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STUDY ON GENETIC DIVERSITY IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) USING MORPHOLOGICAL MARKERS

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ABSTRACT
The 29 breeding lines were evaluated in Randomized Block Design for yield and its component traits grown in *rabi* seasons showed wide variation in all the 12 traits including yield. On the basis of critical D2 values (83.614), 29 genotypes were classified into 8 clusters. Inter cluster distance is the main criterion for selection of genotypes. Maximum inter-cluster distance was observed between cluster VI and cluster VIII (488.13) followed by cluster VI and cluster VII (465.50). In this contest, the genotypes from cluster VI and VIII or cluster VI and VII could be selected as parents for hybridization. The canonical analysis revealed that the important characters responsible for genetic divergence were shelling percentage and kernel yield per plant in the first axis and harvest index and kernel yield per plant in the second axis. Such results indicated that these characters contributed maximum towards total divergence of the genotypes. It was also suggested that attention should be given for these characters for yield improvement of groundnut. Thus this study concluded that the advance breeding line ALG 234 from cluster I and GPBD 5 from cluster IV, exhibiting highest genetic diversity of 640.258 may be selected as parents in the future hybridization programme to give maximum high yielding segregates.

INTRODUCTION

Among the important sources of edible oil and vegetable protein in the world, groundnut occupies fourth and third position respectively. The oil content varies from 45-48 % in the kernel with 44-54% fat and 25-30 % digestible protein. In oil, the content of poly unsaturated fatty acids (PUFA) (40-50%) and mono unsaturated fatty acids (MUFA) like linoleic acid (25-35%) is in right proportion. So the oil is stable and nutritive. The long shelf-life of oil is due to high oleic / linoleic ratio. Thus the groundnut is currently called as "King of Oilseed" specially in India (Mulgir *et al*., 2014). It is rich in 30 essential nutrients, fiber, vitamins (niacin, folate, and vitamin E) and minerals (magnesium, manganese and phosphorus) and free from sodium. Presence of the antioxidant (tocopherol content approximately 0.9 mg/g oil) prevents development of rancidity. It helps to maintain blood cholesterol levels in heart. The foliage and meal are utilized for animal fodder and feed respectively.

To increase productivity, breeders confront with the limitation of relatively low genetic variability in the groundnut germplasm commonly used in the breeding programmes. However, in the groundnut improvement programme, out of total available genetic variability in the germplsm accession very little have been utilized. The most groundnut cultivars have a very narrow genetic base due to lack of sufficient informations about morphological and agricultural characteristics of peanut (Badigannavar *et al*., 2002). Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization (Saundarya *et al*., 2015). So, it is mandatory to evaluate its morphological and agricultural characteristics for exploiting its available diversity in germplasm. The remodeling in plant architecture with erect growth habit, reduced height, more number of pods etc. are the main milestones in the Spanish groundnut improvement. For higher yield in groundnut, Mc Cloud *et al*.(1980) concluded that the physiological criteria responsible for higher yields were short podding phenophase, rapid expansion phenophase, long filling phenophase and a high partitioning of assimilates to pods. Stalker (1990) was presumably the first to characterize 73 wild species accessions from section *Arachis* for 56 morpho-reproductive traits, while Carvalho and Quesenberry (2009) observed morphological diversity among 34 *A. pintoi* accessions. In contrast, wild *Arachis* species have been widely studied for molecular polymorphism, expressing abundant molecular diversity among wild *Arachis* species (Koppolu *et al*., 2010).

