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Molecular characterization of groundnut (*Arachis hypogaea* L.) germplasm lines and varietal set for yield and yield attributing traits

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Abstract

The genetic assessment of 90 germplasm lines and six varieties of groundnut (*Arachis hypogaea* L.) were done with 13 morphological traits and 125 Simple Sequence Repeats markers. Out of 125 molecular markers, 26 were polymorphic and produced 105 alleles. The genetic diversity was found to be 52-83 per cent and Polymorphic Information Content (PIC) was 0.46-0.81 with a mean of 0.42 indicating higher magnitude of genetic diversity in the test genotypes. Analysis of molecular variance showed variation among and within individuals based on allelic variation. Principal Co-ordinate Analysis based on origin of the genotypes formed three major population groups and the genetic analysis determined by population structure divided all the germplasm lines in to 10 populations. Significant and positive correlation was observed between hundred kernel weight and hundred pod weight (r=0.769) and kernel yield (r=0.899); sound mature kernel and pod weight with kernel yield, weight of kernels and harvest index. Genotypes from distinct clusters may be selected in hybridization programme for groundnut improvement. The information on clustering of genotypes will be helpful in identification of novel and superior germplasm for hybridization and development of improved varieties.

Key words: Groundnut, germplasm lines, simple sequence repeat, Polymorphic Information Content (PIC), Principal Co-ordinate Analysis (PCA)

Introduction

Cultivated groundnut or peanut (*Arachis hypogaea* L.) is a self-pollinated oilseed crop and an allotetraploid (AABB, 2n=4x=40) originated from hybridization of two ancient diploid species *viz*., *A. duranensis* (A-genome) and *A. ipaënsis* (B-genome) followed by chromosome doubling (Seijo et al. 2004; Leal-Bertioli et al. 2009). It has a genome size of 2891 Mbp. The groundnut is cultivated in more than 100 countries under different agro-climatic conditions on about 26.5 mha with a total production of 43.9 mt and productivity of 1654 kgha^{-1}. (FAO, 2017). India is the second largest producer of groundnut and its oil after China followed by USA and Nigeria. It is cultivated on about 3.7 mha with the production and productivity of 6.7 mt and 1810 kgha^{-1}, respectively during 2015-16 (Anonymous, 2017). Groundnut is valued as a rich source of energy in form of oil (48-50%) and protein (25-28%) in the kernels. The haulms provide nutritious fodder for livestock which contains protein (8-15%), lipids (1-3%), minerals (9-17%) and carbohydrate (38-45%) higher than cereal fodder. With the rising population world over the demand for groundnut is also increasing hence, greater efforts are needed to increase crop yield from diverse ecological conditions (Guo et al. 2012). The crop improvement by conventional breeding selection, intra-specific or inter-specific hybridization plays an important role to increase groundnut yield (Tang et al. 2007; Guo et al. 2012). The development of groundnut genotypes by identification of genomic regions associated with abiotic stresses such as iron deficiency chlorosis (IDC) tolerance (Naidu et al. 2017) and phosphorous efficient genotypes (Ajay et al. 2017) has led to increased growth and yield. The phenotype based selection procedure followed by selection of promising breeding lines through yield evaluation traits has increased the production more than double in the past.

Groundnut sub-genomes are closely related and contain about 64% repetitive sequences (Dhillon et

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al. 1980; Bertioli et al. 2016). The progenies evolved from parents with diverse origins have a higher probability to produce superior progenies than those of similar ancestry, but it has become increasingly difficult to find high yielding genotypes that do not have common parentage. With the advent of genomic tools, marker assisted breeding (MAB) was deployed to enhance efficiency of selection of target traits in groundnut (Janila et al. 2013; Kanyika et al. 2015). Draft genome sequences for both the diploid progenitors of tetraploid cultivated groundnut are available (Bertioli et al. 2016; Chen et al. 2016) that could help in finding the genes and SNPs present in the QTL regions on the diploid genomes (Zhou et al. 2015). Molecular marker analysis on groundnut germplasm 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) in general has shown very low variation in cultivated gene pool because of the evolutionary genetic bottleneck in the form of polyploidy and self-pollination (Kochert et al. 1996; Subramanian et al. 2000; Varshney et al. 2013). On the other hand, wild diploid *Arachis* species showed relatively higher variation (Hilu and Stalker, 1995; Bravo et al. 2006) providing a rich source of genetic variation for genetic and genomic studies. Among different marker systems analysed in the groundnut SSR markers have been found more informative and useful for genetic analysis and breeding applications (Pandey et al. 2012). The current study was undertaken to analyse genetic diversity at morphological and molecular level in 90 germplasm lines and six released varieties for yield and yield attributing traits using SSR molecular markers.

