent COG 0437 is the major contributor for both of these QTLs. Considering the phenotypic variation explained by these QTLs, identification of more effective QTLs for hundred kernel weight is required to utilise in the marker assisted breeding programme. However the LLS QTL has 37.9 per cent of phenotypic variation explained and hence can be effectively utilised in marker assisted breeding programme. By using these QTLs, plant breeders can effectively monitor the flow of inheritance of the resistance characters along with desirable phenotypic traits.

Key words: Groundnut, Hundred kernel weight, Late leaf spot, QTL, SSR markers.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important oilseed crops in the world with major groundnut growing countries India, China, Nigeria, Sudan and USA. Among the diseases, late leaf spot (LLS) is the major foliar disease that not only reduces pod yield but also severely affects the fodder and seed quality. LLS caused by *Phaeoisariopsis personata* can cause over 50 per cent loss to groundnut production (Subrahmanyam *et al.* 1985; Waliyar, 1991). Though there are many chemical control methods available, development of disease resistant varieties are the best way to control LLS disease. Different sources of LLS have been reported as having digenic recessive basis (Tiwari *et al.* 1984). Molecular markers associated with LLS would improve the process of identification of resistant genotypes. Identification of DNA markers associated with resistance to LLS and their location on a genetic linkage map are pre requisites for the Marker Aided Selection (MAS) in groundnut (Mace *et al.* 2006). Low level of polymorphism in cultivated groundnut has been observed at the DNA level by using RFLPs (Halward *et al.* 1991), RAPDs (Dwivedi *et al.* 2001; Subramanian *et al.* 2000), AFLPs (He and Prakash, 2001; Krishna *et al.* 2004) and ISSRs (Raina *et al.* 2001). These results showed that *A. hypogaea* lack genetic variation and restricted the production of polymorphic profiles using DNA molecular marker techniques. However advanced techniques such as SSRs and AFLP could well reveal polymorphism at molecular level (Singh *et al.* 1998).

Among the molecular markers, SSR has proved to be the most powerful tool for variety identification in groundnut of similar origin and has

*Corresponding Author's e-mail: nmvannan@gmail.com

[1]ICRISAT, Patancheru - 502 324, India

much potential in genetic and breeding studies (Wang *et al.* 2007). High level of polymorphism has been observed in cultivated groundnut by using SSR primers (Mace *et al.* 2006). Molecular markers and genetic linkage maps are pre- requisites for molecular breeding in any crop. Such tools would speed up the process of introgression of beneficial traits into preferred varieties. The first SSR based linkage map and quantitative trait loci for physiological traits in cultivated groundnut were already published by Varshney *et al.* (2008). Positioning of QTLs on linkage map for late leaf spot, rust disease incidence were reported (Khedikar *et al.* 2010). However the percentage of variation explained by these QTLs is less. Hence the utility of these QTLs in marker assisted breeding is little. In the present study, attempts were made to construct a molecular linkage map and to identify Quantitative Trait Loci (QTL) associated with late leaf spot disease resistance.

MATERIALS AND METHODS

Development of mapping population

The 120 F_2:3 mapping population from the cross TMV 2 x COG 0437 was used for this study. TMV 2 originated from mass selection from Gudiatham bunch (AH 32) and COG 0437 from CO 2 x ICGV 94118. TMV 2 belongs to Spanish bunch and a popular variety grown in southern parts of India. It has high susceptiblity to LLS. The genotype COG 0437 belongs to virginia bunch and highly resistant to LLS. All the field experiments were conducted at Oilseeds farm, Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore from 2007 to 2010. The two parents were raised to produce F_1, F_2 and F_3 generations. The F2 population was studied during Dec- Mar, 2009 and used for genotyping. The F2:3 progenies were studied during June-Sep, 2009 and used as phenotype data.

