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Genetic Diversity Analysis of Groundnut Germplasm Lines in Respect to Early and Late Leaf Spot Diseases and Biochemical Traits

Madhurjit Singh Rathore[1], Sushma Tiwari[1], M.K. Tripathi[1], Neha Gupta[1], Sunil Yadav[1], Sangeeta Singh[2], R.S. Tomar[3]

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) is one of the most nutritious crops having versatile use. Its yield is mostly exaggerated by two foliar fungal diseases *i.e.*, early and late leaf spot. In present investigation 96 germplasm lines were screened for early and late leaf spot diseases along with diverse biochemical parameters including protein, oil, fatty acids, proline and sugar contents.

Methods: Early and late leaf spot diseases have been screened under field condition as well as employing SSR molecular markers. Nutritional profiling has also been done for protein, oil, fatty acids *viz.,* oleic acid, linoleic acid, stearic acid and palmitic acid, proline and sugar contents.

Result: In correlation analysis, oleic acid is negatively correlated with linoleic acid (r= -0.911) and palmitic acid (r= -0.688) at 1% level of significance, whereas stearic acid is positively and highly significantly correlated with oil percentage (r= 0. 0.381) at 1% significance level. The gene diversity value varied between 0.1866 to 0.4837 for markers ipahm103 and GM1536 with an average of 0.4014 and polymorphic information content (PIC) values ranged between 0.1692 to 0.3855 for the markers pahm103 to GM1536 respectively with a mean value of 0.3183.

Key words: Early and late leaf spot, Groundnut, Linoleic acid, MAS, Oleic acid, Proline.

INTRODUCTION

Cultivated groundnut (*Arachis hypogaea* L.) is a tetraploid (2n = 40) and highly self-pollinated crop having large genome size (2.7 GB). It is cultivated effectively in more than 110 countries of the world and India is the largest exporter in the world. The average productivity is 1893 kg ha^{-1} from cultivated area 4888 million hectare with a total production of 9253 million ton (FAOSTAT, 2018) in India.

Groundnut yield is mostly affected by different biotic and abiotic stresses. Among diverse biotic stresses, foliar fungal diseases cause serious loss. Amongst the foliar contagious ailments, early leaf spot (ELS) caused by *Cercospora arachidicola* and late leaf spot (LLS) fetched by (*Phaeoisariopsis personata*) having significant importance. The combination of two disease can cause case and feed yield loss of over half to 70%. The yield misfortune because of ELS alone reaches up to 70%. Improvement of high yielding cultivars with disease resistance, increase groundnut production. Chemical control measures are available but they increase production costs by 10% (Coffelt *et al*., 1986) and are beyond the reach of small and marginal farmers. Considering above truths in mind, development and growing of resistant cultivars is the best viable option to minimize economic losses of farmer and maintains good quality of the product. Molecular breeding approaches are one of the promising technologies being applied widely for crop improvement programmes (Adlak *et al*., 2019) in groundnut. Over the last few years, more than 5000 simple Sequence repeat (SSR) markers have been developed and being used widely for groundnut improvement (Pramanik *et al*., 2019; Pandey *et al*., 2017). Screening and identification of germplasm for foliar fungal diseases is one of the primary objectives for resistant breeding. Divyadharsini *et al*., (2017) have used marker assisted selection approach for foliar fungal diseases of groundnut and reported, the marker seq8D09 associated with late leaf spot and rust resistance. Allele-specific PCR based markers have also been developed for late leaf spot and rust using QTL-seq approach (Pandey *et al*., 2017). These markers are cost-effective and

[1]Department of Plant Molecular Biology and Biotechnology, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, College of Agriculture, Gwalior-474 011, Madhya Pradesh, India.
[2]National Institute of Plant Genomic Resources, New Delhi-110 067, India.
[3]Rani Laxmibai Central Agricultural University, Jhansi-284 003, Uttar Pradesh, India.

Corresponding Author: Sushma Tiwari, Department of Plant Molecular Biology and Biotechnology, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, College of Agriculture, Gwalior-474 011, Madhya Pradesh, India. Email: sushma2540@gmail.com

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very easy for developing improved groundnut lines with enhanced resistance to LLS and rust (Bhawar *et al.,* 2020).