Materials and methods

Plant material

The materials consisted of 90 uncharacterized germplasm lines including 53 hypogeal runner type, 34 hypogeal bunch type, 3 *vulgaris* and one *fastigiata* type and six popular varieties developed by Directorate of Groundnut Research (DGR) Junagadh, Gujarat. The diverse germplasm considered for this study included material originating from 17 different countries *i.e.*, United States of America (22), United Kingdom (16), India (7), Sudan (11), Senegal (6), Tanzania (6), Taiwan (1), China (4), Sri Lanka (4), Mexico (3), Nigeria (3), Zambia (2) Argentina (1), Australia (1), Brazil (1), Myanmar (1) and Uganada (1) (Table 1). The cultivated varieties included TG 26 (BARCGI X TG 23), a high yielding variety with fresh seed dormancy and early maturity; GPBD4 and KDG128, resistant to foliar disease and giving high yield; ICGS44 (Selection from Robust 33-1), high yielding variety with drought tolerance; Sunolic 95 R with high oleic acid content and JGN3, sensitive to foliar diseases released from

Table 1. List of groundnut germplasm lines and their place of origin

Country of origin	Germplasm lines	Country of origin	Germplasm lines
USA	S131^{R}, Tifton1133^{R}, US57^{R}, US64^{R}, GAB15112^{B} NC4^{B}, B1280^{B}, B28^{V}, NCAC3033^{B}, AH7864^{B},AH7067^{B}, AH7243^{B}, AH7244^{B}, AH7248^{B}, AH7366^{B}, AH7522^{B}, NC4X^{B},2800^{B}, USA9^{B}, AH7188^{B}, AH7224^{B}	UK	C107^{R}, C108^{R}, A4771^{R}, Tesobunch^{R}, Kanyomabulk^{R}, R713 V^{R}, AH6914^{B}, NC1^{B}, AH7374^{B}, AH7583^{B}, Manfredi88^{B}, R7241^{B}, V53^{B}, 2332^{B}, 6885^{B}
Sudan	R7477^{R} , R7479^{R}, Southeasternru^{R}, USA60^{R}, Venezuela^{R}, Virginiasihit^{R}, S727^{R}, U4713^{R}, S7218^{B}, S722^{B}, S7116^{V}	India	RR90^{R}, C14512P7^{R}, M145^{R}, MF53^{R} VRR222^{B}, Robut331^{B}, GPBD4^{Var}, KDG124^{Var}, TG26^{Var}, JGN3^{Var}, Sunolic^{Var}, ICGS44^{Var} RS1^{V},
Senegal	R7475^{R}, R4195^{R}, 2813^{R}, 2839^{R}, AH7457^{B}, 69101^{F}	Tanzania	R749^{R}, R7410^{R}, R7123^{R}, R7245^{R}, R7247^{R}, Bansaino.1^{B}
Sri Lanka	AH6993^{R}, AH17^{R}, AH7245^{R}, AH7295^{R}	China	AH7339^{R}, A4^{R}, EC7585^{R}, MD351^{R}
Mexico	Morilose^{R}, Newberryrunner^{R}, Spanishpeanut^{R}	Nigeria	S724^{R}, S719^{R}, AH7218^{B}
Zambia	AH678^{R}, AH5^{R},	Brazil	S72413^{R}
Australia	Cotedeivorie^{R}	Argentina	US6^{R}
Uganda	R7246^{R}	Mexico	AH7140^{R}
Taiwan	Penghu2^{R}		

where R = Hypogeal runner type; B = Hupogeal branch type; Var = Variety, V = *vulgaris* and F = *fastigiata*

Madhya Pradesh. All the six varieties taken in the present study were developed in India for different ecologies and cultivated regions.

Growth conditions for field evaluation

The field experiment was conducted at Research Farm, Department of Plant Breeding and Genetics, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) in augmented design with checks at regular intervals over two years. The material was planted in rows with inter and intra row spacing of 30 and 10 cm. Prior to sowing, the seeds were disinfected with fungicide (Dithane M-45 @2.0gkg^{-1} seed + Bavistin@1.0gkg^{-1} seed). The crop was raised following the recommended cultural practices with application of fertilizer NPK in a ratio of 20:60:20 and essential dose of gypsum. The experimental land is situated at 26^{o} 13 N latitude and 78^{o} 14' E longitudes at an altitude of 211.5 m above sea level in Gird belt (MLS). It has a subtropical climate with hot and dry summer where maximum temperature exceeds 45^{o}C during May and June. The winters are cold and the minimum temperatures reaches as low as 2^{o}C in the month of December and January.

Measurement of morphological traits and statistical analysis

A set of 90 germplasm lines and six varieties were evaluated for 13 morphological traits namely, initial plant stand, final plant stand, days to 50% flowering, days to maturity, fresh weight/plant (g), dry weight/plant (g), number of pods/plant, hundred pod weight (g), hundred kernel weight (g), kernel yield (g), sound mature kernel, shelling percentage and harvest index were recorded. The initial plant stand and final plant stand have been counted to see sensitivity of particular lines by counting plant number at seedling stage and before harvesting. Three plants were taken at random when plant were green and weighing was done to get fresh weight and dry weight of plants were recorded after drying under sun light. The other yield contributing traits viz., number of pods/plant, hundred kernel weight (g), kernel yield (g), number of sound mature kernel, shelling percentage and harvest index were also recorded. The analysis of variance (ANOVA) of morphological traits was carried out to calculate standard error (S.E.), critical difference (CD) and coefficient of variation (CV). The coefficient of correlation among all morphological traits at maturity was calculated using SPSS ver19.0 software.

DNA extraction and genotyping with SSR markers

The 20 days old young leaves from each genotype were sampled from field. The genomic DNA was extracted using CTAB method (Murray and Thompson, 1980) with minor modification. The quality of the DNA was checked on 1% agarose gel and the DNA concentration was estimated with the micro volume spectrophotometer (Helix Biosciences, New Delhi, India). The DNA concentration for use in polymerase chain reaction (PCR) was diluted to 20ng/µl.

