AFLP Diversity Among Selected Foliar Diseases Resistant Groundnut (*Arachis hypogaea* L.) Germplasm

SL Dwivedi[1], S Gurtu[2], SN Nigam*[3]

[1] *102 Imperial Manor Apartments, Begumpet, Hyderabad – 500 016 (Andhra Pradesh)*
[2] *F-5, B-Block, Brindavan Apartments, Begumpet, Hyderabad 500 016 (Andhra Pradesh)*
[3] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O.-502 324, (Andhra Pradesh)*

Knowledge of genetic diversity facilitiates the judicious use of germplasm in crop improvement programs. The amplified fragment length polymorphism (AFLP) technique was employed to identify foliar diseases resistant accessions with distinct DNA profiles for mapping and genetic enhancement in groundnut (*Arachis hypogaea* L.). This study revealed 93.3% polymorphic AFLP fragments and 7.2 unique AFLP markers per primer pair. Primer pairs E-ACA + M-CAG, E-AAC + M-CAT, E-ACA + M-CTG, E-AAC + M-CTT, and E-ACC + M–CTC detected between 11-12 unique markers. Accessions with distinct DNA profiles (ICG 6284 with ICGV 99001; TMV 2 with ICG 405, ICGV 1705, ICG 6284, ICGV 99004, and ICGV 99005) and with more number of unique markers (ICG 405, ICG 1705, ICG 6284, ICGV 9904, and ICGV 99005) were identified to diversify breeding populations and identify AFLP markers/quantitative trait loci linked with resistance to ELS, LLS, and rust in groundnut.

Key Words : AFLP Markers, Genetic Diversity, Leaf Spots, Peanut, Rust

Knowledge of genetic diversity, identification of molecular markers linked with specific traits, and availability of genetic linkage map are pre-requisite to marker-assisted genetic enhancement. Amplified fragment length polymorhism (AFLP) has been recently used to study genetic diversity and phylogentic relationships in a wide range of crop species (Sharma *et al.*, 1996; Tohme *et al.*, 1996; Maugham *et al.*, 1996; Singh *et al.*, 1998; Aggarwal *et al.*, 1999; Caidedo *et al.*, 1999; Sanchez *et al.*, 1999). AFLP analysis required no prior sequence knowledge of the target genome, detects greater polymorphisms, and the markers are robust, reliable, and reproducible (Vos *et al.*, 1995; Maughan *et al.*, 1996; Tohme *et al.*, 1996). Recent studies, using DNA amplification fingerprinting (DAF) and AFLP (He and Prakash, 1997), random amplified polymorphic DNA (RAPD) (Bhagawat *et al.*, 1997; Subramanian et al., 2000; Dwivedi *et al.*, 2001), and simple sequence repeats (SSRs) (Hopkins *et al.*, 1999) assays revealed polymorphism in cultivated groundnut (*A. hypogaea* L.).

Rust (*Puccinia arachidis* Speg.), late leaf spot (LLS) (*Phaeoisariopsis personata*) (Berk. & Curt.) v. Arx), and early leaf spot (ELS) (*Cercospora arachidicola* Hori) are widely distributed foliar fungal diseases of groundnut in the semi-arid tropic regions. They cause yield losses in excess of 50per cent to groundnut production (Subrahmanyam *et al.*, 1984, 1985; Waliyar, 1991), and reduce seed quality (Dwivedi *et al.*, 1993). Research at ICRISAT led to the identification of several sources of resistance to rust, LLS, and ELS (Singh *et al.*, 1997), however, the extent of genetic diversity among these accessions is not known. There is a need to identify genetically distinct accessions for diversification of breeding populations with enhanced resistance to rust, ELS, and LLS in groundnut.

The present study was initiated to determine molecular diversity among selected foliar diseases resistant and susceptible accessions to identify those with distinct DNA profiles and unique AFLP markers for mapping and genetic enhancement in groundnut.