Groundnut is one of the significant palatable oilseed crops developed in world. Oil content is a significant part and associated with yield potential. The oil substance of groundnut may differ from 40 to 65% contingent on assortment, season and development. Since unsaturated fats make up the significant segment of the heaviness of an oil atom, the physical and compound properties of the oil will in general be dictated by the properties of the unsaturated fats which prevail in their makeup. In spite of the fact that up to 12 unsaturated fats have been accounted for in groundnut, yet in our work the oleic (18:1) and linoleic corrosive (18:2) are the two primary boundaries. Consequently, unsaturated fat organization and sucrose are considered as the significant determinant of groundnut quality (Bera *et al.,* 2018). Germplasm characterization of groundnut, particularly for foliar fungal diseases and fatty acid profiling, is one of the prime objectives to improve groundnut productivity worldwide. Selection of superior germplasm and its utilization in hybridization programme provide basis to get foliar disease resistant lines and high-quality varieties.

MATERIALS AND METHODS

Plant material

In present investigation 89 groundnut germplasms lines (50 local groundnut germplasm lines collected from Shivpuri, Madhya Pradesh and 39 groundnut germplasm ICGV series collected from DGR Junagadh) along with 7 check varieties *viz.,* TG26, GPBG4, KDG128, JGN3, SUNOLIC 95-R, ICGS-44 and Gangapuri were chosen for morpho-physiological traits evaluation and disease indexing under field conditions was done during *Kharif* 2019-20 at Research Farm, Rajmata Vijayaraje Scindia Agricultural University, Gwalior. Molecular characterization for foliar fungal diseases was done by employing gene-specific SSR markers. Among 96 genotypes, GPBD4 and KDG128 are known as resistant to foliar fungal diseases and others are sensitive varieties; while JGN3 and Gangapuri are local check varieties; and Sunoleic 95R is high oleic acid containing genotype.

Screening for early and late leaf spot at field condition

Groundnut germplasms were evaluated for resistance to early and late leaf spot on a 1 to 9 scale (no disease symptoms = 1 and 81 to 100% diseases severity = 9) at 35 and 45 days after sowing for ELS and at 75 and 85 days after sowing for LLS (Subrahmanyam *et al.,* 1995).

Screening foliar fungal disease resistant genotypes using gene-based marker

Plant genomic DNA isolation

The young foliar leaves of 20-days-old seedlings from each groundnut germplasm lines were sampled from field. The genomic DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) with minor modification (Tiwari *et al.,* 2017). The quality of the DNA was checked on 0.8% agarose gel electrophoresis and the DNA concentrations were estimated with the micro volume spectrophotometer. The DNA was diluted to 20 ng/µl concentration to be used in polymerase chain reaction (PCR).

Polymerase chain reaction using SSR markers

Total 16 simple sequence repeat (SSR) markers were used for screening of foliar fungal diseases in groundnut germplasms (Divyadarshini *et al.*, 2017). Out of 16 markers only 5 were found highly polymorphic (Table 1); hence used for screening of all the 96 germplasms (Varma *et al.,* 2005; Sujay *et al.*, 2012; Khedikar *et al.,* 2010). The SSR primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was performed in 10il reaction mixture comprising of 1X PCR buffer, 0.1 U *Taq* DNA polymerase (Fermentas), 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 20 ng/µl of genomic DNA in a thermocycler (Bio-Rad, USA). The PCR protocol comprised of initial denaturation step of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, annealing at 55°C for 30 sec, elongation at 72°C for 1 min with final extension at 72°C for 10 min. The PCR products were resolved on 3% agarose gel at 120V for 2-3 hrs and documented using Syngene, Gel Documentation System (USA).

Molecular markers analysis

The major allele frequency, number of alleles per locus, gene diversity and polymorphism information content (PIC) was analyzed using Power Marker v3.25 software (Liu and Muse, 2005). The dendrogram based on unweighted pair group method for arithmetic average (UPGMA) and bootstrap value of 1000 permutations was constructed using MEGA 6.0 software (Tamura *et al.* 2007).

Biochemical estimation and nutritional profiling

Proline content estimation

According to method suggested by Bates *et al.* (1973) seed samples were homogenized with aqueous sulphosalicyclic acid and 10 min centrifugation at 5000 rpm. In the clear supernatant, acetic acid and ninhydrin was mixed. The reaction was heated to a temperature of 100°C for 1 hour and the reaction was terminated by keeping on ice bath. Toluene was mixed and stored for 10 min in dark. The intensity of colour was read at 520 nm and equated with standard proline.

Total sugar estimation

The total sugar extraction was estimated using Dubois *et al.* (1956) method. A standard graph of sugars was plotted for the determination of sugar content.

Total protein%, oil% and fatty acid profiling

Total protein%, oil% and fatty acid% was estimated by NIRS Spectroscopy at ICRISAT, Hyderabad. Heat map and double dendrogram analysis was done by using R software.