A total of 125 SSR primers reported by Pandey et al. (2012) were used for genetic diversity study. Out of these primers, 26 were found to be polymorphic between two contrasting groundnut genotypes (KDG128, foliar disease tolerant and high yielding and JGN3, foliar disease sensitive and low yielding). These selected primers were used for molecular characterization of 96 genotypes which included germplasm lines and varieties. The SSR primers were synthesized by Eurofins Genomics India Pvt. Ltd. Polymerase chain reaction was performed in 10 µl reaction mixture comprising of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pico Molar) and 2 µl (conc. 20ng/µl) of genomic DNA in a thermocycler (Bio-Rad, USA). The PCR protocol comprised of initial denaturation step of 94^{o}C for 3 min followed by 35 cycles of 94^{o}C for 1 min, annealing at 55^{o}C for 30 sec, elongation at 72^{o}C for 1 min with final extension at 72^{o}C for 10 min. The PCR products were resolved on 3% agarose gel at 120V for 2-3 hrs and documented using Zenith, Gel Documentation System (India).

Genetic diversity analysis

The genetic profile of 96 groundnut genotypes was scored on the basis of difference in allele size using 26 SSR 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). Based on the banding pattern data was recorded with allele pattern A/A and B/B homozygous condition and A/B for heterozygous condition and in case of no amplification (-/-) was used. The population structure for 96 groundnut genotypes comprising both germplasm lines and 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 et al. 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. The principal co-ordinate analysis (PCA) and relation between genetic similarity identified by SSR markers and taxonomic distance measured by mean genetic distance and yield were analyzed using Jaccard's similarity index, calculated by NTSYS-pc v2.1 software. Duncan's Multiple Range Test (DMRT) (P = 0.05) was used to evaluate differences among clusters for significance by using SPSS ver. 19.0 software.

Results

Morphological characterization

The days to 50% flowering varied from 38 to 61 and days to maturity from 90-120. The fresh weight of plants ranged from 120-574g, number of pods per plant 12-66, weight of pods per plant 18-120g, hundred pods weight ranged from 40-110g, kernel yield ranged from 6-94g, hundred kernel weight 28-84 and harvest index 7-69%. The weight of sound mature kernel was in the range of 32-82 and average shelling percent was observed 12-85. Significance of correlation of different traits was analyzed using SPSS ver. 19.0 software at 1% and 5%, probability levels respectively (Table 2). Significant and positive correlation was found between hundred kernel weight and hundred pod weight (r=0.769) and kernel yield (r=0.899) at 1% significant level. Similarly, significant and positive correlation was detected between sound mature kernel and pod weight; kernel yield and kernel shelling percent found correlated with weight of pods, kernel yield and weight of kernels. Sound mature kernel and harvest index were also significantly correlated to pod weight, kernel yield, kernel weight and shelling percent at 1% significant level (Table 2). However, a negative correlation was also observed for fresh weight (r=-0.621) and dry weight (r=-0.528) with harvest index at 1% significance level (Table 2). This shows that fresh weight and dry weight of the plant conversely affect harvest index.

Phylogenetic analysis and PIC information

A set of 125 SSR primers were screened in two genotypes, out of which, 26 showed polymorphism and were selected for molecular characterization of 90 germplasm lines and six varieties. A total of 89 alleles were identified with an average of 4 alleles per locus for 18 highly polymorphic SSR markers (Table 3). The number of alleles per locus ranged from 3

Table 2: Pearson correlation coefficients among thirteen yield attributing traits recorded under field conditions

Trait	IPS	FPS	DF	DM	FW	DW	NPP	HPW	KYD	HKW	MK	SP	HI
IPS	1	0.923**	–.135	–.034	.006	–.010	.047	–.010	0.065	.144	.131	.087	.009
FPS	.	1	–.095	–.004	.110	.079	.032	–.034	0.025	.082	.100	.041	–.067
DF50			1	.086	–.011	.014	–.187	–.204	–.188	–.108	–.127	–.167	–.050
DM				1	0.143	0.185	0.192	–.077	–.017	–.028	–.092	–.030	–.126
FW					1	0.819**	0.025	–.058	–.004	–.029	.025	–.019	.628**
DW						1	-.068	–.110	–.044	–.106	–.027	–.078	–519**
NPP							1	0.283**	0.276**	0.183	0.179	0.250*	0.197
HPW								1	0.938**	0.769**	0.496**	0.913**	0.527**
KYD									1	0.899**	0.614**	0.989**	0.444**
HKW										1	0.716**	0.953**	0.339**
MK										*	1	0.661**	0.181
SP												1	0.428**
HI													1

** = Correlation is significant at the 0.01 level (2-tailed), * = Correlation is significant at the 0.05 level (2-tailed), IPS = Initial plant stand; FPS = Final plant stand; DF = Days to 50% flowering; DM = Days to maturity; FW = Fresh weight of plants; DW = Dry weight of plants; NPP = Number of pods per plant; HPW = Hundred pod weight; KYD = Kernel yield; HKW = Hundred kernel weight; MK = Sound mature kernel; SP = Shelling %; HI = Harvest Index

Table 3. Allelic variation and PIC values for microstallite loci (SSR) identified in 96 groundnut genotypes

