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Study on genetic diversity of terminal drought tolerant groundnut (*Arachis hypogaea* L.) genotypes based on morphological and molecular data

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Abstract
Genetics divergence using D^2 analysis of 49 terminal drought yolerant genotypes of groundnut (*Arachis hypogaea* L.) revealed existence of considerable diversity for fourteen different characters and genotypes were grouped into seven clusters. The cluster I was the largest containing 23 genotypes followed by cluster II and cluster IV consisted 11 and 9genotypes respectively. The cluster V, VI and VII were solitary in nature. Among the 27 groundnut specific SSR markers, only seven primer pairs showed polymorphic among test genotypes. The polymorphic markers amplified a total of 20 alleles with an average of 2.86 alleles per loci and polymorphism information content ranged from 0.47 to 0.66. The dendrogram was obtained from the binary data deduced from the DNA profiles of thesamples analyzed, and at 82 per cent similarity coefficient six major clusters were formed. Cluster III consists of maximum number of genotypes followed by Cluster I and Cluster II. The genotype included in the diverse clusters can be used as promising parents for hybridization programme for obtaining high heterotic response and developing drought tolerant genotypes.

Keywords: Groundnut, Terminal drought, diversity, morphological, polymorphism, Simple Sequence Repeats (SSRs)

Introduction
Groundnut (*Arachis hypogaea* L.) also known as peanut is a self pollinated crop an allotetrapoloid (2n=4x=40), which originated in the region of eastern foothills of Andes (Southern Bolivia and Northern Argentina). It is an unpredictable crop due to its underground pods development. Groundnut yield is not only polygenically controlled, but also influenced by its component characters. Groundnut seeds are valued both for its oil and protein contents (Desai *et al*., 1999) [4]. Groundnut crop is predominantly grown as a rain fed crop in India (>80 %) where drought is a major constraint of crop production.

Genetic diversity is one of basic requirements of development in plant breeding. The high genetic diversity in germplasm of groundnut has not been used sufficiently in improvement programs of groundnut due to lack of sufficient information's about morphological and molecular characteristics of groundnut (Badigannavar *et al.*, 2002) [2]. So in order to exploit available diversity in genotypes of groundnut, it is necessary to evaluate its morphological and molecular characteristics.

Germplasm characterization both at the phenotypic and molecular level is important in all plant breeding programs. Morphological characters are sometimes influenced by the environment and are labour intensive. In recent times, molecular markers have been employed as an alternative to the use of morphological traits. Even then, using a combination of the two (that is, morphological characters and DNA markers) has been found to offer a more comprehensive characterization of plant genotypes. Among all DNA markers SSR markers appear to have wider application because of their presence in genomes of all living organisms, high level of allelic variation, co-dominant way of inheritance and potential for automated analysis (Li *et al*., 2001) [6].

The genetic variability has to be looked for planning suitable measures for the crop improvement. Microsatellite-based markers represent a useful tool for dissecting genetic variations in groundnut. These markers showed a good level of PIC value in cultivated germplasm and therefore would be very useful for germplasm analysis, linkage mapping, diversity studies and phylogenetic relationships in cultivated groundnut. The objective of this study was to determine the genetic diversity of terminal drought tolerant groundnut genotypes by using both morphological and SSR markers approaches.

Material and Methods

The experimental material consist of forty nine terminal drought tolerant groundnut genotypes includes ICGV series from ICRISAT, released varieties, pipeline varieties, advance breeding lines and germplasm lines collected from different institutes across India (Table 1) were sown during *kharif* 2016 in Simple Lattice Design. In each replication every genotype was sown in two rows of 5 m length with a spacing of 30 cm x10 cm. Standard agronomic practices as per recommendations in package of practices were followed. Data on yield and its important attributing traits were recorded and subjected to statistical analysis.

The data collected on 14 different characters were analyzed through Mahalanobis's D^2 statistic (Mahalanobis, 1936) [7] for assessing the genetic divergence between genotypes. Grouping of the genotypes into different clusters was done by using Tocher's method as described by Rao (1952) [11].