Materials and Methods

Eleven accessions were selected for the study. ICG 10881 and ICG 10890 are resistant to rust and ICG 405, ICG 1705, ICG 6284, and ICG 6886 to ELS. They are land races and native of South America. Except ICG 6284, they belong to subsps *fastigiata*. ICG 6284 belongs to subsps hypogaea. ICGV 99001, ICGV 99002, ICGV 99004 and ICGV 99005 are interspecific derivatives originating from crosses between *A hypogaea* and foliar diseases resistant wild *Arachis* species, and are resistant to rust and/or LLS. TMV 2 is highly susceptible to ELS, LLS, and rust. Prior to study, these accessions were grown under field conditions for two seasons to

*Author for correspondence (email: s.nigam@cgiar.org; Fax 91-40-3241239).

detect for any phenotypic variation within each accession. All the accessions included in the study, were phenotypically uniform.

Young unfolded leaves from two week greenhouse grown old plants were bulk harvested for each accession and immediately placed in liquid nitrogen to extract DNA using CTAB method (Saghai-Maroof *et al.*, 1984). The quality and concentration of DNA was assessed by spectrophotometer and also by gel electrophoresis using 0.8% agarose with known concentration of uncut lamda DNA.

Seventeen AFLP assays, using primer pairs E-ACT with M-CAT; E-ACC with M-CAC and M-CTC; E-ACA with M-CAG, M-CTC, and M-CTG; E-AGC with M-CCA; E-AAC with M-CAT, M-CAA, M-CAC, M-CTG, M-CTA, and M-CTT; and E-AGG with M-CAA, M-CTT, M-CAG, and M-CTA, were conducted on 11 accessions following Vos *et al.* (1995) with minor modification. *Genomic DNA (500 ng) was double digested* with 1μl *EcoR* 1/*Mse* 1,5 μl of 5X reaction buffer and AFLP grade water added to a total reaction volume of 15 μl. The reactions were incubated for 3 h at 37°C and then denatured to inactivate the restriction endonucleases at 70°C for 15m. The digested DNA fragments were ligated using 0.5 μl of T4 DNA ligase and 12 μl of adapter ligation solution containing *EcoR* 1 and *Mse* 1 adapters at 20°C for 2 h, The adapter ligated DNA was diluted 10-fold with TE buffer and used as template DNA for pre-amplification. The pre-amplification reaction mixture consisted of 5 μl of template DNA (1:10 diluted ligation mixture), 40 μl of pre-amp primer mix, 5 μl of 10 X PCR buffer containing Mg^{2+}, and 1 μl of Taq DNA polymerase (1 unit/μl). Twenty cycle PCR programme was performed at 94°C for 30s, 56°C for 60s, and 72°C for 60s. The pre-amplified DNA was diluted in a ratio of 1:50 in TE buffer and used as template for selective amplification with *EcoR* 1 and *Mse* 1 primers, each having three selective nucleotides at their 3' ends. Prior to selective amplification, the *EcoR* 1 primer was end labelled with [γ^{33}P] ATP (2000Ci/mmol). 10μl of labelled primer along with 10μl of 5 X kinase buffer and 2 μl of T4 kinase were made to 50 μl with AFLP grade water. The reactions were incubated at 37°C for 1 h and inactivated at 70°C for 10m. The 20μl PCR reaction for selective amplification contained 5 μl of 1:50 diluted pre-amplification products, 0.5 μl of labelled *EcoR* I primer, 4.5 μl of *Mse* 1 primer (containing dNTPs), 2.0 μl of 10 X PCR buffer, 0.1 μl of Taq DNA polymerase (5 units μl) and 7.9 μl of AFLP grade water. The cycling profile for selective amplification was one cycle of 30s at 94°C, 30s at 65°C, and 60s for 72°C. The annealing temperature was lowered by 0.7°C per cycle during the first 12 cycles. This gave touch down phase of 13 cycles. The remaining 23 cycles were performed at 94°C for 30s, 56°C for 30s, and 70°C for 60s. PCR reaction was stopped by adding 20μl of formamide dye (98% formamide, 10mM EDTA, 0.025% bromophenol blue, and 0.025% of xylene cyanol). The reaction mixtures were then incubated for 3m at 90°C and placed immediately on ice. 3μl of sample was loaded on a 6% polyacrylamide gel. Electrophoresis was performed on Sequi Gen sequencing unit of BIORAD at constant power of 1500 volts for 3h. After electrophoresis, the gels were covered with Saran wrap and Whatmann # 3 paper and dried in vacuum on a gel drier (Model 583 biorad) for 2h. The dried gels were than exposed to X-ray films for 6 to 10 days, depending upon the intensity of the radiation signal, for developing good autoradiographs.

