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SCREENING OF GROUNDNUT GENOTYPE FOR LATE LEAF SPOT RESISTANCE USING ISSR MARKERS

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ABSTRACT

Groundnut *(Arachis hypogea* L.*)* belongs to the subfamily papilionaceae of the family leguminaceae. The narrow genetic base is the major problem in groundnut breeding and to overcome this problem the present investigation was under taken to analyze the genetic diversity at molecular level. The DNA was extracted from leaf portion of 8 groundnut genotypes. Quantities of all DNA samples were found between 400-3000 ng/ml. Seventeen ISSR primers were used to generate molecular data for screening of Groundnut genotypes for Late Leaf Spot Resistance. In all total 784 bands were amplified from which 593 bands were found polymorphic (%). Mean bands (46.11) were amplified per primer. Primer ISSR 809, ISSR 811, ISSR 817, ISSR 836, ISSR 841 were produced maximum i.e. 100 % polymorphism. Primer ISSR 844 able to amplify and produce 37.25 % lowest polymorphism. The resulted molecular data were pooled for computing screening analysis based on similarity index by using NTSYS software. The overall polymorphism percentages obtained by ISSR primers were 74.10%. The similarity coefficient for 8 genotypes of groundnut ranged from 0.4160 to 0.7280 in ISSR. The present study may be useful for detection of highly diverse and vigorous genotypes for improvement of some major morphological, agronomical and quantitative traits.

INTRODUCTION

Groundnut is a native of South American leguminous oil seed (Hammons, 1982). The botanical name of groundnut *Arachis hypogaea* L. is derived from Greek word Arachis meaning a legume and *hypogaea* means below ground. Groundnut belongs to the genus *Arachis* of subfamily Papilionaceae of the family leguminosae. Groundnut seeds are widely used as principle source of cooking oil, digestible protein, mineral and vitamin in many countries and significantly to food security and alleviating poverty (Savage and Keenam, 1994).

Problem related to leaf spot disease causes nearly complete defoliation and yield loss up to 50 percent or more depending upon disease severity. The leaf spot disease epidemics are affected by wheatear parameters such as hot and wet conditions (Shew *et al.,* 1988). Control of leaf spot diseases has depended on some cultural practices and on multiple applications of fungicides.

DNA fingerprinting is an important tool for characterization of germplasm and establishment of identify variety/hybrids/parental sources etc. ISSR is a PCR based method, which involves amplification of DNA present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellite, it can be either unanchored or more usually anchored at 3′ or 5′ end with 1 to 4 degenerate basses extended into the flanking sequences.

ISSRs have been used in many crop species including legumes (Ratnaparkhe *et al.,* 1998, Bornet and Branchard., 2001, Iruela *et al.,* 2002, Tahir *et al.,* 2011, Bhareti *et al.*, 2012, and Joshi *et al.,* 2013).

Comparative studies on genetic makeup of isolates in resistant and susceptible genotypes have often helped in understanding the nature and mechanism of resistance which could be used as guide in search for disease resistance genotypes and hence could be utilized in resistance breeding program.

The objective of the present study was to explore the genetic diversity among resistant and susceptible genotypes of groundnut and computing diversity analysis on the basis of similarity index using NTSYS-PC software.

MATERIALS AND METHODS

Plant material and DNA extraction

Experimental material comprised of 8 elite cultivars of groundnut viz. R-2001-3, VG-9816, GPBD-4, JL 220, JL24, TAG 24, LGN-1 and LGN-117, which were obtained from Oil Seed Research Station, Latur (Maharashtra). Total genomic DNA was extracted from the 7 days young leaves of groundnut following the method suggested by Saghai-Maroof *et al.,* (1984) with slight modifications. DNA quantification as well as quality assessment was carried out spectrophometrically using Biophotometer (Eppendorf-AG 22331, Germany) and checked by comparing DNA samples with known DNA. The quality of DNA was also checked by 1% agarose gel electrophoresis (Sambrook and Russell, 2001). The isolated DNA was diluted to 25 ng/ml and stored at -20ºC for further application.