In order to obtain desirable genotypes, genetic diversity is the pre-requisite for hybridization programme. Genetic diversity is very much indispensable to meet the different goals in plant breeding such as for producing cultivars with increased yield (Joshi and Dhawan, 1966), desirable quality and pest resistance (Nevo *et al.,* 1982), wider adaptation. Using of more diverse parents is very significant to obtain the high heterotic F_1 and broader spectrum of variability in succeeding segregating generations (Arunachalm, 1981). Tomooka (1991) stated that the evaluation of diversity is important to know the source of genes for particular trait within the available germplasm. So, it is necessary to know the genetic diversity of the existing genotypes before undertaking any crop

improvement programme. Hence, the present investigation was made to study the genetic divergence in 29 germplasm lines of groundnut *(Arachis hypogaea* L.) to identify potential lines for various yield traits which could be utilized in the hybridization programme as parents to improve yield.

MATERIALS AND METHODS

Materials for the present study included 27 advance breeding lines and 2 high yielding standard varieties of groundnut. The field trial on 29 entries was conducted in randomized block design (RBD) with three replications during 2013. Each entry was characterize by 1 row of 3 m length with a spacing of 30 cm x l0 cm. A fertilizer dose of 20:40:40 kg NPK/ha was applied. At the time of top dressing, hoeing and hand weeding were done and need based plant protection measures were followed. In this study, Twelve characters related to growth, vigour and yield were recorded. Days to 50% flowering was taken on plot basis. For other characters like plant height (cm), branches per plant, number of pods/plant , number of kernels/ plant, 100 kernel weight, sound mature kernel percent, shelling percent, harvest index, kernel yield/plant, haulm yield per plant and pod yield/plant observations were recorded on five consecutive plants per genotype in each replication and average data were used in multi-variable statistical analysis. Genetic divergence analysis with regard to twelve quantitative traits among 29 groundnut genotypes was done by following two methods.

(i)D^2 analysis of genetic divergence

(ii)Canonical analysis

Mahalanobis' D^2 statistic (Rao, l952) was used for assessment of genetic divergence among the 29 genotypes of groundnut for twelve characters. On the basis of D^2 values, the studied genotypes were grouped into clusters according to the Tocher's method (Rao, 1952). The methods of Singh and Chaudhary (1985) were used for estimating the intra and inter cluster distances. Canonical analysis was done according to Anderson (1958). The divergences of 29 groundnut genotypes were represented in two dimensional graph using first two canonical vectors (Z_1 and Z_2) as coordinates. Statistical analyses were carried out by software SPAR 2.0.

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among the genotypes for all the characters under study thereby indicating the presence of ample variability. On the basis of magnitude of D^2 values, all the 29 genotypes of groundnut for 12 characters, showed that the generalized distance (D^2) between two populations varied from 9.044 (ALG 234 and AG 2006-15) to 640.258 (ALG 234 and GPBD 5) which were indicators of considerable diversity available in the material evaluated. The smallest D^2 estimate (9.044) was observed between ALG 234 and AG 2006-15, so these genotypes were much similar in many traits. The largest D^2 estimate (640.258) was obtained between ALG 234 and GPBD 5, which indicated the maximum diversity. In the present study, the advance breeding lines showed considerable amount of diversity for the morphological traits. Study on genetic diversity in cultivated groundnut accessions based on morphological trial in one or more seasons was done by Upadhyaya (2003), Mahalaxmi *et al.*(2005), Upadhyaya *et al.* (2006), Laxmidevamma *et al.* (2006), Aalami *et al.* (2007), Khote *et al.* (2010), Dolma *et al.* (2010), Venkataravana *et al*.(2010), Sudhir Kumar *et al.* (2010), Upadhyaya *et al.* (2011) and Sonone *et al.* (2011). They observed wide range of D^2 values suggesting the presence of considerable amount of genetic diversity in the genotypes studied. Genetic relationships among cultivated and wild accessions of groundnut were studied based on microsatellite markers which showed that cultivated groundnut presents a relatively reduced variation at the DNA level (Moretzsohn, 2004; Tang *et al.,* 2007; Wang *et al*.,2015). Similar little variation has also been noticed at the DNA level using techniques such as RFLPs, RAPDs, and AFLPs (Subramanian *et al.*, 2000; Suneetha *et al*., 2015).

