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Genetic Diversity, Population Structure and Biochemical Parameters Estimations Driving Variations in Groundnut Germplasm

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) is an exceedingly nutritious legume being employed for farming globally. The characterization of groundnut for genetic diversity and population structure can be used for unswerving detection of phenotype x genotype relations.

Methods: The investigation was carried out with groundnut germplasm lines for nutritional and anti-nutritional profiling *i.e*., total sugars, proline, total amino acid, DPPH, phenol, phytic acid, flavonoid, lipid peroxidation and superoxide dismutase activity (SOD) along with molecular characterization and population structure analysis for early and late leaf spot diseases.

Result: The heat map ranged between (-4 to 4) and represents the level of expression of diverse biochemical parameters. The genetic diversity for markers S008 and S053 ranged between 0.2293 to 0.5269 and PIC values ranged between 0.203 to 0.4224 for S008 and S053 molecular markers, respectively. The number of sub populations (K) was constructed based on maximum likelihood and delta K (dK) values subdivided into three subgroups. Employing a membership probability threshold, 31 genotypes were allocated to subgroup (SG) 1, 15 genotypes to SG 2 and rest of the genotypes was mixed proportions.

Key words: Anti-nutritional profiling, Genetic diversity, Groundnut, Nutritional profiling, Population structure.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) locally recognized as “moongphali” in India was introduced in early 16th century and gained agricultural status and emerged as major oilseed crop in 19th century in the country (John *et al.,* 1955). It is a leguminous cash crop which is highly self-pollinated auto-tetraploid (2n=4x=40) with the extent of out crossing up to 3.9% only owing to very small insects such as ants. This crop is lengthily cultivated in tropical and sub-tropical region originated in South America with a genome size of 2891 Mbp, originated through a single hybridization and polyploidization event (Bhawar *et al.*, 2020). In Indian subcontinent peanut is compulsively consumed as cooking oil and in forms of an array of food products. It is valued as an opulent source of energy in form of oil (48-50%) and protein (25-28%) in the kernels. It provides 564 kcal of energy from 100 g of kernels (Jambunathan *et al.,* 1991). Along with this many health beneficial nutrients such as minerals, it is also very rich in vitamins and antioxidants. Its haulms can be used as nutritional fodder for livestock which contains protein (8-15%), lipids (13%), minerals (9-17%) and carbohydrate (38-45%) higher than cereal fodder.

In groundnut there are several factors which affects its productivity, these factors can be biotic or abiotic. Biotic stress factors mainly are early and late leaf spot, rust, mottle virus caused by *Cercospora spp, Phaeoisariopsis personata, Puccinia arachidis* and *Peanut mottle virus* respectively on the other hand major abiotic stress is imposed by drought or salinity conditions. Sometimes severe heat and cold also affects the crop production adversely. During stress condition, crops try to cope up with it through various biochemical and molecular mechanisms. Certain stress parameters such as chlorophyll content, total sugar content, proline content, phytic acid content, superoxide dismutase (SOD) assay, reactive oxygen species (ROS) assay helps to demonstrate how do a plant react biochemically to overcome such stress and to sustain against them. Thus, these biochemical estimation helps in identifying the genotype which try to withstand with diseases by adopting such mechanisms. Marker assisted selection employing SSR molecular markers are preferred as, SSR markers are size and sequence specific, co-dominant, springs reproducible results and are user friendly and hence are

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widely used worldwide for different crops (Janila *et al.,* 2013; Tiwari *et al*., 2014; Adlak *et al*., 2019; Pramanik *et al*., 2019; Sahu *et al.,* 2020; Mishra *et al.,* 2022; Upadhyay *et al.,* 2020a; Pramanik *et al*., 2022; Yadav *et al*., 2021; Mandloi *et al*., 2022). Thus, screening of foliar fungal disease in groundnut using SSR markers is of great convenience. Keeping all facts in mind, present investigation was conducted for screening of groundnut germplasm lines on the basis of different biochemical traits and SSR molecular markers in context to foliar fungal diseases.