ISSR analysis and PCR Reaction

Seventeen ISSR primers (Imperical Life Science) listed in Table-2. PCR amplification was carried out in 25 ml reaction vol-

ume containing PCR tubes containing 2.5 μl 10X PCR buffer, 1.5 μl 25mM MgCl2, 0.5 ìl 10 mM dNTP each, 0.2 ìl 1.0 U Taq DNA polymerase (Bangalore Genie, India), 1ìl primer, 1ìl genomic DNA and 18.3 ìl distilled water . The amplification was performed in an Eppendorf thermo cycler, with reaction conditions programmed as initial pre-denaturation at 94°C for 7 min. followed by 40 cycles of denaturation at 94°C for 30 Sec., annealing at optimized temperature for 45 Sec., and extension at 72°C for 2 min. and 30Sec. A final 10 min. extension at 72°C followed the completion of 40 cycles. Aliquots of the amplification products were separated by electrophoresis on 1.5% agarose gels stained with ethidium bromide at 100 V for 3 h, and bands were visualized and documented in Gel Documentation System (Alpha Innotech).

Data analysis

The amplified product of ISSR from agarose gel images were scored for presence (1), absence (0), missing and doubtful case was scored as 9. The data was stored in Microsoft Excel file for further analysis. Band size was also determined by comparison with 100bp DNA ladder (Banglore Genei). Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02, Rohlf, 1993). The SIMQUAL program was used to calculate the Jaccard's coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on Jaccard's coefficient. The polymorphic percentage of the obtained bands were calculated by using following formula,

Polymorphic % = (No. of polymorphic bands /Total bands) x 100

RESULTS AND DISCUSSION

8 elite groundnut genotype were selected for the present study, from which three genotypes viz., R-2001-3, VG-9816, GPBD-4 Gowda *et al*. (2002) were resistant to Rust and Late Leaf Spot (LLS) another three genotypes viz., JL-220, JL-24, TAG-24 were susceptible to Rust and Late Leaf Spot (LLS) and two genotypes viz. LGN-1 were tolerant to Rust and Late Leaf Spot (LLS) and LGN-117 Tolerant to Rust and resistant to Late Leaf Spot (LLS).

Extraction of genomic DNA

Bulk DNA was extracted from leaf portion of each 8 genotypes of groundnut by the method suggested by Saghai-Maroof *et al.,* (1984). The modifications in the original protocol produced superior results. The results on agarose gel electrophoresis showed a good quality of DNA. Through electrophoresis neither lagging and unbinding band nor impurity was found in each lane. The amount of DNA of all DNA samples were found between 400-3000 ng/ml.

ISSR analysis and PCR Reaction

17 primers out of 31 primers were selected for their clarity, repetition and relatively high polymorphism. Conditions for ISSR reaction were optimized using various concentrations of

Table 1: List of ISSR primers with polymorphism

ISSR Primer	Sequence	Total no. of nucleotides	Total no. of amplicon	No. of polymorphic amplicons	% polymorphism
ISSR 809	AGA GAG AGA GAG AGA GG	17	38	38	100
ISSR 810	GAG AGA GAG AGA GAG AT	17	56	48	85.71
ISSR 811	GAG AGA GAG AGA GAG AC	17	59	59	100
ISSR 816	CAC ACA CAC ACA CAC AT	17	38	30	78.94
ISSR 817	CAC ACA CAC ACA CAC AA	17	38	38	100
ISSR 818	CAC ACA CAC ACA CAC AG	17	36	20	55.55
ISSR 824	TCT CTC TCT CTC TCT CG	17	62	46	74.19
ISSR 827	ACA CAC ACA CAC ACA CG	17	39	31	79.49
ISSR 830	TGT GTG TGT GTG TGT GG	17	46	38	82.61
ISSR 834	AGAGAGAGA GAG AGA GYT	18	63	39	61.90
ISSR 836	AGAGAGAGA GAG AGA GYA	18	41	41	100
ISSR 841	GAGAG GAG AGA GAG AYC	18	36	36	100
ISSR 842	GA AGA GAG AGA GAG AYG	18	49	41	83.67
ISSR 844	CTC TCT CTC TCT CTC TRC	18	51	19	37.25
ISSR 845	CTC TCT CTC TCT CTC TRG	18	26	10	38.46
ISSR 856	ACA CAC ACA CAC ACA CYA	18	27	3	11.11
ISSR 859	TGT GTG TGT GTG TGT GRC	18	79	56	70.89
Total			784	593	75.63

Table 3: Similarity Matrix of 8 Groundnut genotypes obtained from ISSR marker.