RESULTS AND DISCUSSION

Screening for early and late leaf spot diseases

Early leaf spot at 35 days is found highly significantly and positively correlated with ELS at 45 days (r=0. 963), LLS at 75 days (r=0.762) and LLS at 85 days (r=0.795) at 1% significant level (Table 1). Based on disease scoring at field conditions, out of 96 genotypes, 10 were found highly resistant, 26 moderate resistance, 38 susceptible while 23 displayed highly susceptible expression for the early and late leaf spot during disease scoring (Fig 1).

Phylogenetic cluster analysis and PIC information

A total of 14 alleles were identified with an average of 2.40 alleles per locus for different markers. The gene diversity was documented 0.1866 to 0.4837 for the markers ipahm103 and GM1536 respectively with an average of 0.4014 and Polymorphic Information Content (PIC) values varied between 0.1692 to 0.3855 for the markers ipahm103 and GM1536 correspondingly with a mean value of 0.3183 (Table 2). The primer which showed highest gene diversity and PIC values was GM1536 while the lowest gene diversity and PIC values was observed for the primer ipahm103. The major allele frequency varied between 0.60 (GM2301) to 0.89 (ipahm103) with a mean worth of 0.6979 (Table 2).

The genetic relationships among groundnut genotypes are presented in molecular based UPGMA tree. All the genotypes were grouped into 4 clusters and among them cluster 3 is grouped with resistance for foliar disease and showing high fatty acids composition (Fig 2). Cluster 1 included 37 genotypes including Shivpuri-local collection and two check verities KDG128 and GPBD 4. These genotypes represented resistance or moderately resistance under field condition. They may have resistance gene for early and late leaf spot based on our morphological and molecular data interpretation. Cluster 2 included 17 genotypes and cluster 3 included 37 genotypes. The second largest group was represented by cluster 3 having most of the groundnut germplasm lines collected from DGR Junagadh. It included sensitive check varieties JGN-3, ICGS-44, SUNOLEIC 95-R and Gangapuri so, it can be predicted that all these genotypes may be sensitive or highly sensitive to early and late leaf spot diseases at molecular level as well as under indexing under field conditions. Current study, identified 10 local groundnut germplasms as highly resistant to foliar fungal diseases *i.e.*, Shivpuri Local-21, Shivpuri Local-26, Shivpuri Local-30, Shivpuri Local-31, Shivpuri Local-32,

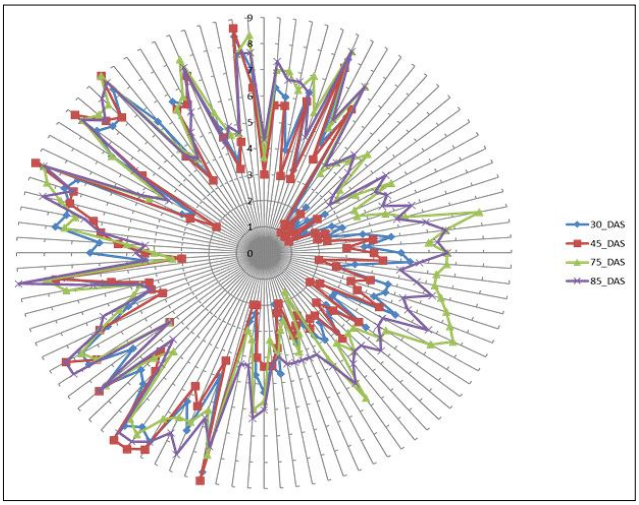

Fig 1: Scatter chart representing number of groundnut genotypes for early and late leaf spot diseases.

Table 1: Details of primers used for screening of groundnut genotypes for foliar fungal diseases.

Primer name	Marker type	Sequence of primer	References
IPAHM103	Dominant	F: GCATTCACCACCATAGTCCA	Khedikar *et al.*, 2010
		R: TCCTCTGACTTTCCTCCATCA	
GM1536	Co-dominant	F: AAAGCCCTGAAAAGAAAAGCAG	Sujay *et al.*, 2012
		R: TATGCATITGCAGG1TCTGGT	
GM2079	Co-dominant	F: GTAACCACAGCMGCATGAAC	Sujay *et al.*, 2012
		R: TCITCAAGAACCCACCAACAC	
GM2301	Co-dominant	F: GGCCAAGGAGAAGAAGAAAGA	Sujay *et al.*, 2012
		R: GAAGGAGTAGTGGTGCTGCTG	
SEQ13A07	Co-dominant	F: AACTCGCTTGTACCGGCTAA	Varma *et al.*, 2005
		R: AGGAATAATAACAATACCAACAGCA	

Table 2: Allele specific SSR markers presenting major allele frequency, number of alleles, gene diversity and polymorphic information content (PIC) in groundnut.