Primer name	Major allele frequency	Allele number	Gene diversity	PIC
PM15	0.6042	4.00	0.5488	0.4848
PM42	0.5104	6.00	0.6497	0.5994
PM73	0.3750	6.00	0.7563	0.7217
PM204	0.2083	7.00	0.8398	0.8193
PM346	0.2500	6.00	0.7886	0.7550
S009	0.2500	6.00	0.7913	0.7580
S026	0.8542	3.00	0.2563	0.2357
S048	0.6250	4.00	0.5297	0.4666
S050	0.2552	6.00	0.7901	0.7569
S052	0.8438	3.00	0.2715	0.2478
S057	0.6146	4.00	0.5447	0.4848
S059	0.4896	4.00	0.6056	0.5285
S076	0.3125	6.00	0.7925	0.7629
S078	0.3698	7.00	0.7594	0.7247
S084	0.4271	4.00	0.7027	0.6527
S107	0.8542	3.00	0.2563	0.2357
S110	0.6146	4.00	0.5447	0.4848
GM724	0.2708	6.00	0.7984	0.7679
Mean	0.6286	4.03	0.4596	0.4296

(S026, S052 and S107) to 7 (PM204, S078). The gene diversity and PIC values varied between 0.25 to 0.83 and 0.23 to 0.81 with an average of 0.45 and 0.42. The primer PM204 showed highest gene diversity (0.839) and PIC (0.819) values while primer S026 displayed lowest gene diversity (0.25) and PIC (0.23). The major allele frequency varied between 0.2 (PM 204) to 0.62 (S080) with the mean value of 0.63 (Table 3).

Cluster analysis using molecular markers and morphological traits

The genetic relationships among germplasm lines and varieties are presented in SSR based Unweighted Neighbor Joining (UNJ) dendrogram (Fig. 1). All the genotypes were grouped into 7 major clusters. The germplasm lines GAB15112R, GA20734, AH7374, AH7366, S7116, AH7522 and NC1 were distinct and not included into these clusters. Cluster 1 contains 19 genotypes while five genotypes were grouped in Cluster 2. Cluster III was formed with maximum number of genotypes (30) including high yielding and drought tolerant variety, ICGS 44; high yielding and fresh seed dormancy variety, TG26 and high oleic acid containing variety, Sunolic 95 R. Sixteen sensitive genotypes were grouped with JGN 3, which is sensitive to foliar disease in cluster 7. Clusters 4, 5 and 6 consisted of genotypes which showed intermediate banding pattern for yield attributing traits and drought tolerance. The clusters based on SSR markers are found to have relationship with the degree of drought tolerance, sensitivity and resistance to foliar disease. Most of the genotypes with the similar degree of tolerance were clustered into same groups. Further, contrasting genotypes for yield were separated when correlation between genetic similarity index and taxonomic distance for average yield was evaluated using Jaccard's similarity index using NTSYS ver 2.1 (Rohlf 2000). Most of the germplasm lines were characterized bearing similar morphological traits. The variety in the coordinate KDG128 a high yielding consist germplasm lines which may possess traits contributing higher yield (Fig. 2)

Population structure analysis

The population structure of the 96 genotypes was constructed using STRUCTURE v2.3.3c software based on 26 SSR markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 10. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values into ten subgroups (Fig. 3). Using a membership probability threshold of 0.75, seven genotypes were assigned to subgroup SG 1, 10 genotypes to SG 2, 17 to subgroup 3, 21 to subgroup 4, four to subgroup 5, 30 to subgroup 6 and six genotypes were assigned to subgroup 7. The relationship between subgroups derived from STRUCTURE explained that SG 1 to SG 10 comprised of distinct types. These results indicated that the population structure was in accordance with clustering of groundnut genotypes formed using UPGMA tree based on SSR data.

Principal Co-ordinate analysis and AMOVA for SSRs

Principal Co-ordinate analysis (PCA), based on origin of accessions formed three major population groups. Group I included accessions from India, Sudan, Nigeria, USA, Senegal and Gambia while group II contained accessions mainly from India, Taiwan, Sudan, Australia and Senegal. Group III consisted of accessions from India, Nigeria, Sudan, Senegal,

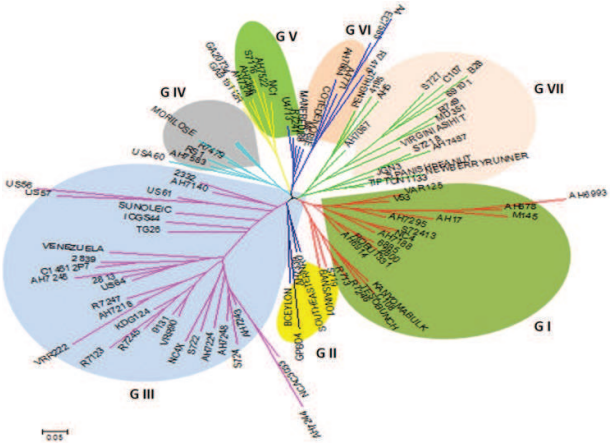

Fig. 1. UPGMA tree based on similarity index of 26 SSR markers for 96 groundnut genotypes

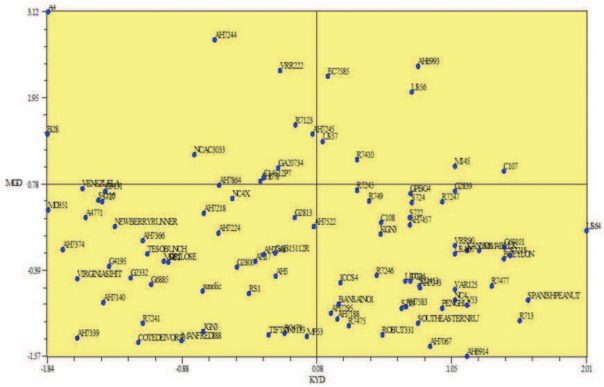

Fig. 2. Correlation between genetic similarity index and taxonomic distance for average yield