	R-2001-3	VG-9816	GPBD-4	JL-220	JL-24	TAG-24	LGN-1	LGN-117
R-2001-3	****							
VG-9816	0.664	****						
GPBD-4	0.672	0.528	****					
JL-220	0.416	0.496	0.520	****				
JL-24	0.632	0.600	0.624	0.592	****			
TAG-24	0.448	0.544	0.472	0.616	0.672	****		
LGN-1	0.544	0.608	0.552	0.616	0.672	0.728	****	
LGN-117	0.584	0.616	0.592	0.448	0.600	0.672	0.656	****

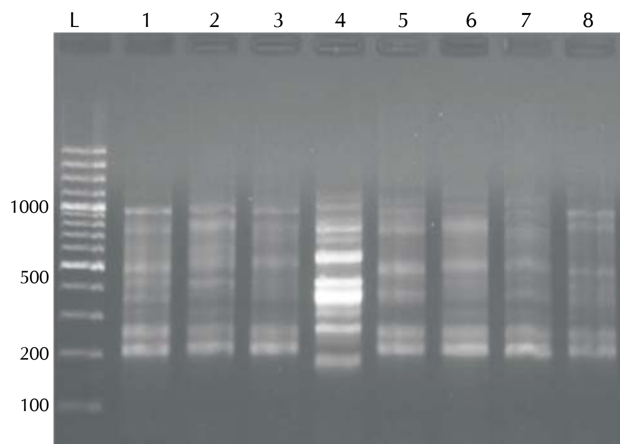

Figure 1: ISSR profile of groundnut genotype with primer ISSR-811

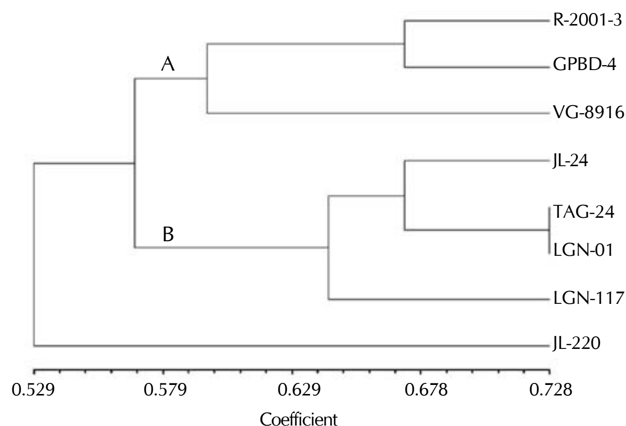

Figure 2: UPGMA dendrogram of Groundnut genotype for ISSR analysis

different ingredients of the reagents. 1 ml of 15 ng/ml DNA, 10 pmol primer, 1.5 mM of $MgCl_2$ concentration, 0.2 mM dNTPS and 1U/ul of *Taq* DNA polymerase produced good results.

The amplified DNA product was resolved on 2 % agarose gel by staining with ethidium bromide. Total 784 bands were amplified from which 593 bands were found polymorphic (%). Mean bands (46.11) were amplified per primer. Primer ISSR 809, ISSR 811, ISSR 817, ISSR 836, ISSR 841 were produced maximum i.e. 100 % polymorphism (Fig 1), where as primer ISSR 844 was able to produce 37.25 % lowest polymorphism. In all 75.63 % polymorphism was calculated (Table-1).

The amplification generated by 17 ISSR primers for screening 08 groundnuts was scored across the lanes. Presence of band was scored as "1", absence of band as "0". Each of the bands was treated as an independent character regardless of its intensity. The collected data was analyzed by using the NTSYS-pc version 2.02 25. The SIMQUAL program was used to calculate the Jaccard's similarity coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) and saved as JPGE format. Cluster analysis revealed two clusters among 8 genotypes with one out group. The two clusters i.e. cluster A and cluster B were formed at 56.0 percent genetic similarity and one out group at 52.0 per cent genetic similarity (Fig. 2). Jaccard's similarity coefficient ranged from 0.4160 to 0.7280. The cluster 'A' grouped three genotypes (i.e. R-2001-3, GPBD-4, and VG-9816) at 58.70 percent genetic similarity (Table - 3). These three genotypes found resistant to late leaf spot (LLS) and rust respectively. The cluster 'B' comprised of four genotypes, (JL-24, TAG-24, LGN-1, and LGN-117) which are susceptible to rust and LLS except LGN-1 and LGN-117, which are tolerant to rust and LLS disease of groundnut. In this group LGN-1 (tolerant) and TAG-24 (susceptible) showed highest genetic similarity (72.80) as these genotypes closely resemble with each other in general character. Maximum similarity was found in between LGN-1 and TAG-24 at 72.80 percent and lowest similarity in between R-2001-3 and JL-220 at 41.60 percent and hence are genetically most distant indicating if these two varieties are used in hybridization program by the breeder a wide range of genetic variability will be observed in F2 and subsequent segregating generations thereby providing scope of selecting desirable genotypes (Joshi *et al.,* 2013).