The amplified fragments on the autoradiographs were manually scored as '1' for the presense and '0' for the absence of band from higher to lower molecular weight products. Pair-wise genetic similarities (S_{ij}) between accessions I and j were estimated using the similarity coefficient of Nei and Li (1979) as Sij = 2 Nij/(Ni +N_j), where N_{ij} is the number of bands in common in accessions i and j, and N_i and N_j are the totals of bands in accessions i and j, respectively, S_{ij} represents the proportion of bands in common between any two accessions and may range from '0' (no common bands) to '1' (identical band profile for the two accessions). S_{ij} values were used to estimate genetic dissimilarity, as $D_{ij} = 1 - S_{ij}$, and D_{ij} values were used to determine the relationships among accessions using principal co-ordinate analysis (PCoA) (Sneath and Sokal, 1973). All computations were performed using statistical computing package Genstat 5 Release 4.1. A band is identified as a unique molcular marker if present in one accession at specific molecular weight but absent in the remaining accessions studied together for a given AFLP primer pair.

Results and Discussion

Seventeen AFLP assays, performed on 11 accessions, produced 8656 fragments of which 8074 (93.3%) were polymorphic (Table 1). Polymorphism ranged between

Table 1. Analysis of polymorphism(%) and unique markers in foliar diseases resistant and susceptible accessions as revealed by AFLP analysis in groundnut

Primer pair*	#amplified Bands+	# polymorphic bands	Polymorphism (%)	# of bands per primer Pair++	# of unique markers per primer pair	Accessions with unique AFLP markers
E-ACT+M-CAT	399	323	80.9	36.3	5	1 each in ICG#6284 and 10881, and ICGV# 99001, 99002, and 99005
E-ACC+M-CAC	285	259	90.8	25.9	5	1 each in ICG#6284 and 10881, and ICGV# 99001, 99002, and 99005
E-ACC+M-CTC	518	518	100.0	47.1	22	12 in ICG 1705, 6 in ICG 6284, and I each in ICGV# 99002, 99004, and 99005 and ICG 10881
E-ACA+M-CAG	474	434	91.6	43.1	11	5 each in ICG 6284 and ICGV 99005 and I in ICGV 99004
E-ACA+M-CTC	604	604	100.0	54.9	3	1 each in ICGV# 99001, 99002, and 99005
E-ACA+M-CTG	593	593	100.0	53.9	12	11 in ICG 1705 and I I ICG 6284
E-AGC+M-CAA	479	383	79.9	43.5	1	1 in ICG 10881
E-ACC+M-CAT	598	598	100.0	54.4	11	7 in ICG 405, 2 in ICGV 99001, and I each in ICGV 99004 and ICG 1705
E-AAC+M-CAA	615	577	93.8	55.9	4	2 in ICG 405 and 1 each in ICGV 99005 and ICG 6284
E-AAC+M-CAC	539	494	91.6	49.0	3	2 in ICG 405 and 1 in ICG 10890
E-Aac+M-CTG	524	462	88.2	47.6	1	1 in ICG 405
E-AAC+M-CTA	689	689	100.0	62.6	4	2 each in ICG# 405 and 10881
E-AAC+M-CTT	682	682	100.0	62.0	17	7 in ICGV 99001, 6 in ICG 405, 3 in ICGV 99004, and 1 in ICG 10890
E-AGG+M-CTT	453	413	91.2	41.2	5	3 in ICG 405, amd 1 each in ICG 6886 and IMV 2
E-AGG+M-CTT	447	405	90.6	40.6	4	3 in ICG 405 and 1 in ICG 1705
E-AGG+M-CAG	275	206	74.9	25.0	8	7 in ICG 405 and 1 in ICGV 99004
E-AGG+M-CTA	483	434	89.8	43.9	7	3 in ICG 405 and 2 each in ICG 10881 and ICGV 99001