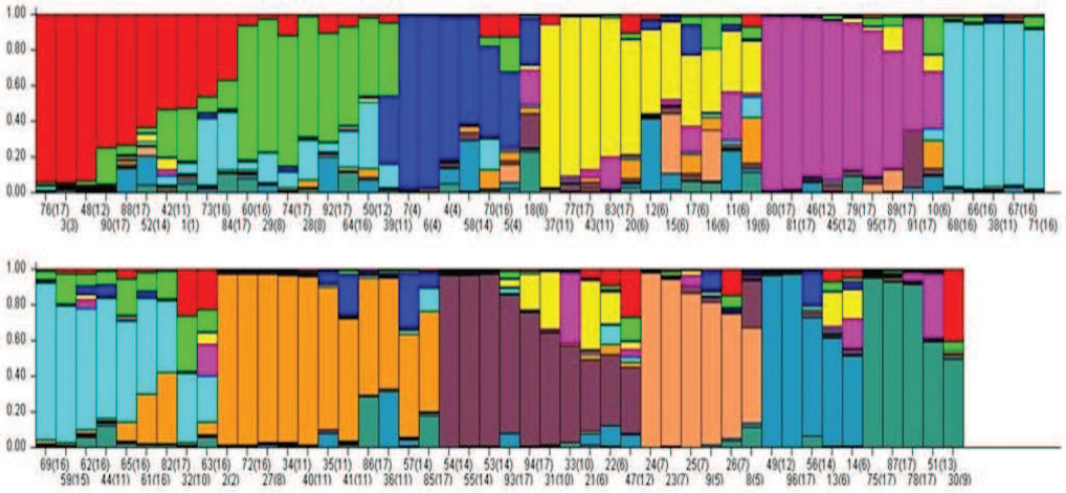

Fig. 3. Model based population structure plot with K = 10, using 26 polymorphic SSR markers

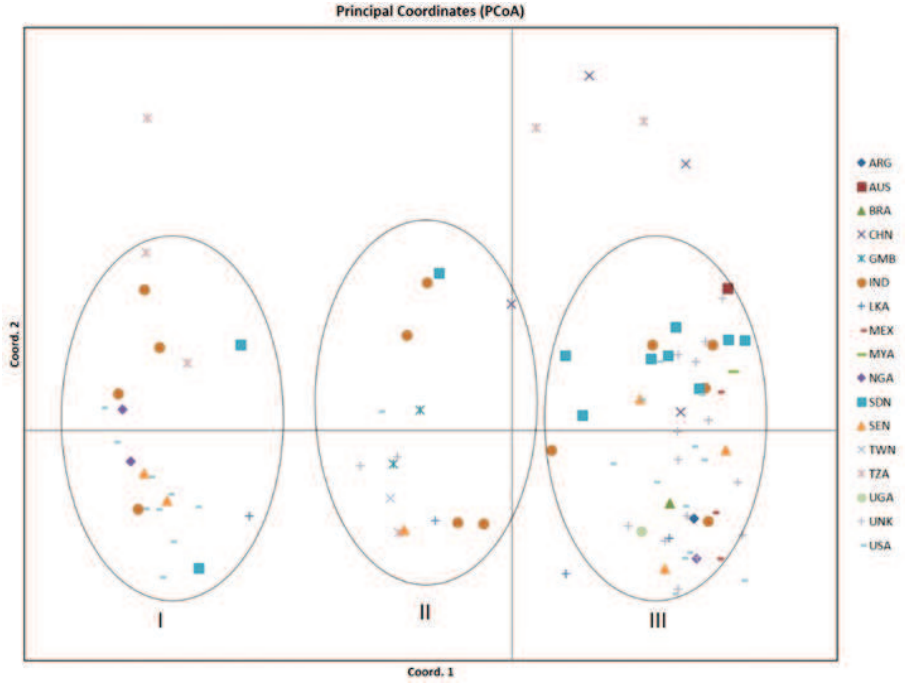

Fig. 4. Principal Co-ordinate Analysis (PCoA) of all genotypes from 17 countries based on SSR data

Gambia, Argentina, Mexico and Myanmar. The accessions from China were distinct and therefore not included in these 3 groups. However, released cultivars from India were commonly found in all the three groups. It means all the varieties of Indian origin are having highly diversified characteristics (Fig. 4). Analysis of molecular variance (AMOVA) represented only 8% variation among various populations, 30% variation within individuals and 62% variation among individual based on allelic variation indicated by SSR markers.

Discussion

The conventional breeding procedures employ hybridization, phenotype based selection followed by selection of promising breeding lines through yield evaluation traits. With the advent of advance genomic tools, marker assisted breeding (MAB) was deployed to enhance efficiency of selection of target traits in groundnut (Janila et al. 2013; Kanyika et al. 2015). Recent advances in the area of crop genomics including marker assisted selection (MAS), array based genotyping, genotyping by sequencing (GBS) and next generation sequencing (NGS) have offered molecular techniques to assist breeding for the development of improved varieties of groundnut.