Screening for LLS

The parents TMV 2 and COG 0437 along with their F_3 progenies were screened artificially for LLS resistance during June-Sep, 2009. Augmented design I was used to raise the population and each progeny was raised in 4 m row with 30 x 10 cm spacing. Highly susceptible cultivar for LLS, COGn 4 was raised after for every fifth row for effective screening. LLS leaf symptoms usually appear between 30 to 50 days after planting so the infected leaf debris from the fields at harvest in the early season crop was collected in cloth bags and spore suspension was prepared and sprayed on 50 days old plants. Haemocytometer was used to count spores to obtain desired inoculum concentration (approximately 10_6 spores/ ml). Mini sprinkler irrigation was given in the field during evening 4 to 6 pm regularly to increase the disease pressure. The conidial suspension was prepared and inoculated the infector rows. Several plants of each entry were examined for disease severity for accuracy. All leaves on the main stem were examined and care was taken to eliminate damage due to factors other than LLS. Nine point disease scale (Subrahmanyam *et al.* 1995) was used to screen the progenies for sources of resistance to LLS. Disease score of 1 for 0%; 2 for 1-5%; 3 for 6-10%; 4 for 11-20%, 5 for 21-30%; 6 for 31-40%; 7 for 41-60% and 8 for 61-80% and 9 for 81-100% disease severity were recorded and plants with a disease score of 1-3 were designated as being resistant; 7-9 were susceptible according to Pande and Rao (2001).

DNA extraction and SSR analysis

Genomic DNA of the two parents was extracted by CTAB method (Doyle and Doyle,1987) and the quality was checked by using 0.8% (w/v) agarose gel electrophoresis. Seventy seven SSR primer pairs specific to cultivated groundnut were selected from the previous study (Selvaraj *et al.* 2009) and used. The polymerase chain reaction (PCR) mixtures (10 µl) contained 2 µl template DNA (10 ng), 1 µl of 10 X Taq buffer + $MgCl_2$ (15mM), 1 µl of dNTP (2 mM), each 0.5 ìl of forward and reverse primers (10 M), 0.1 µl of taq polymerase (Genei 3IU/ l) and 4.9 µl of sterile double distilled water. Amplification was performed in 0.2 ml (each tube) thin walled PCR plates (96 wells / plate) in a thermal cycler (Applied Biosystems). The samples were initially incubated at 94.0°C for 3 min and then subjected to 20 times of the following cycle: 94.0 °C for 30 Sec (-0.5° C per cycle), 63.0°C for 30 sec, 72.0°C for 1 min. This was followed by another 20 cycle: 94.0°C for 15 sec, 55.0°C for 30 sec, 72.0°C for 1 min. Final Extension was 72.0°C for 10 min. Amplified products were analyzed using 6% non denaturing polyacrylamide gel at constant power 350 volts for about 4 h and silver stained (Benbouza *et al.* 2006). The segregation pattern for SSR markers in the selected F_2 individuals were scored as 1, 2 and 3 which corresponds to the banding pattern for

TMV 2 (P_1), heterozygotes and COG 0437 (P_2) respectively. Markers were subjected to single marker analysis to identify the marker-trait association using single factor regression analysis. All the marker data and the mean traits value of F_3 progenies were used for calculating three marker classes (TMV 2, heterozygotes and COG 0437) and their variances. The significant threshold for association of marker to the trait was set at $P \leq 0.05$ for single marker analysis. The adjusted R^2 (phenotypic variance) value was used as per cent of variance explained by the marker on the particular trait of test.

Linkage map and QTL construction

MAPMAKER Version 3.0 was used to analyze the data of F_2 mapping population of the cross TMV 2 x COG 0437. Genotyping and phenotyping data obtained were analyzed for mapping QTLs by using the method composite interval mapping (CIM), proposed by Zeng (1993, 1994) in the WinQTL Cartographer, version 2.5 (Wang *et al.* 2007). CIM analysis was performed using the Model 6, scanning the genetic map and estimating the likelihood of a QTL and its corresponding effects at every 2 cM, while using significant marker cofactors to adjust the phenotypic effects associated with other positions in the genetic map. Thresholds were determined by permutation tests (Churchill and Doerge ,1994; Doerge and Churchill, 1996) using 1000 permutations and a significance level of 0.05. Graphic presentation of the Linkage Groups (LGs) and the QTLs were obtained by using MapChart, version 2.1(Voorrips, 2002).