*E= *EcoR*1 primer and M = *MSE*1 primer; +Total number of bands recorded in 11 accessions; ++Average number of bands per primer per accession

74.9% with primer pair E-AGG+M-CAG to 100.0% with primer pairs E-ACC+M-CTC, E-ACA+M-CTC, E-ACA+M-CTG, E-ACC+M-CAT, E-AAC+M-CTA, and E-AAC+M-CTT. The amplified fragments ranged from 25.0 to 62.6, with an average of 46.3 per primer pair. The AFLP analysis of 17 primer pairs on 11 accessions identified 123 unique molecular markers. With an average of 7.2 markers per primer pair (Table 1). The unique markers detected in each accessions were 1 each in ICG 6886 and TMV 2; 2 in ICG 10890; 4 in ICGV 99002; 7 in ICGV 99004; 8 in ICG 10881; 10 in ICGV 99005; 14 in ICGV 99001; 15 in ICG 6284; 25 in ICG 1705; and 36 in ICG 405. All the 11 accessions could be differentiated based on the presence of unique makrer(s) at specific molecular weight DNA fragments. The AFLP primer pairs E-ACA+M-CAG, E-AAC+M-CAT, E-ACA+M-CTG, E-AAC+M-CTT, and E-ACC+ M-CTC detected between 11-22 markers.

The genetic dissimilarity (D_{ij}) ranged from 0.0632 to 0.2844 with an average of 0.1703 (Table 2). The D_{ij} matrice was used to determine the genetic relationships among accessions using principla co-ordinate analysis (PCoA). Accessions TMV 2 (quadrant II), ICG 6284 (quadrant III), and ICGV 99004 (quadrant IV) were well separated from each other, and the remaining formed two distinct clusters: ICG 6889, ICG 10881, ICG 10890, and ICGV 99002 in cluster 1 and ICG 405, ICG 1705, ICGV 99001,

Table 2. AFLP dissimilarity matrix for foliar diseases resistant and susceptible accessions as revealed by AFLP analysis in groundnut

	ICG 10890	ICG 10881	ICGV 99001	ICGV 99002	ICGV 99004	ICGV 99005	ICG 1705	ICG 405	ICG 6284	ICG 6886	TMV 2
ICG 10890	0.0000										
ICG 10881	0.0692	0.0000									
ICGG 99001	0.1674	0.1208	0.0000								
ICGG 99002	0.1202	0.0850	0.1310	0.0000							
ICGG 99004	0.2032	0.1763	0.1693	0.1813	0.0000						
ICGG 99005	0.1748	0.1687	0.2194	0.1368	0.21909	0.0000					
ICG 1705	0.1970	0.1745	0.2210	0.1839	0.2429	0.1278	0.000				
ICG 405	0.1644	0.1374	0.1944	0.1294	0.1960	0.1366	0.1743	0.0000			
ICG 6284	0.2442	0.2214	0.2265	0.2399	0.2844	0.2335	0.2621	0.2315	0.0000		
ICG 6886	0.0991	0.0632	0.1351	0.0845	0.1745	0.1622	0.1740	0.1286	0.2055	0.0000	
TMV 2	0.1532	0.1093	0.1934	0.1296	0.2024	0.1980	0.2024	0.1531	0.1852	0.0687	0.0000