In present study, 90 germplasm lines and six check varieties were evaluated by means of morphological and molecular characterization using highly polymorphic SSR markers. As expected, the fresh weight of plants was significantly higher to dry weight and harvest index indicating that these traits are directly proportional to each other. Shelling per cent was directly related to other yield parameters such as weight of pods, weight of kernels and harvest index. The days to 50% flowering is documented as highly variable depending upon the growth conditions and various environmental factors. Amara et al. (2016) reported variation in days to flowering ranging from 42 to 67 days, while 64 to 76 days were reported in the Free State Province in South Africa by Massawe et al. (2005) and 43-80 days in Loughborough region by Masindeni (2006). In the present study, days to 50% flowering ranged from 38 to 61 supporting the earlier findings. Morphological and physiological traits have been influenced due to domestication and later on by the changes in agricultural practices not only in groundnut but in other species too (Muñoz et al., 2017; Blanc and Wolfe, 2004; Chen 2010).

The study demonstrated wide variation for yield and yield attributing traits, which may be useful for selection of elite germplasm lines/genotypes as desirable parents in groundnut improvement. The number of pods per plant is also an important trait for improving seed yield in groundnut (Luz et al. 2011). In the present investigation, number of pods per plant indicated positive correlation with seed yield and hundred kenel weight (Table 2). Similar relationship was reported in Chinese groundnut mini-core collection (Jiang et al. 2014) and Asian groundnut core collection (Swamy et al. 2003) showing number of pods per plant is one of the important selection criteria to obtain higher seed yield of groundnut. The genotypes with higher number of pods per plant could be used as a useful genetic resource for obtaining higher yield potential. Shelling percentage which shows an index of percentage of grains or seeds (Dapaah et al. 2014) is one of the important selection criteria in groundnut breeding (Anothai et al. 2008). However, Minimol (2001) reported shelling percentage is highly influenced by genotypic×environment interactions. In the present study, this trait is positively and significantly contributed with weight of pods, kernel yield, weight of kernels, sound mature kernel and harvest index. The two traits *viz.*, seed size and seed weight greatly differ due to production environment and cultivation practices (Upadhyaya et al. 2014). Hundred-seed weight showed significant and positive correlations with important yield attributing traits in the present study (Table 2). Similarly, the results indicated positive correlation with number of pods per plant (Nath and Alam 2002; Dapaah et al. 2014), and flowering time (Upadhyaya 2003) in different studies. Such common relationships obtained from above studies suggest that consideration of reduced number of traits in characterization using easily measurable traits in groundnut may be more rewarding. To obtain higher seed yield under different environmental conditions is one of the most important challenges in plant breeding (Yol et al. 2018). In the present study, seed yield showed wide variation among germplasm lines and varietal set obviously due to diverse origin and ecological growing conditions under which it was grown. However, the genotypes with higher number of pods per plant and high yield tend to be late flowering. These genotypes are ideally good to be used as parents in crossing programs to obtain superior recombinant with early maturity and higher seed yield characteristics. The present study identified superior germplasm lines for yield and yield attributing traits providing better opportunities for developing high seed yielding cultivars in future breeding programme.

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 (Kochert et al. 1996; Subramanian et al. 2000; Varshney et al. 2013). However, Chopra et al. (2018) using SNPs developed SSR markers from transcriptomic data, and further utilized them to identify QTLs for plant architectural traits in groundnut. Several reports are avaiable, where SSR markers revealing more information and useful results for genetic analysis, gene tagging and mapping (Zhao et al. 2017). Pandey et al. (2012) reported highly informative genetic and genomic markers (4485) on a set of 20 genotypes with only 1351 (37.3%) markers showed polymorphism. In contrast to the previous studies, the present study highlighted initial polymorphism screening using 125 primers among two contrast groundnut genotypes and was followed by genotyping of 96 accessions using selected polymorphic markers. The PIC value recorded in the range of 0.23 (S026) to 0.81 (PM204). While, 13 highly polymorphic SSR markers showed 0.5 or above PIC values. The genetic diversity observed by SSR markers was similar to Cuc et al. (2008) with very high PIC value of more than 0.5. Markers with high PIC vaues can be further utilized for characterization of another set of groundnut genotypes or germplasm lines. The microsatellite markers help in estimation of phylogenetic inferences and the genetic variation exist between germplasm lines (Moretzsohn et al. 2004). Previously, Varshaney et al. (2009) reported the genetic variation among 189 groundnut (*Arachis hypogaea* L.) accessions comprising landraces, cultivars, a mutant, advanced breeding lines and others representing 29 countries and 10 geographical regions assessed at 25 microsatellite or simple sequence repeat loci.

In the present study 90 germplasm lines and six varieties originated from 17 different countries were gouped into 7 clusters based on 13 morphological traits analysis. The Principal Coordinate anaysis revealed that groundnut germplasms originated from India were highly diverse. In some of the studies clustering pattern do not reflect on the origin of the genotypes as reported in *Vigna* species (Bangar et al. 2018). The present study indicates that there is low genetic variation among various populations but displaying higher genetic variation among individuals. Bangar et al. (2018) also reported the presence of moderate genetivc differentiation among groups compared to higher differentiation among varieties within populations of *Vigna radiata*. The highly polymorphic 26 SSR markers identified in the present study can be used for molecular characterization and genetic diversity analysis for yield attributing traits in other set of groundnut germplasms. However, the study identified diverse clusters of germplasm lines with varieties of known characteristic features 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 germplasms with superior characters could be used in hybridization programme for crop improvement.

Authors' contribution

Conceptualization of research (AP, ST); Designing of the experiments (ST); Contribution of experimental materials (AKS, MKT, ST); Execution of field/lab experiments and data collection (AP, ST); Analysis of data and interpretation (ST, AP, RST); Preparation of manuscript (ST, AP, RST).

Declaration

The authors declare no conflict of interest.