Marker	Major allele frequency	Genotype no	Allele no	Gene diversity	PIC
GM1536	0.6250	4.0000	4.0000	0.4837	0.3855
GM2079	0.6354	2.0000	2.0000	0.4633	0.3560
ipahm103	0.8958	2.0000	2.0000	0.1866	0.1692
GM2301	0.6042	2.0000	2.0000	0.4783	0.3639
Seq13A07	0.7292	2.0000	2.0000	0.3950	0.3170
Mean	0.6979	2.4000	2.4000	0.4014	0.3183

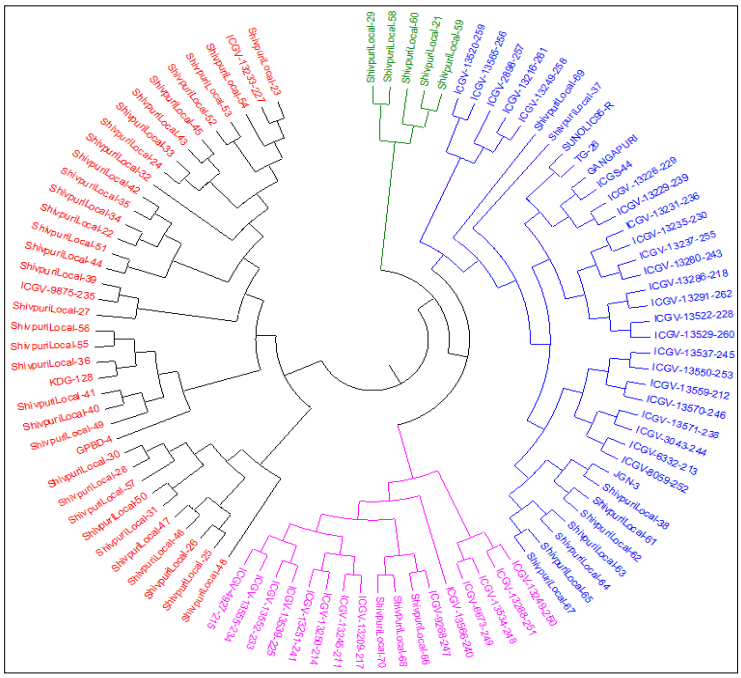

Fig 2: Dendrogram of groundnut genotypes showing clusters based on similarity using UPGMA relationship.

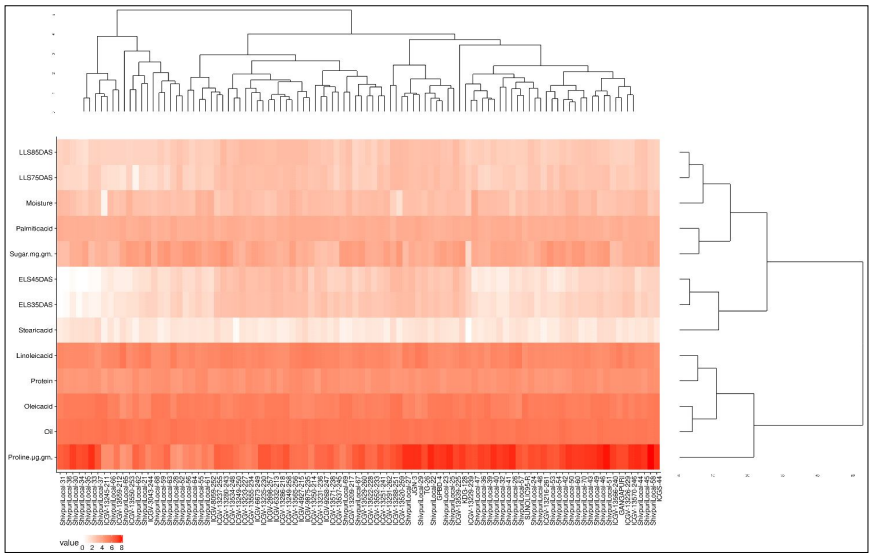

Fig 3: Heat map and double dendrogram of groundnut genotypes between oleic acid, linoleic acid, protein, oil, proline, stearic acid, palmitic acid, early and late leaf spot diseases.

Shivpuri Local-33, Shivpuri Local-34, Shivpuri Local-35, Shivpuri Local-36 and Shivpuri Local-66.