MATERIALS AND METHODS

Plant material

In present investigation, five check varieties including JGN 3, GPBG4, SunOleic 95 R, KDG-128 and Gangapuri and fifty-one groundnut germplasm lines collected from Junagarh, Gujarat (24), Shivpuri, Madhya Pradesh (11), Dhar, Madhya Pradesh (8), Badwani, Madhya Pradesh (5) and Jhabua, Madhya Pradesh (04) were taken. Temperature between 25°C-30°C suits best for the crop with an average rainfall ranging between 50-75 cm. The experimental field of College of Agriculture, Gwalior is located at 26 13″ N latitude, 78 14″ E longitude and at an altitude of 211.5 m above the sea level in gird belt. It has hot weather conditions and during summers the temperature rises beyond 45°C.

Methodology

Biochemical estimation for nutritional and anti-nutritional parameters

Ten different biochemical parameters including chlorophyll content, total sugar, total amino acid, proline, phenol, DPPH, phytic acid, flavonoid, lipid peroxidation and H_2O_2 peroxidation were estimated from the leaves at 35 Days after sowing for investigate the nutritional and antinutritional values of the groundnut genotypes. Based on the estimated values grouping of genotypes was done along with heat map to study the effect of level of expression in genotypes. Total chlorophyll was calculated as per method suggested by Arnon *et al*. (1949), total sugars as per protocol described by Dubois *et al*. (1956), proline as per method of Bates *et al*. (1973), total amino acid by the method described by Moore and Stein (1948), DPPH as per Sultana *et al*. (2007), phenol as per method employed by Swain and Hills (1959), Phytic acid as per the method given by Wilcox *et al*., (2000), Flavonoid given by Zhu *et al*. (2010), Lipid peroxidation by the protocol proposed by Hodges *et al*. (1999). Assaying for superoxide dismutase activity (SOD) was calculated by the method of Beyer *et al*. (1987).

Molecular characterization

Genomic DNA was isolated from 20-30 days young leaves of groundnut germplasms by modified CTAB method (Murray and Thompson, 1980; Tiwari *et al*., 2017). Twenty-six SSR primers unveiling polymorphism between two contrasting genotypes were employed for analysis of genetic diversity among 56 genotypes included germplasm lines and check varieties (Pramanik *et al*., 2019). Out of 26 only 11 primers were found to be polymorphic. The SSR primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was accomplished in 10µl reaction mixture encompassing 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 tracked 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).

Genetic diversity and population structure assessment

The genetic profile of groundnut genotypes was scored on the basis of difference in allele size using 11 highly polymorphic SSR molecular markers. The major allele frequency, number of alleles per locus, polymorphism information content (PIC) and gene diversity 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). 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). The population structure for groundnut genotypes comprising both germplasm lines and released check varieties was inferred using Structure 2.3.4 software (Pritchard *et al*., 2000). The structure outputs were visualized using Structure Harvester from which Evanno plots were constructed (Earl and Von Holdt, 2012). An assumed admixed model with independent allele frequency and a uniform prior probability of the number of populations, K was used in structure. All the runs were conducted for K= 1 to 10 with 50,000 Markov Chain Monte Carlov (MCMC) replicates after a burn-in of 50,000 replicates. For each value of K, 3 independent runs were done to generate an estimate of the true number of sub-populations.

RESULTS AND DISCUSSION

Biochemical estimation for nutritional and antinutritional parameters

Minimum chlorophyll content at 470 nm absorption was documented for the genotype Talun1 (0.891) and maximum for the genotype ICGV13574. Total sugar was measured minimum in genotype Shivpuri local 75 (0.035) while maximum in genotype ICGV- 13269 (0.076). Phenol was minimum for the genotype DHGN7 (0.059) and maximum for the genotype ICGV13574 (0.159). Lipid peroxidation was maximum in genotype ICGV13245 (0.156) and minimum in genotype ICGV13236 (0.045). Flavonoid was observed to

be maximum and minimum in genotypes GPBD4 (0.044) and JCGN4, (0.781) respectively. DPPH had the minimum value of (0.169) for the genotype Shivpuri Local-37 and maximum for the genotype Shivpuri local 65. Phytic acid was found maximum in genotype DGGN4 (0.343) and minimum in genotype JGN3 (0.169). Proline accumulation was minimum in genotype Shivpuri local 82 and maximum in genotype ICGV7988 (0.281). SOD was documented minimum for the genotype GPBD4 (0.109) and maximum for the genotype ICGV9249 (0.412).