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References

Ajay B. C., Meena H. N., Singh A. L., Bera S. K., Dagla M. C., Kumar N. and Makwana A. D. 2017. Response of different peanut genotypes to reduced phosphorous availability. Indian J. Genet., **77**(1):105-111. DOI: 10.5958/0975-6906.2017.00014.1.

Amara D. G., Ferdjani M., Bokandi M., Khaled K., Mohammed A. M., Ahmed E. and Mohammad M. S. 2017. Comparison of the quantitative and chemical characteristics of the three varieties of Peanut (*Arachis hypogeae* L). Direct Res. J. Agric. Food Sci., **5**: 244-250.

Anonymous. 2017. Directorate of Economics & Statistics, Department of Agriculture, Cooperation & Farmers Welfare.

Anothai J., Patanothai A., Jogloy S., Pannangpetch K., Boote K. J. and Hoogenboom G. 2008. A sequential approach for determining the cultivar coefficients of

peanut lines using end-of-season data of crop performance trials. Field Crops Res., **108**: 169-178.

Bangar P., Chaudhury A., Umdale S., Kumari R., Tiwari B., Kumar S., Gaikwad Ambika B. and Bhat K. V. 2018. Detection and characterization of polymorphic simple sequence repeats markers for the analysis of genetic diversity in Indian mungbean [*Vigna radiata* (L.) Wilczek]. Indian J. Genet., **78**(1): 111-117 DOI: 10.5958/0975-6906.2018.00013.5.

Bertioli D. J., Cannon S. B., Froenicke L., Huang G., Farmer A. D., Cannon E. K. et al. 2016. The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. Nat. Genet., **48**: 438-446.

Blanc G. and Wolfe K. H. 2004. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell, **16**: 1667-1678.

Bravo J. P., Hoshino A. A., Angelici C., Lopes C. R. and Gimenes M. A. 2006. Transferability and use of microsatellite markers for the genetic analysis of the germplasm of some Arachis section species of the genus Arachis. Genet. Mol. Biol., **29**: 516-524.

Chen X., Li H., Pandey M. K., Yang Q., Wang X., Garg V., *et al.* 2016. Draft genome of the peanut A-genome progenitor (*Arachis duranensis*) provides insights into geocarpy, oil biosynthesis, and allergens. Proc. Natl. Acad Sci. USA, **113**: 6785-6790.

Chen Z. J. 2010. Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci., **15**: 57-71.

Chopra R., Charles S. E., Hillhouse A., Payton P., Sharma J. and Burow M. D. 2018. SNP genotyping reveals major QTLs for plant architectural traitsbetween A-genome peanut wild species. Mol Genet and Genomics. https://doi.org/10.1007/s00438-018-1472-z.

Cuc L. M., Mace E., Crouch J., Quang V. D., Long T. D. and Varshney R. K. 2008. Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol., **8**: 55.

Dapaah H. K., Mohammed I. and Awuah R. T. 2014. Growth and yield performance of goundnuts (*Arachis hypogaea* L.) in response to plant density. Int. J. Plant and Soil Sci., **3**: 1069-1082.

Dhillon S. S., Rake A. V., Miksche J. P. 1980. Reassociation kinetics and cytophotometric characterization of peanut (*Arachis hypogaea* L). *DNA Plant Physiol*, 65:1121-1127.

Earl D. A., von Holdt B. M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet. Resour., **4**: 359 https://doiorg/101007/s12686-011-9548-7.

FAO. 2017. FAO statistical database. FAO, Rome, Italy. http://www.fao.org/faostat.

Guo B. Z., Chen C. Y., Chu Y., Holbrook C.C., Ozias-Akins P. and Stalker H. T. 2012. Advances in genetics and genomics for sustainable peanut production In: Benkeblia N, ed Sustainable Agriculture and New Biotechnologies CRC Press, Boca Raton, FL pp 341-367.

Hilu K. W. and Stalker H. T. 1995. Genetic relationships between peanut and wild species of Arachis sect Arachis (Fabaceae): Evidence from RAPDs. Plant Syst. Evol., **198**: 167-178.

Janila P., Nigam S. N. and Varshaney R. K. 2013. Groundnut improvement: Use of genetic and genomic tools. Front Plant Sci., **4**: 23.

Janila P., Murali T., Khare D. and Chaudhari S. 2017. Genetic analysis of foliar disease resistance, yield and nutritional quality traits in groundnut. Elect. J. Plant Breed., **8**(2): 485-493.

Jiang H., Huang L., Ren X., Chen Y., Zhou X., Xia Y., Huang J., Lei Y., Yan L., Wan L., Liao B. 2014. Diversity characterization and association analysis of agronomic traits in a Chinese peanut (*Arachis hypogaea* L.) mini-core collection. J. Integ. Plant Biol., **56**: 159-169.

Kanyika B. T. N., Lungu D., Mweetwa A. M., Kaimoyo E., Njunge V. M., Monyo E. S., Siambi M., He G., Prakash C. S., Zhao Y. and de Villiers S. M. 2015. Identification of Groundnut (*Arachis hypogaea* L.) SSR marker suitable for multiple resistance traits QTL mapping in Africa germplasm. Elect. J. Biotech., **8**: 61-67.

Kochert G., Stalker H. T., Gimenes M., Galgaro L., Lopes C. R. and Moore K. 1996. RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (leguminosae). Am. J. Bot., **83**: 1282-1291.