RESULTS AND DISCUSSION

Parental polymorphism

The cross TMV 2 x COG 0437 was surveyed with 77 SSR primers to identify polymorphic markers that would discriminate susceptible genotype TMV 2 and the resistant genotype COG 0437. Among SSR markers, nine out of 77 primers (11.7 per cent) were found polymorphic between the parents TMV 2 and COG 0437. Similar low level of polymorphism in groundnut was reported by many authors (Selvaraj *et al.* 2009; Varshney *et al.* 2007). Low level of genetic polymorphism in cultivated groundnut has attributed to its origin from a single polyploidization event that occurred relatively recently on an evolutionary time scale (Young *et al.* 1996). Varshney *et al.* (2007) suggested that the low level of polymorphism was due to the marker techniques used. They also emphasized the importance of development of SSR markers from longer SSR enriched libraries, BAC-end sequences and SNP (single nucleotide polymorphism) markers. The true F1's were confirmed by polymorphic markers.

LLS screening

The advent of molecular markers has enabled to dissect quantitative traits into their single genetic components (Dudley, 1993) and it assists in the selection and pyramiding of the beneficial QTL alleles through marker-assisted breeding (Ribaut *et al.* 2002). For mapping QTLs, each plant of a large mapping population (normally in multiples of hundred) should be genotyped with numerous molecular markers. In the present study, a total of 120 F_2s were developed from a cross between TMV 2, a LLS susceptible cultivar with a LLS resistant genotype COG 0437. The F3 populations were tested for LLS resistance under artificial screening and based on disease severity, disease score ranging from 1-9 was recorded.

Linkage map and QTL construction

One of the most important uses of QTL mapping is to apply them in marker-assisted selection (MAS) for genetic improvement of quantitative traits. Once the tightly linked markers have been identified, the traits can be selected indirectly using MAS. Genotype and phenotype data were obtained for eight quantitative characters *viz.*, plant height, number of branches per plant, number of pods per plant, pod yield per plant, kernel yield per plant, shelling percentage, hundred kernel weight and LLS severity score. The yield component traits and reaction for LLS were analyzed using the method composite interval mapping (CIM). The molecular and phenotypic data obtained from 120 F_2 individuals were subjected to single marker analysis using one way regression analysis (Sax,1923). In single marker analysis (Table 1), the marker PM 3 had association with number of branches, number of pods per plant, kernel yield per plant, hundred kernel weight and LLS severity score. Markers *viz.,* PM 384, pPGPseq5D5, PM 3, PMc 588 and PM 343 had associated with characters namely number of branches, hundred kernel weight and LLS severity score. The marker PM 375 had association with

TABLE 1: Single marker analysis for SSR primers linked to yield and yield components in the cross TMV 2 x COG 0437.

SSR markers	b value
Number of pods per plant	
PM 3	-1.45*
Kernel yield per plant	
PM 3	-1.189*
100 kernel weight	
PM 384	-2.497**
pPGPseq5D5	-3.008**
PM 137	-2.124*
PM 3	-4.139**
PMc 588	-4.143**
PM 343	-4.076**
PM 377	-4.282**
LLS severity score	
PM 375	1.105**
PM 384	1.765**
pPGPseq5D5	1.666**
PM 137	1.78**
PM 3	2.2**
PMc 588	1.794**
PM 343	2.421**
PM 377	2.205**

*, ** Significant at 5 and 1% levels respectively

LLS severity score only. The marker PM 377 had linked with hundred kernel weight and LLS severity score.