Diversity and expression analysis among biochemical parameters

Phylogenetic analysis between groundnut germplasm lines for nutritional, anti-nutritional and antioxidant profile revealed three major clusters. Based on dendrogram (Fig 1) we can classify 56 genotypes in two major groups A and B having 14 and 45 genotypes respectively. Group A is further divided into A_1 and A_2 having 10 genotypes and 4 genotypes respectively, whereas group B is divided into B_1 and B_2 which consisted of 19 and 26 genotypes respectively and further division goes on. Genotype ICGV13574 is most diverse to genotype Dhar1. The heat map is ranged between (-4 to 4) and it represents the level of expression of different biochemical parameters (Fig 1). The expression of proline seems to be maximum in genotype KDG128, a check variety for disease resistance and minimum in Shivpuri local28, the chlorophyll expression seems to be maximum in genotype DGGN-6 and minimum in genotype ICGV13562. The expression for lipid peroxidation is most expressed in genotype ICGV-13523 and least in genotype Gangapuri a sensitive check variety (Fig 1). Flavonoid seems to be most expressed in genotype ICGV-13523 and least in genotype ICGV13264. DPPH was seemed to expressed minimum in genotype Shivpuri local-37 and maximum in genotype Dhar-1. Phenol expression seems to be maximum in genotype Shivpuri local-6 and minimum in genotype Bajjatakhurd-2. The expression for total amino acid has been documented maximum in genotype Shivpuri local-28 and minimum in genotype ICGV-13264. Phytic acid expression was seen maximum in genotype Shivpuri local-42 and minimum in genotype ICGV-13264. Total sugar expression seems to be maximum in genotypes JCGN4 and DHGN6 and minimum in genotype ICGV13264 and SOD activity seems to be mostly expressed in genotype ICGV-9112 and least in genotype GPBD-4. Recently, Rathore *et al.* (2023) have studied different groundnut germplasms and represented heat map for different expression levels of oleic acid, linoleic acid, protein, oil, proline, stearic acid, palmitic acid, early and late leaf spot diseases. Nutritional and antinutritional profiling is important aspects of crop improvement and varietal development (Sahu *et al*., 2022; Upadhyay *et al*., 2023; Mishra *et al*., 2021; Sharma *et al*., 2021).