Relative contributions of 12 characters to D^2 among the genotypes were estimated by average D^2 (table 1). On the basis of average D^2, shelling percentage contributed maximum divergence followed by harvest index, kernel yield per plant, hundred kernel weight and all other characters. Sound mature kernel percent, plant height, pod yield per plant, days to 50% flowering, number of branches per plant, haulm yield per plant, number of pods per plant and number of kernels per plant contributed least to D^2 estimates. Days to first flowering followed by shelling percentage contributed maximum to the

Table 1: Relative contribution of each character to total genetic divergence in groundnut

Character	Avearage D^2 value	% Contribution
Days to 50% flowering	2.959	1.711
Plant height (cm)	2.268	1.312
No of branches/plant	2.968	1.716
No of pods/plant	4.190	2.423
Sound mature kernel (%)	1.558	0.901
100 kernel weight (g)	18.503	10.700
No of kernels/plant	5.141	2.973
Kernel yield/plant (g)	22.065	12.759
Shelling %	73.455	42.476
Haulm yield/plant (g)	3.452	1.996
Harvest index (%)	33.787	19.538
Pod yield/plant (g)	2.589	1.497

N.B.: Data in parentheses indicate the order of contribution to total genetic divergence

Table 2: Composition of genetic clusters by D^2 values

Clusters	No. of genotypes	Name of genotypes
1.	6	ALG-234, AG-2006-15, KGN-34, Dh-204, UG-5, Dh-107.
2.	5	K-1371, Dh-216-2, DRT-53, Dh-209, GG-6.
3.	10	R-2001-1, ICGV-95401, CSMG-2014, ICGV-9921 , Dh-8, Dh-108, TCGS-159, JL-575, AG-2240, Dh-206.
4.	2	R-8892, GPBD-5.
5.	2	K-1336, Dh-216-1.
6.	2	OG-86-5-2, JNDB-14.
7.	1	UG-3.
8.	1	DhS-102.

Table 3: Average inter and intra cluster D2 values among total clusters of groundnut genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII
I	54.34	237.35	143.01	441.08	89.13	271.64	367.68	168.34
II		64.40	197.07	159.57	104.04	332.16	94.98	136.40
III			63.22	192.99	105.23	89.53	308.86	280.11
IV				24.93	238.00	201.55	196.07	391.38
V					73.01	227.57	211.11	95.66
VI						79.63	465.50	488.13
VII							0.00	213.52
VIII								0.00

Table 4: means of Clusters of different characters of groundnut

Sl. No.	Character	Z1	Z2
1	Days to 50% flowering	-0.0209	0.0592
2	Plant height (cm)	0.0031	0.0571
3	No of branches/plant	0.0089	-0.0135
4	No of pods/plant	-0.0850	-0.0897
5	Sound mature kernel (%)	0.0428	-0.0349
6	100 kernel weight (g)	0.1946	0.04733
7	No of kernels/plant	0.0291	-0.0012
8	Kernel yield/plant (g)	0.2415	0.4709
9	Shelling %	0.9433	-0.2650
10	Haulm yield/plant (g)	0.0029	-0.0620
11	Harvest index (%)	0.0559	0.6801
12	Pod yield/plant (g)	-0.0186	-0.0362
	Variances accounted for (%)	44.5%	39.1%

Table 5: The coefficient of first two canonical vectors (Z1 and Z2) for 12 characters of 29 groundnut genotypes