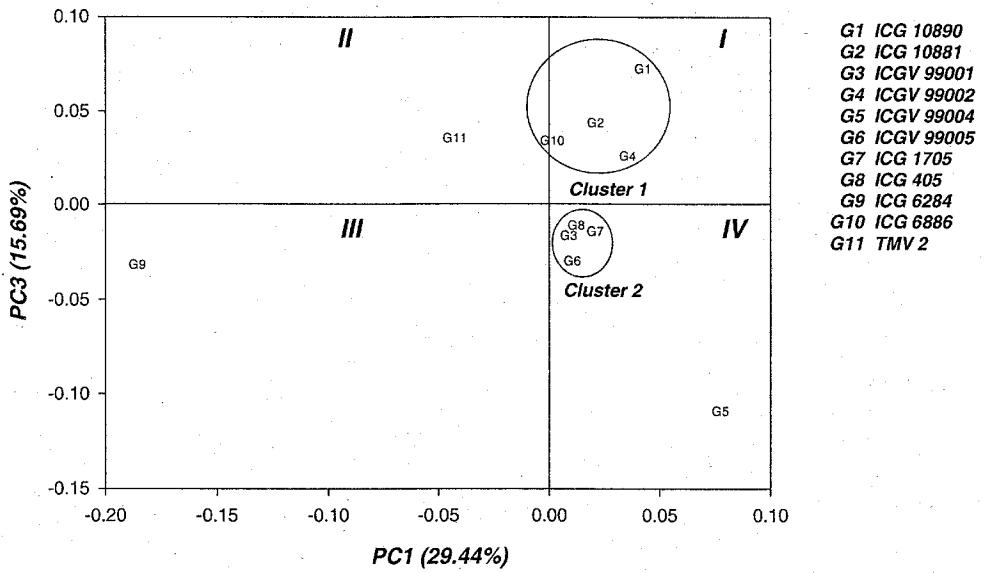

Fig.1. Relationships among 11 accessions as visualized by principal co-ordinate analysis (PCoA) using AFLP based dissimilarity values in groundnut

and ICGV 99005 in cluster 2 (Fig. 1). ICG 6284 and ICGV 99004 are the ideal accessions, as they showed maximum dissimilarity, to develop diverse breeding populations with combined resistance to ELS, LLS and rust, Accessions ICG 405, ICG 1705, ICG 6284, ICGV 99001, and ICGV 99005 with TMV 2 would be the better cross combinations to develop recombinant inbred lines (RILs) for mapping quantitive trait loci (QTL) associated with resistance to ELS, LLS, and rust in groundnut. These accessions possess a large number of unique markers that are absent in TMV 2.

With the use of DNA profiles, the genetic uniqueness of each accession relative to all other accessions can be determined and quantified. Accessions with DNA profiles most distinct are likely to contain the greatest number of novel alleles. It is in these accessions that one is most likely to uncover the largest number of unique and potentially agronomically useful alleles. This strategy has resulted in high proportion (~50%) of new and useful QTL alleles in rice and tomato (Tanksley and McCouch, 1997). With similar studies initiated in groundnut, it is proposed to determine the chromosomal position of the key loci and allelic variants at these loci controlling resistance to ELS, LLS, and rust and yield enhancing QTL for targeted genetic enhancement in groundnut. Few accessions with high genetic

dissimilarity and with greater number of unique molecular markers have been crossed for mapping and genetic enhancement in groundnut at ICRISAT. The enhanced breeding populations and RILs will be freely accessible to researchers for further studies in groundnut.

References

Aggarwal RK, DS Brar, S Nandi, N Huang and GS Khush (1999) Phylogentic relationships among *Oryza* species revealed by AFLP markers. *Theor. Appl. Genet.* **98:** 1320-1328.

Bhagawat A, TG Krishna and CR Bhatia (1997) RAPD analysis of induced mutants of groundnut (*Arachis hypogaea* L.) *J. Genet.* **76:** 201-208.

Caicedo AL, E Gaitan, MC Duque, OT Chica, DG Debouck and J Tohme (1999) AFLP fingerprinting of *Phaseolus lunatus* L. and related wild specied from South America. *Crop Sci.* **39:** 1497-1507.