Genotyping for identified polymorphic markers was carried out on 120 F_2:3 progenies of the cross TMV 2 x COG 0437. The identified nine polymorphic markers *viz.,* PM 3, PM 375, PM 137, PMc 588, PM 343, PM 377, PM 384, pPGPseq5D5 and Ah4-26 were used for profiling of F_2 progenies. Genotyping data obtained for nine marker loci was used to establish groups. Using a minimum LOD score of 3.0, one linkage group was formed with eight SSR markers. One SSR marker PM 343 was found as unlinked to the linkage group. Thus a total length of 37.2 cM was covered with this linkage group with an average 4.65 cM. Genetic linkage map and QTL positions in the cross TMV 2 x COG 0437 are depicted in Fig.1. The CIM analysis resulted into two QTLs *viz.*, one each for hundred kernel weight and LLS severity score (Table 2). All these QTLs had the LOD of 3.7 and 29.2 respectively. The QTL for LLS had very high phenotypic variation in terms of R^2 (37.9 per cent) while the hundred kernel weight had low R^2 (6.1 %). The parents COG437 and TMV 2 contribute to high hundred kernel weight and high LLSseverityrespectively. The nearest marker for QTLs of hundred kernel weight and LLS severity score were Ah 4-26 and PM 384 respectively. The markers PM 384 (4.3 cM) and pPGPseq5D05 (4.3 cM) are the flanking markers for Ah 4-26 and these markers can be utilised for marker assisted breeding programme for hundred kernel weight. The markers PMc 588 (3.9 cM) and Ah 4-26 (4.3cM) are the flanking markers for PM 384 and hence these flanking markers can be used for marker assisted breeding for LLS resistance. The parent COG 0437 is the major contributor for both of these QTLs. Considering the phenotypic variation explained by these QTLs, identification of more effective QTLs for hundred kernel weight is required to utilise in the marker assisted breeding programme. However the LLS QTL has 37.9 per cent of phenotypic variation explained and hence can be effectively utilised in

FIG. 1. Genetic Linkage map and QTL positions in the cross TMV 2 x COG 0437 HUN – Hundred kernel weight; LLS – Late leaf spot severity score.

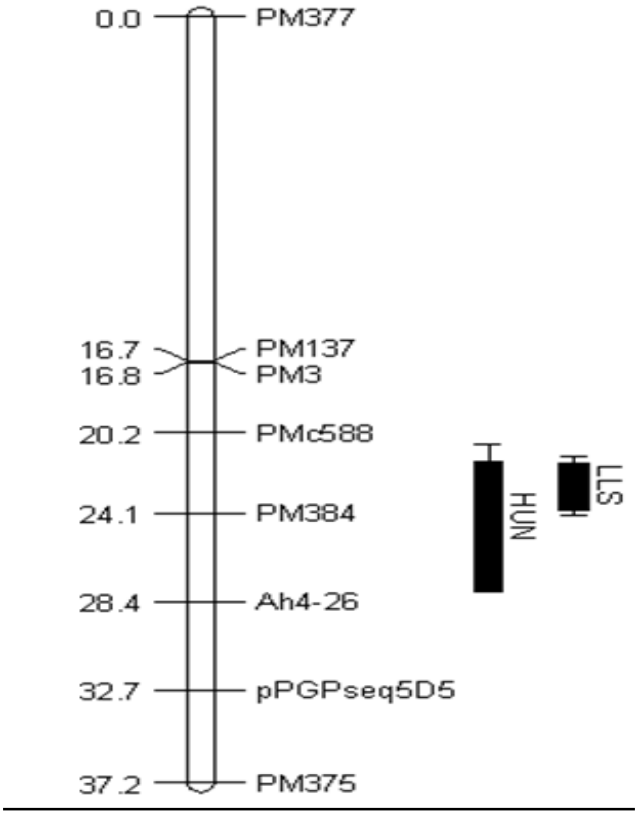

TABLE 2. Quantitative trait loci for yield component traits by composite interval mapping (CIM). method.

Trait	QTL	Nearest marker	Position (cM)	Highest LOD score	Additive effect	Donor parent	Phenotypic variation (R^2) %
Hundred kernel weight	HUN	Ah4-26	25.8	3.7	-3.3	COG 0437	6.1
LLS severity score	LLS	PM 384	23.8	29.2	2.0	TMV 2	37.9

marker assisted breeding programme. Khedikar *et al.* (2010) reported that the marker PM 137 was presented in the linkage group 2 for LLS and rust resistance; marker PM 377 was presented in the linkage group 10 for LLS and resistance and marker PM 3 was grouped in linkage group 8 for rust resistance.

From the fore going discussion, it may be concluded that the linkage and QTL analysis resulted in one linkage group and two QTLs for important traits namely, hundred kernel weight, and LLS incidence score. The major bottle neck in the resistance breeding programme is the tight linkage between undesirable phenotypic traits like poor shelling percentage with resistance. Many times the breeder ultimately ends with resistant entries with poor phenotypic traits. By employing these QTLs, the breeder can effectively monitor the flow of inheritance of the resistance characters along with desirable traits.

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