The 49 genotypes were screened with 27 groundnut specific SSR markers used for

Molecular diversity study. Twenty day old healthy leaf samples were used for DNA extraction following 4% CTAB method (Sambrook *et al*. 2001) [14]. The quantification of the DNA was carried out by using electrophoreses in 0.8% (w/v) agarose with known concentration of lambda DNA.

Table 1: List of terminal drought tolerant groundnut (*Arachis hypogaea* L.) genotypes used for investigation

Source	Genotypes	No.
ICRISAT, Patancheru, Hyderbad, Telangana. India	Spanish types: series of ICGVs-97058,02242,01274,01464,03043,95440,13245,05198,13241,07235,06188,05057,07390,97092,3343,98184,4729,97182,07213,3102,99161,99206,96155,91114,07408,02317,07120,07273,05193,00351.	31
	Virginia type: CS-39.	
BARC, Trombay, Mumbai, India	Spanish types: TAG-24, TG-37A, TG-47, TG-72, TG-80,and TG36.	7
	Virginia type: Somanatha.	
PAU, Ludhian, Punjab, India	Spanish types: 49-M-16.	1
ARS, Kadiri India	Spanish types: Kadiri-6.	1
UAS Dharawad, Karnataka, India	Spanish types: GPBD-5, Mutant-3 and Dh-216.	5
	Virginia types: DSG-41 and TDG-39.	
UAS Raichur, Karnataka, India	Spanish types: R-2001-2, Kadiri-9, R-8808 and TMV-2.	4
Total		49

The PCR profile consisting of 34 cycles, and amplication condition for SSR consisted of initial denaturation 94 °C for 1min, denaturation at 94 °C for 30 seconds, primer annealing vary with each primer, final extention at 72 °C for 10 min, hold at 4 °C for 10 min.

Amplification products were resolved on 3% agarose gel containing Ethidium bromide (0.5 µg/ml) using 1X TBE buffer (Sambrook *et al*. 2001). 8.5 µl of PCR products were mixed with 2 µl of loading buffer and 10.5 µl was loaded in the wells in agarose gel. A 100bp DNA ladder was spotted on each gel as a fragment length standard. The gels were visualized under UV light and documented by using gel doc unit in the computer. Fragment length was determined visually by comparison with the ladder.

Each fragment size was treated as a unique characteristic and scored as present (1) or absent (0). Genetic similarity index was used to construct a dendrogram which illustrated the genetic relationship among the forty nine genotypes of groundnut used in the study. A dendrogram was constructed using similarity index adopting Sequential Hierarchial and Nested (SAHN) using the NTSYS-pc program.

Results and Discussions

Forty nine genotypes of groundnut possessing 14 different characters have been characterized both at morphological and molecular level.

Cluster analysis – Using Phenotypic data

The 49 genotypes were grouped into seven clusters (Table 2 and Fig. 1) based on the Mahalanobis's D^2 statistics. Clustering pattern showed appreciable amount of divergence among the genotypes. Among the clusters, cluster I contained maximum number of genotypes (23 genotypes) might be due to free flow or exchange of breeding material from one place to another and the unidirectional selection practiced by breeders of different locations. Cluster II and IV contained 11 and 9 genotypes respectively, were as cluster III had three genotypes. Clusters V, VI and VII contained one genotype each. Three genotypes were not included with any other cluster as they maintained separate identity from all others and each exhibiting high genetic diversity with most of the other clusters. Yadav *et al*. (2014) [16] observed 12 clusters in 60 groundnut genotypes and Amarasinghe *et al*. (2016) [1] screened 19 groundnut genotypes for divergence studies and genotypes grouped into 3 distinct clusters.Mahalanobis's D^2 statistic is a powerful tool for quantifying the degree of divergence and technique gives an insight into the most genetically divergent parents that could be used for heterotic hybridization (Makinde and Ariyo, 2013) [8].