Leal-Bertioli S. C., Jose A. C., Alves-Freitas D. M., Moretzsohn M. C., Guimaraes P. M., et al. 2009. Identification of candidate genome regions controlling disease resistance in Arachis. BMC Plant Bio., **I9**: 112.

Liu K. and Muse S. 2004. Power Marker: new genetic data analysis software, version 27 Available: http:// wwwpowermarkernet.

Luz L. N., Santos R. C. and Filho P. A. M. 2011. Correlations and path analysis of peanut traits associated with the peg. Crop Breed. and App. Biotech., **11**: 88-93.

Masindeni D. R. 2006. Evaluation of Bambara groundnut (*Vigna subterranea*) for yield stability and yield related characteristics (Master's Thesis). University of the Free State, Bloemfontein, South Africa.

Massawe F. J., Mwale S. S., Azam-Ali S. N. and Roberts J. A. 2005. Breeding in bambara groundnut (*Vigna subterranea* (L.) Verdc.): Strategic considerations.

African Journal of Biotechnology, 4, 463-471.

Minimol J. S., Datke S. B., Deshmukh S. N. and Satpute G. N. 2001. Genotype×environment interaction in groundnut (*Arachis hypogaen* Linn.). Annals of Plant Phys., **14**: 74-79.

Moretzsohn M. C., Hopkins M. S., Mitchell S. E., Kresovich S., Valls J. F. and Ferreira M. E. 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol., **4**: 11.

Muñoz N., Liu A., Kan L., Li M-W. and Lam H. M. 2017. Potential uses of wild germplasms of grain legumes for crop improvement. Int. J. Mol. Sci., **18**(2): 328.

Murray M. G. and Thompson W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., **8**: 4321-4325.

Naidu G. K., Pattanashetti S. K., Boodi I. H., Singh O. K., Prakyath K. V., Biradar B. D. and Wali M. C. 2017. Genetic analysis of recombinant inbred lines for iron deficiency chlorosis and productivity traits in groundnut. Indian J. Genet., **77**(3): 414-421. DOI: 10.5958/0975-6906.2017.00056.6

Nath U. K. and Alam M. S. 2002. Genetic variability, heritability and genetic advance of yield and related traits of groundnut (*Arachis hypogaea* L.). J. Biol. Sci., **2**: 762-764.

Pandey M. K., Bhimana G., Thandoniappan J., Sriswathi M. and Varshney R. K. 2012. Highly informative genic and genomic ssr markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea* L.). Plant Breed., **131**(1).

Pritchard J. K., Stephens M. and Donnelly P. J. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**: 945-959.

Rohlf F. J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 21 Exeter Software, Setauket, NY.

Seijo J. G., Lavia G. I., Fernandez A., Krapovickas A., Ducasse D. A. and Moscone E. A. 2004. Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that Arachis duranensis and A ipaënsis are the wild diploid progenitors of *A. hypogaea* (Leguminosae) Am. J. Bot., **9**(1): 1294-1303.

Subramanian V., Gurtu S., Nageswara Rao R. C. and Nigam S. N. 2000. Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Genome, **43**: 656-660.

Swamy B. P. M., Upadhyaya H. D., Goudar P. V. K., Kullaiswamy B. Y. and Singh S. 2003. Phenotypic variation for agronomic characteristics in a groundnut core collection for Asia. Field Crops Res., **84**: 359-371.

Tamura K., Dudley J., Nei M. and Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.Molecular Biology and Evolution, 24:1596-1599.

Tang R., Gao G., He L., Han Z., Shan S., Zhong R., Zhou C., Jiang J., Li Y., Zhuang W. 2007. Genetic diversity in cultivated groundnut based on SSR markers. J. Genet. Genomics, **34**: 449-459.

Upadhyaya H. D. 2003. Phenotypic diversity in groundnut (*Arachis hypogaea* L.) core collection assessed by morphological and agronomic evaluations. Genetic Resources and Crop Evolution, **50**: 539-550.

Upadhyaya H. D., Dwivedi S. L., Vadez V., Hamidou F., Singh S., Varshney R. K. and Liao B. 2014. Multiple resistance and nutritionally dense germplasm identified from mini core collection in groundnut. Crop Sci., **54**: 679-693.

Varshney R. K., Mohan S. M., Gaur P. M., Gangarao N. V., Pandey M. K., et al. 2013. Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol. Adv., **31**: 1120-1134.

Varshney R. K., Mahendar T., Aruna R., Nigam S. N., Neelima K., Vadez V. and Hoisington D. A. 2009. High Level of Natural Variation in a Groundnut (*Arachis hypogaea* L.) Germplasm Collection Assayed by Selected Informative SSR Markers, Plant Breed., **128**(5): 486-494.

Yol E., Furat S., Upadhyaya H. D. and Uzun B. 2018. Characterization of groundnut (*Arachis hypogaea* L.) Collection using quantitative and qualitative traits in the Mediterranean Basin. J. Integ. Agric., **17**(1): 63-75.

Zhou Z., Jiang Y., Wang Z., Gou Z., Lyu J., Li W., et al. 2015. Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean. Nat. Biotechnol., **33**: 408-414.

Zhao C., Qiu J., Agarwal G., Wang J., Ren X., Xia H., Guo B., Ma C., Wan S., Bertioli D. J., Varshney R. K., Pandey M. K. and Wang X. 2017. Genome-wide discovery of microsatellite markers from diploid progenitor species, *Arachis duranensis* and *A ipaensis* and their application in cultivated peanut. Frontiers in Plant Sci, DOI 103389/fpls201701209.