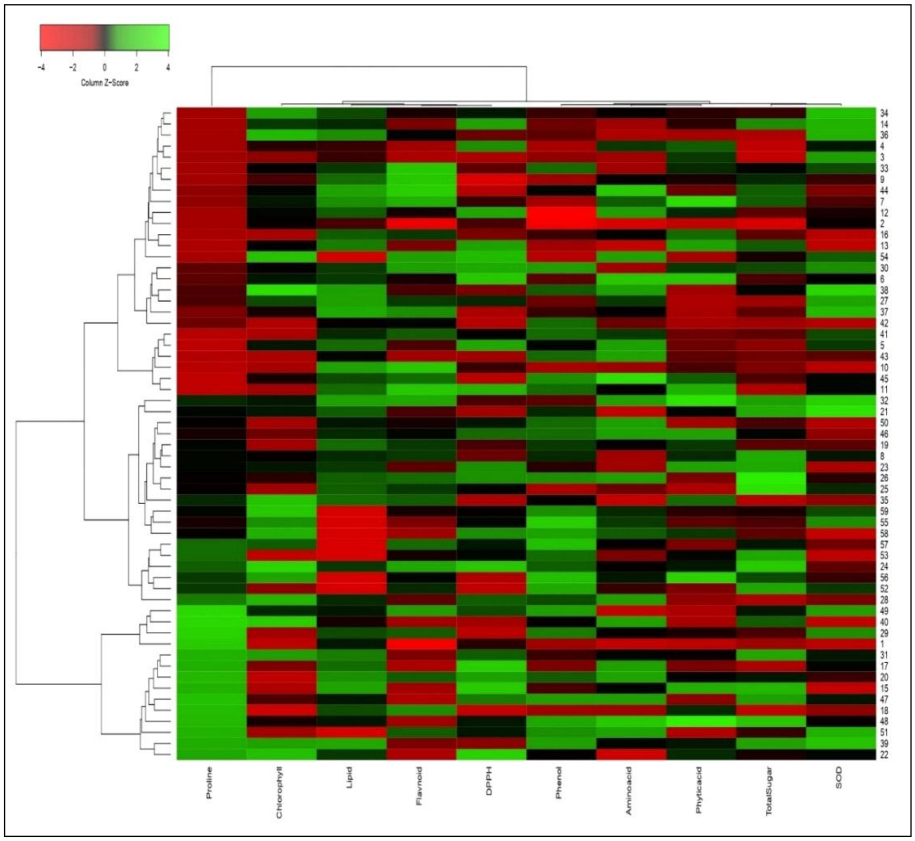


Fig 1: Double dendrogram representing diversity among groundnut germplasm lines based on biochemical observations and expression pattern of different biochemical parameters.

Phylogenetic analysis of SSR markers and PIC information

The number(s) of alleles identified were 28 in total with an average of 2.55 allele per locus. The range of number(s) of alleles per locus was found between 2 to 3. The genetic diversity varied from 0.2293 to 0.5269 for markers S008 and S053 respectively. PIC values ranged between 0.2030 for marker S008 to 0.4224 for marker S053. The mean genetic diversity was documented 0.3711 and mean PIC value comes out to be 0.3068. The major allele frequency varied between 0.8679 for marker S008 and S046 and 0.5472 for marker S053 with a mean of 0.7376 (Table 1). The UGMA tree shows the genetic relationship between the groundnut germplasm lines collected from different parts of Madhya Pradesh (Fig 2). All the genotypes were grouped in 9 clusters. In groundnut very low variation has been reported using a variety of molecular markers such as microsatellites or simple sequence repeats (SSRs), randomly amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) analysis because of the evolutionary genetic bottleneck (Varshney *et al.,* 2013). In our study also we found only 11 polymorphic markers.

Population structure analysis

The population structure of the 56 groundnut genotypes was estimated using STRUCTURE v2.3.4 software based on SSR molecular markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K= 3. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, into three subgroups. Using a membership probability threshold of 0.8, 31 genotypes were assigned to subgroup (SG) 1, 14 genotypes to SG 2 and rest of the genotypes was mixed proportions (Fig 3).

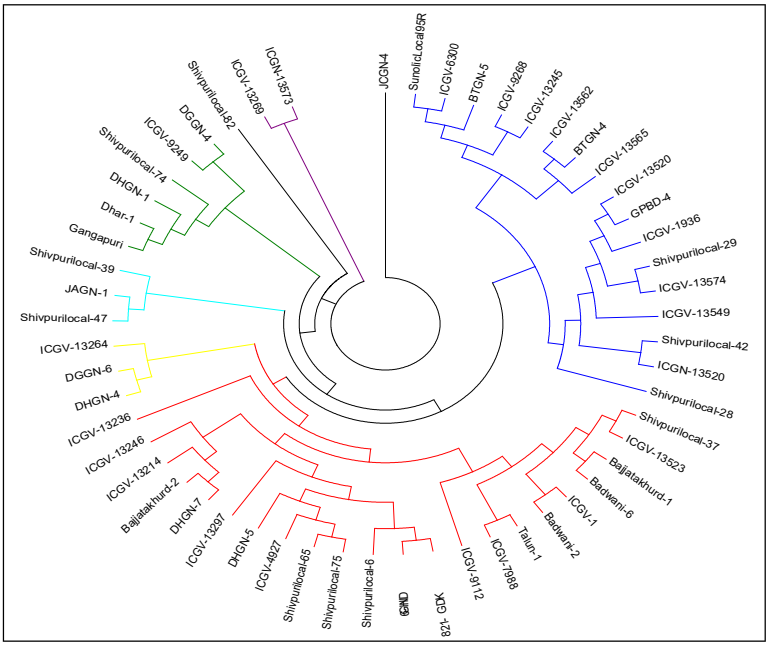


Fig 2: Diversity among groundnut germplasm lines based on SSR markers profiling.