SSR allelic diversity

A total of 27 groundnut specific SSR markers were used to screen the
49 groundnut genotypes for studying the diversity among the genotypes. Among 27 markers used, 7 markers were polymorphic and 20 markers showed monomorphic bands. Seven were used for final analysis in scoreable amplified bands. DNA amplification profile for marker GM 2301 is presented in Fig. 2.

Table 2: Clustering pattern of 49 groundnut genotypes, based on the 14 quantitative traits

Clusters	No. of genotypes	Genotypes
I	23	Kadiri-9, ICGV-07213, ICGV-3102, ICGV-99161, ICGV-07235, ICGV-05057, TG-80, ICGV-97092, ICGV-98184, ICGV-02317, SOMANATHA, TG-47, ICGV-96172, GPBD-5, ICGV-96155, ICGV 3343, R-2001-2, ICGV-04729, DH-216, ICGV-05193, MUTANT-3, TAG-24, ICGV-13241.
II	11	R-8808, TMV-2, ICGV-00351, TG-37A, Kadiri-6, ICGV-99206, ICGV-91114, ICGV-13245, ICGV-97182, ICGV-95440, ICGV-07390.
III	3	TG-72, TDG-39, TG-36.
IV	9	49-M-16, CS-39, DSG-41, ICGV-07120, ICGV-02242, ICGV-03043, ICGV-01274, ICGV-01464, ICGV-07408.
V	1	ICGV-07273.
VI	1	ICGV-05198.
VII	1	ICGV-06188.

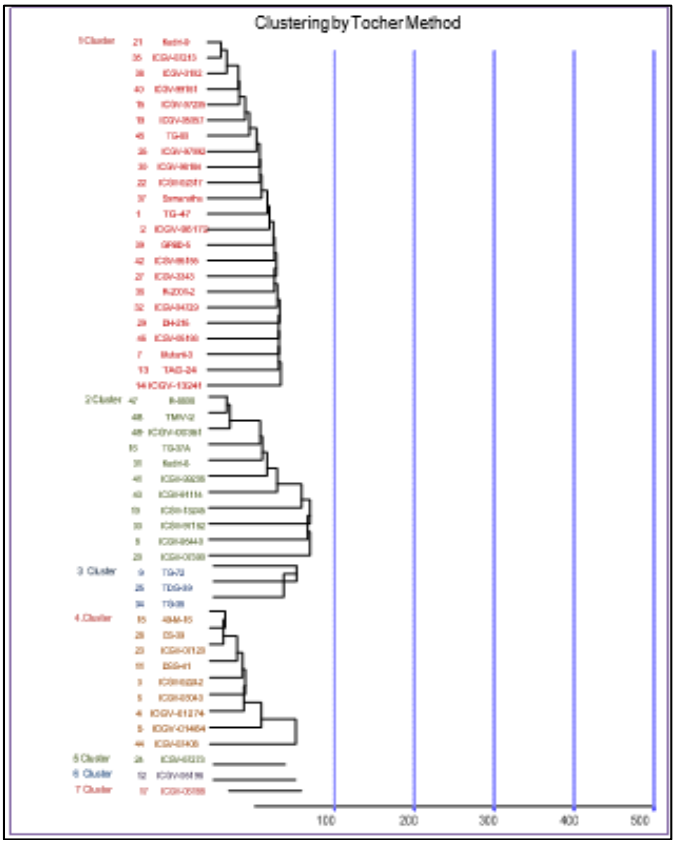
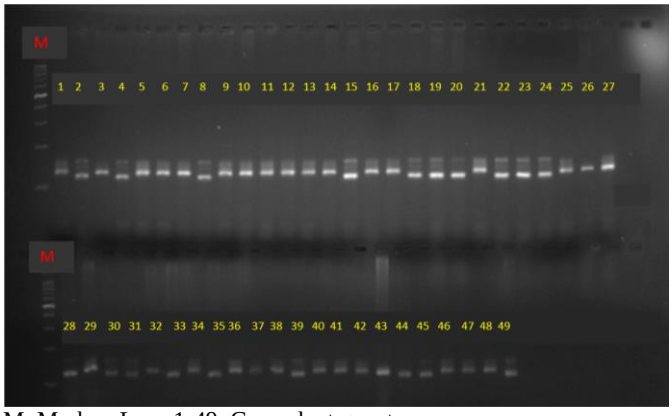