DNA isolation protocol of Saghai-Maroof *et al.,* (1984) with some modification was found useful for the sufficient DNA isolation. The isolated DNA produced successful amplification. ISSR primers have advantages with high annealing temperature and repetition and lower cost, and widely used in study of crop plants. In the previous study carried out by Mondal *et al.,* (2008) on 2 varieties viz. VG 9515 (resistant to LLS) and TAG 24 (susceptible to LLS) of groundnut by using 21 ISSR markers resulting 74.5 per cent polymorphism. Further similar result i.e. 74.67 percentage polymorphism was obtained by Mondal *et al.* (2009), after analyze genetic diversity among twenty cultivated groundnut (*Arachis hypogaea* L.) genotypes using 21 ISSR primers. The obtained results were found similar to the results obtained by Azzam *et al.* (2007).

REFERENCES

Azzam Clara, R., Azer, S. A., Khaleifa, M. M. A. and Abol-Ela, M.F. 2007. Characterization of peanut mutants and molecular markers associated with resistant to pod rot disease and aflatoxin contamination by RAPD and ISSR. *Arab J. Biotech.* **10(2):** 301-302.

Bhareti, P., Singh, D. P. and Khulbe, R. K. 2012. Genetic diversity in urdbean [*Vigna mungo* (L.) Hepper] revealed by ISSR markers. *J. Food Legumes.* **25(2):** 89-93.

Bornet, B. and Branchard, M. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter.* **19:** 209-215.

Gowda, M. V. C., Motagi, B. N. Naidu G. K. Diddimani, S. B. and Sheshagiri, R. 2002. GPBD 4: a spanich bunch groundnut genotype resistant to rust and late leaf spot. *International Arachis Newsletter.* **22:** 29-32.

Hammons, R. O. 1982. Origin and early history of the peanut. In peanut Science and Technology, ed H. E. Pettee and C. T. Young 1-20.

Iruela, M., Rubio, J., Cubero, J. I. and Millan, T. 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theor Appl Genet.* **104:** 643-651.

Joshi, M., Verma, S. K., Singh, J. P. and Barh, A. 2013. Genetic

diversity assessment in lentil (*Lens culinaris* medikus) genotypes through ISSR marker. *The Bioscan*. **8(4):** 1529-1532.

Mondal, S., Sutar, S. R. and Badigannavar, A. M. 2008. Comparison of RAPD and ISSR marker profile of cultivated peanut genotype susceptible or resistant to foliar diseases *J. Food Agriculture and Environment*. **6(2):** 181-187.

Mondal, S. Sutar, S. R. and Badigannavar, A. M. 2009. Assessment of genetic diversity in cultivated groundnut (*Arachis hypogaea* L.) with differential responses to rust and late leaf spot using ISSR marker. *Indian J. Genet*. **69(3).**

Ratnaparkhe, M. B., Santra, D. K., Tullu, A. and Muehlbauer, F. J. 1998. Inheritance of inter-simple sequence repeat polymorphisms and linkage with *Fusarium* wilt resistance gene in chickpea. *Theor. Appl. Genet*. **96:** 348-353.

Rohlf, F. G. 1993. NTSYS-PC Numerical Taxonomy and Multivariate analysis: version 1.8 Applied Biostatistics, New York.

Sambrook, J. and Russell, D. W. 2001. Rapid isolation of yeast DNA In: Molecular Cloning: A laboratory manual (Sambrook, J. and Russell, D. W. eds.) Cold Spring Harbor Laboratory Press. New York. 631-632.

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. Proceedings of National Academy of Science, USA 81: 8014-8018.

Shew, B. B. Beute M. K. and Wynne, J. C. 1988. Effect of temperature and relative humidity on expression of resistance to *Cercosporidium personatum* in peanut. *Phytopathol.* **78:** 493-498.

Tahir, N. A. and Karim, H. F. H. 2011. Determination of Genetic Relationship among Some Varieties of Chickpea (*Cicer arietinum* L.) in Sulaimani by RAPD and ISSR Markers. *J. Biological Sciences*. **4(2):** 77-86.