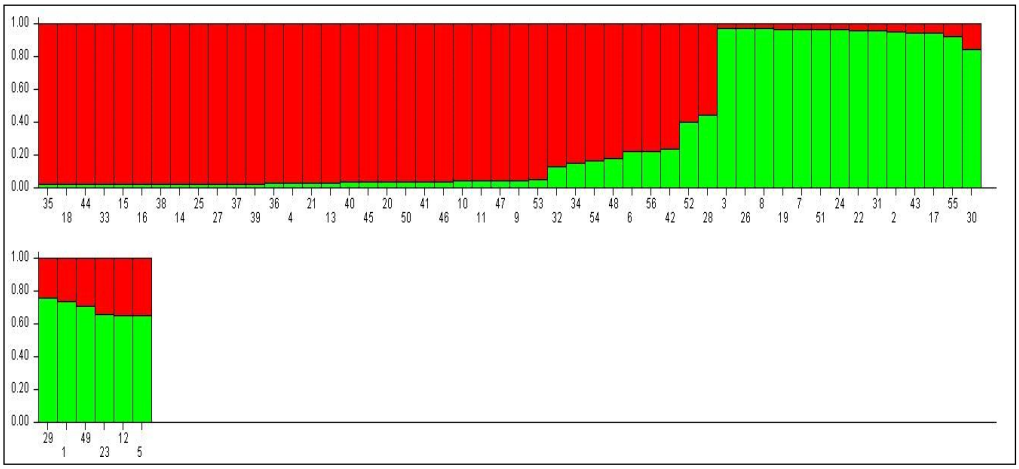


Fig 3: Model based population structure plot with K = 3, using polymorphic SSR markers.

Table 1: Allele specific SSR markers presenting major allele frequency, number of alleles, gene diversity and polymorphic information content (PIC).

Name of SSR	Marker code	Major allele frequency	Allele no.	Gene diversity	PIC
PM238	HB569420-21	0.5849	2.0000	0.4856	0.3677
PM346	HB569422-23	0.8302	3.0000	0.2876	0.2557
PM419	HB569430-31	0.6604	3.0000	0.4607	0.3705
S001	HB569448-49	0.7547	3.0000	0.3859	0.3351
S003	HB569453-54	0.7736	2.0000	0.3503	0.2889
S007	HB569458-59	0.7170	2.0000	0.4058	0.3235
S008	HB569460-61	0.8679	2.0000	0.2293	0.2030
S046	HB569498-99	0.8679	3.0000	0.2335	0.2137
S050	HB569504-05	0.8491	2.0000	0.2563	0.2235
S053	HB569508-09	0.5472	3.0000	0.5269	0.4224
S083	HB569544-45	0.6604	3.0000	0.4607	0.3705
	Mean	0.7376	2.5455	0.3711	0.3068

The relationship between subgroups derived from STRUCTURE explained that SG 1 comprised of disease resistant ELS and SG 2 comprised of sensitive types LLS germplasm lines, respectively. This indicated that the population structure was in accordance with clustering of groundnut genotypes formed using UPGMA tree based on SSR data. Similar study was conducted by Pramanik *et al.* (2019) estimated population structure of the 96 groundnut genotypes using STRUCTURE v2.3.3c software based on 26 SSR markers and the highest peak was observed at delta K= 10.

CONCLUSION

Identified highly polymorphic SSR markers can be employed for analysis of genetic diversity in other set of groundnut germplasms. The study identified diverse clusters of germplasms with known check varieties and some unique clusters representing new traits in germplasm lines. The morphological and molecular characterization of peanut (*Arachis* spp.) will be helpful in formation of strategies for collection, conservation and development of new varieties using various germplasm lines. Also, identified groundnut germplasm lines with superior characters could be used in hybridization programme for crop improvement.

Conflict of interest

All authors declared that there is no conflict of interest.

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