Fig 1: Dendrogram of 49 groundnut genotypes based on phenotypic data



M: Marker, Lane 1-49: Groundnut genotypes

Fig 2: Amplification profile of 49 groundnut genotypes with GM2301 marker on Agarose gel

These polymorphic markers amplified a total of 20 alleles with an average of 2.86 alleles per markers in groundnut genotypes. Maximum 3 alleles were amplified by 6 markers (PM377, PM436, 3RG-173Fb, GM1536, PM325, PM343) fallowed by primer GM2301 amplified 2 allele. Remaining all primers were amplified with single allele per locus. Tang *et al.* (2007) [15] observed 108 polymorphic allele with an average of 8.3 marker in 24 groundnut genotypes. Ren *et al.* (2014) [12] screened 196 groundnut cultivars using 146 SSR markers that amplified 440 polymorphic bands with an average of 2.99 alleles per locus and Roomi *et al.* (2014) [13] recorded 2.6 loci per primer in 70 groundnut genotypes.

The PIC value ranged from 0.47 to 0.66 with an average of 0.57 per marker (Table 3). PM436 marker recorded highest PIC (0.66) and MI value (1.98) among 27 markers used in diversity analysis. Lower PIC values results from the closely related genotypes and the vice-versa.

Frimpong *et al.* (2015) [5] reported PIC value of 0.57 in 48 groundnut genotypes with 53 SSR markers, Molosiwa *et al.* (2015) [9] screened 1230 groundnut collections with 68 polymorphic SSR markers and PIC value ranging from 0.08 to 0.89 with an average of 0.42 and Bhad *et al.* (2016) [3] obtained PIC values ranging from 0.04 to 0.95 in 44 groundnut genotypes. Based on the PIC values and number of alleles amplified the primer PM436, 3RG-173Fb, PM325, PM377 were found to be more informative in the present set of genotypes. Hence, the four primers used for genetic diversity studies in groundnut breeding program.

Table 3: Polymorphic information content of SSR primers used in the analysis of the 49 groundnut genotypes.

S. No.	SSR marker	No. Allele produced	Allele frequency	PIC. Value	MI value
1	PM377	3	0.14, 0.28, 0.57	0.57	1.71
2	PM436	3	0.30, 0.32, 0.36	0.66	1.98
3	3RG-173Fb	3	0.26, 0.30, 0.42	0.65	1.95
4	GM2301	2	0.38, 0.61	0.47	0.94
5	GM1536	3	0.10, 0.26, 0.63	0.52	1.56
6	PM325	3	0.10, 0.42, 0.46	0.59	1.77
7	PM343	3	0.10, 0.32, 0.57	0.56	1.68
Total		**20**			
Mean		**2.86**		**0.57**	**1.66**

Where, PIC = polymorphic information content, MI = Marker index

Cluster analysis

The genetic dissimilarity estimates for 49 genotypes were employed to generate dendrogram by using tree construction with NTSYS software (Table 4 and Fig. 3). At 0.82 coefficients of similarity all the genotypes were grouped into six clusters and these clusters were further divided into sub clusters.

Cluster III consists of maximum number of 18 genotypes followed by Cluster I and Cluster II consists of nine genotypes in each. The cluster III showed maximum similarity with each other when compared with the rest of genotypes. Cluster IV and Cluster V consists of five and six

genotypes respectively, only two genotypes are found in cluster VI. A similar study by Tang *et al*. (2007) [15], Mondal and Badigannavar (2010) [10] and Ren *et al*. (2014) [12] observed two major clusters in groundnut genotypes. Roomi *et al.* (2014) [13] recorded six clusters at 0.67 coefficients of similarity in 70 groundnut genotypes.