Marker-assisted selection is an important tool to enhance tolerance/resistance to these stresses and has the potential to enable faster and larger gains through genetic improvement of popular varieties (Adlak *et al.*, 2019; Mishra *et al.*, 2020; Upadhyay *et al.*, 2020a). The transfer of targeted traits has been completed in 2-3 years through marker assisted backcrossing (MABC) as opposed to 6-8 years needed with conventional methods (Varshney, 2016; Tiwari *et al.*, 2014). Over the last few years, about 5000 SSR markers have been developed for groundnut (Mondal *et al.*, 2009; Pandey *et al.*, 2017). Recently, Pramanik *et al.* (2022) has identified groundnut germplasm lines for foliar disease resistance and high oleic traits using SNP and gene-based markers. Molecular markers play major role in selecting ELS and LLS resistant genotypes in groundnut.

Biochemical analysis and fatty acid profiling of groundnut genotypes

Proline ranged from 20 (SL-52) to 284 (SL-58) with a mean value of 114.57 (ug g^{-1}), sugar ranged from 4 (ICGV-13229)

Table 3: Correlation coefficient among proline, sugar, moisture, protein, oil, linoleic acid, oleic acid, palmitic acid, steric acid, early and late of groundnut germplasm lines.

	Proline	Sugar	Moisture	Protein	Oil	LA	OA	PA	SA	ELS_{35}	ELS_{45}	LLS_{75}	LLS_{85}
Proline	1	-0.066	-0.088	0.109	0.118	-0.197	0.199	-0.062	0.223*	-0.052	-0.047	0.202*	0.007
Sugar		1	0.047	0.069	-0.070	-0.098	0.026	-0.054	0.037	0.000	-0.014	0.069	0.026
Moisture			1	0.323**	-0.452**	0.000	-0.248*	0.388**	-0.058	-0.026	-0.086	0.077	0.053
Protein				1	-0.597**	0.011	-0.095	0.086	0.031	0.209*	0.168	0.169	0.157
Oil					1	0.181	-0.013	0.059	0.381**	-0.078	-0.049	0.025	-0.015
LA						1	-0.911**	0.606**	0.171	0.148	0.119	-0.053	0.054
OA							1	-0.688**	-0.184	-0.107	-0.059	0.064	-0.004
PA								1	-0.034	0.011	-0.026	-0.003	0.007
SA									1	0.001	-0.020	0.100	0.006
ELS_{35}										1	0.963**	0.762**	0.795**
ELS_{45}											1	0.765**	0.838**
LLS_{75}												1	0.864**
LLS_{85}													1

**: Correlation is significant at the 0.01 level (2-tailed). *: Correlation is significant at the 0.05 level (2-tailed).

Chl_{45} = Chlorophyll at 45 days, Chl_{70} = Chlorophyll at 70 days, CA = carotenoid at 45 days, CA = carotenoid at 70 days, LA = Linoleic acid, OA = Oleic acid, PA = Palmitic acid and SA = stearic acid, ELS_{35} = Early leaf spot at 30 days, ELS_{45} = Early leaf spot at 45 days, LLS_{75} = Late leaf spot at 75 days and LLS_{85} = Late leaf spot at 85 days.

to 25 (ICGV-13280) with a mean value of 14.65 (ugg^{-1}), protein from 18 (SL-65) to 32 (ICGV-4927) with a mean worth of 24.69%, oil between 43 (ICGV-13555) to 58 (Sl-65) with an average of 50.51%, linoleic acid ranged between 23 (SL-37) to 51 (SL-65) with a mean value of 33.73%, oleic acid from 28 (SL-29) to 54 (SL-37) with a mean of 42.12% (Table 1). In correlation analysis, oleic acid is negatively correlated with linoleic acid (r= -0.911) and palmitic acid (r= -0.688) at 1% level of significance, while, stearic acid is positively and highly correlated with oil percentage (r=0. 0.381) at 1% significance level (Table 3).

Phylogenetic analysis of biochemical nutritional parameters

Heat map represented different expression levels of oleic acid, linoleic acid, protein, oil, proline, stearic acid, palmitic acid, early and late leaf spot diseases (Fig 3). Based on these biochemical traits four major clusters has been formed between groundnut genotypes showing similarity between closely related germplasm lines. Nutritional and antinutritional profiling is important aspects of crop improvement and varietal development (Sahu *et al.*, 2020; Upadhyay *et al.*, 2020b; Sharma *et al.*, 2021).

CONCLUSION

Groundnut is considered as cash crop due to its nutritional values. Current study identified groundnut genotypes with foliar disease resistance and high nutritional values. Based on nutritional profiling SL 65 has been identified with highest oil and linoleic acid and 10 germplasms with foliar disease resistance. These genotypes could further be used in groundnut crop improvement programme.

Conflict of interest: None.

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