Dwivedi SL, S Gurtu, S Chandra, W Yuejin and SN Nigam (2001) Assessment of genetic diversity among selected groundnut germplasm. I. *RAPD* Analysis. *Plant Breeding* **120:** 345-349.

Dwivedi SL, SN Nigam, P Subrahmanyam, R Jambunathan, GVS Nagabhushanam, PM Reddy, K Raghunath and D McDonald (1993) Effect of foliar diseases control by Chlorothalonil on pod yield and quality characteristics of confectionery groundnuts (*Arachis hypogaea* L.) *J. Sci. Food Agric.* **63:** 265-271.

He G and S Prakash (1997) Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica* **97:** 143-149.

Hopkins MS, AM Casa, T Wang, Se Mitchell, RE Dean, GD Kochert and S Kresovich (1999) Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut, *Crop Sci.* **39:** 1243-1247.

Maughan PJ, MA Saghai Maroof, GR Buss and GM Huestis (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis *Theor. Appl. Genet.* **93:** 392-401.

Nei M and WH Li (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc.Natl. Acad Sci.* USA **76:** 5269-5273.

Saghai-Maroof MA, KM Soliman, RA Jorgensen and RW Allard (1984) Ribosomal DNA spacer length polymorphism in barley, Mendelian inheritance, chromosomal locationa and population dynamics. *Proc. Natl. Acad. Sci. USA* **81:** 8014-8018.

Sanchez, G S Restrepo, Myriam-Cristina Duque, M Fregene, M Bonierbale and V Verdie (1999) AFLP assessment of genetic variability in cassava accessions (*Manihot esculenta*) resistant and susceptible to the cassava bacterial blight (CBB). *Genome* **42:** 163-172.

Sharma SK, MR Knox and THN Ellis (1996) AFLP analysis of the diversity and phylogeny of Lens and its comparision with RAPD analysis. *Theor. Appl. Genet.* **93:** 751-758.

Singh AK, VK Mehan and SN Nigam (1997) Sources of resistance to groundnut fungal and bacterial diseases: an update and appraisal. *Information Bulletin No. 50.* Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, 48 pp.

Singh S, JS Sidhu, SK Uberoi, M Sodhi, Y Vikal and HS Dhaliwal (1998) Genetic diversity analysis of Oryza using amplified fragment length polymorphism. *Crop Improv.* **25:** 15-20.

Sneath PHA and RR Sokal (1973) Numerical Taxonomy. WH Freeman and Company, San Fransisco, USA.

Subramanian V, S Gurtu, RCN Rao, and SN Nigam (2000) Indentification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome* **43:** 656-660.

Subrahmanyam P, LJ Reddy, D McDonald and RW Gibbons (1985) Peanut rust: a major threat to peanut production in the semi-arid tropics. *Plant Dis.* **69:** 813-819.

Subrahmanyam P, JH Williams, D McDonald and RW Gibbons (1984) The influence of foliar diseases and their control by selective fungicides on a range of groundnut (*A. hypogaea* L.) genotypes *Ann. Appl. Biol.* **104:** 467-476.

Tanksley SD and SR McCouch (1997) Seed banks and molecular maps: Unlocking genetic potential from the wild. *Science* **277:** 1063-1066.

Tohme J, DO Gonzalez, S Beebe and C Duque (1996) AFLP analysis of gene pools of a wild bean core collection, *Crop Sci.* **36:** 1375-1384.

Vos P, R Hogers, M Bleeker, M Reijans, T van de Lee, M Hornes, A Frijters, J Pot, J Peleman, M Kuiper and M Zabeau (1995) AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* **23:** 4407-4414.

Waliyar F (1991) Evaluation of yield losses due to groundnut leaf diseases in West Africa. Summary P*roc. of the second ICRISAT Regional Groundnut Meet for West Africa, 11-14 September 1990.* ICRISAT Sahelian Center, Niamey, Niger. ICRISAT, Patancheru, India pp. 32-33.