Table 4: Clustering pattern of 49 groundnut genotypes based on SSR markers analysis (0.82 coefficient of similarity)

Clusters	No. of genotypes	Genotypes
I	9	TG-47, ICGV-04729, TG-36, ICGV-02242, Kadiri-6 ICGV-97182, Somanatha, ICGV-3102, R-2001-2.
II	9	ICGV-96172, 49-M-16, ICGV-00351, ICGV -13241, GPBD-5, ICGV-07235, ICGV-07408, TG-72, ICGV-07213.
III	18	ICGV-03043, Mutant-3, ICGV-13245, DSG-41, ICGV-05198, TAG-24, TG-37A, ICGV-06188, ICGV-3343, CS-39, DH-216, ICGV-98184, ICGV-99161, ICGV-99206, ICGV-96155, ICGV-91114, ICGV-05193, R-8808.
IV	5	ICGV-01464, TMV-2, ICGV-07273, TDG-39, ICGV-97092.
V	6	ICGV-01274, TG-80, ICGV-95440, ICGV-05057, ICGV-07390, ICGV-07120.
VI	2	Kadiri-9, ICGV-02317.

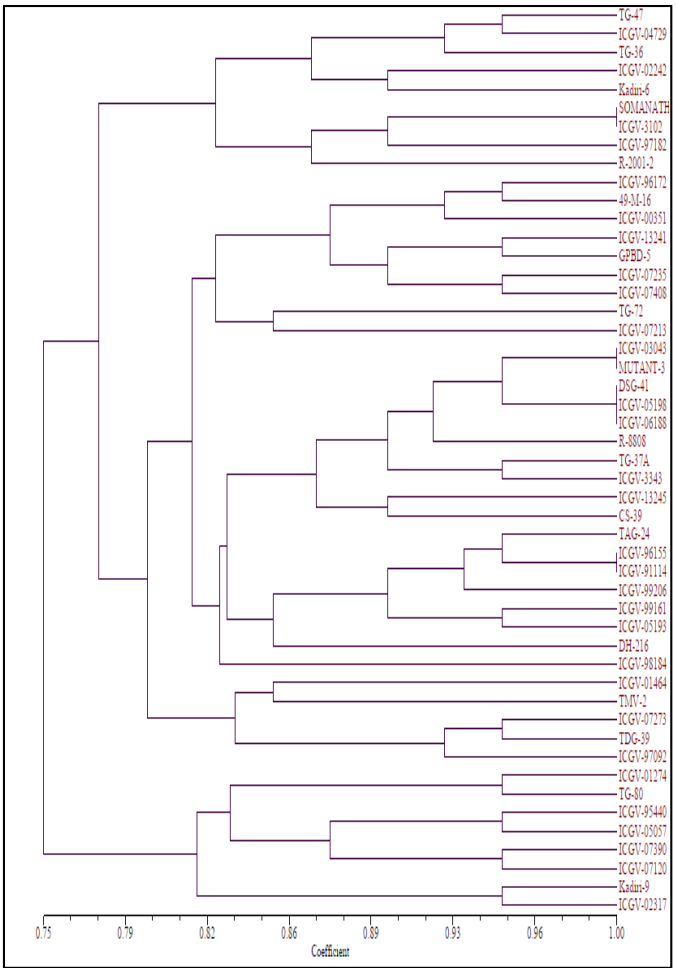


Fig 3: Dendrogram of 49 genotypes based on SSR marker analysis

Conclusion

From this study concluded that molecular level genetic diversity is superior as compared to morphological and biochemical polymorphism. Morphological characters are sometimes influenced by the environment and are labour intensive. The assessment of molecular level diversity is more important for preservation of genetic assets, recognition of best germplasm resources and the collection of superior cultivars for hybridization purpose.

PM436, 3RG-173Fb, PM325, PM377 primers used for genetic diversity studies in groundnut breeding program. Genotypes grouped into different clusters used as parents for heterotic hybridization.

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