Cluster	Days to 50% flowering	Plant height (cm)	No. of branches / plant	No. of pods/ plant	Sound mature kernel(%)	100 kernel weight (g)	No. of kernels /plant	Kernel yield/ plant (g)	Shelling (%)	Haulm yield/ plant(g)	Harvest index (%)	Pod yield/ plant(g)
I	30.45	21.00	4.67	12.20	93.65	42.00	19.66	8.30	80.72	24.94	29.74	10.64
II	30.93	19.22	4.41	12.00	91.91	40.40	18.72	7.56	67.47	23.21	29.65	9.86
III	31.94	22.85	4.69	11.95	93.52	48.49	18.89	9.13	75.63	24.67	33.43	12.35
IV	36.34	22.87	4.64	12.50	90.04	49.26	18.93	9.31	65.00	24.62	33.70	12.49
V	32.83	20.37	4.87	12.20	95.72	41.75	18.34	7.67	74.00	25.02	29.48	10.44
VI	33.50	21.17	4.64	10.90	87.51	44.9	17.47	9.32	74.00	23.54	35.28	12.81
VII	31.67	24.80	4.60	16.07	82.98	38.00	24.27	9.16	71.33	23.60	35.57	13.03
VIII	37.33	21.93	5.13	15.80	92.96	37.36	20.60	7.69	76.00	27.87	26.10	9.84

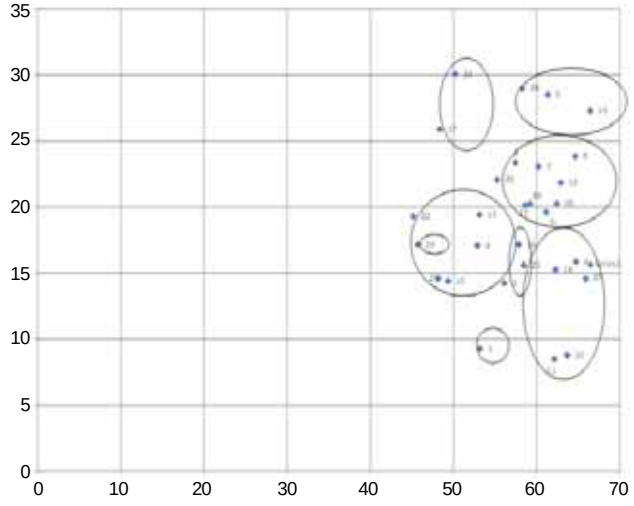

Figure1: Two dimensional representation of divergence of 29 genotypes of groundnut using the first two canonical vectors (Z_1 andZ_2) as coordinates, the grouping byD^2 super imposed.

total divergence as reported by Mahalaxmi *et al.* (2005). Laxmidevamma *et al.* (2006) observed test weight, days to maturity and oil content as the most potential traits contributing towards the total divergence. Sonone *et al.* (2011) observed contribution of various characters towards the expression of genetic divergence on pooled performance in twelve different environments indicated that pod length (25.6 %), plant height (15.1 %), shelling percent (11.7 %), seed weight (8 %) and number of kernels per plant (7.2 %) contributed maximum (67.5 %) towards total divergence in the material.

On the basis of critical D^2 values (83.614), 29 genotypes were classified into 8 clusters (table 2). In the present study, advance breeding lines of major groundnut growing states of India were grouped into eight different clusters. This indicated the huge diversity existing in the advance breeding lines of groundnut giving the opportunity for additional progress in groundnut. Thus recent released varieties contain enough diversity though groundnut itself has narrow genetic base. Cluster III was the largest, accommodating as many as 10 genotypes and cluster VII and VIII was smallest with one genotype each. Cluster IV, V and VI possessing two genotypes

Table 6: Mean canonical values of 29 genotypes of groundnut

Genotype name	Z(1)	Z(2)
1	53.170	9.282
2	57.441	23.371
3	56.099	14.239
4	52.872	17.075
5	61.347	28.510
6	64.705	15.866
7	60.203	23.066
8	64.616	23.845
9	61.133	19.612
10	63.659	8.791
11	62.114	8.510
12	62.877	21.854
13	53.100	19.429
14	62.249	15.268
15	49.342	14.409
16	57.861	17.168
17	48.326	25.897
18	62.385	20.270
19	66.418	27.285
20	65.901	14.566
21	58.426	15.589
22	45.149	19.299
23	50.180	30.082
24	58.599	20.152
25	48.202	14.590
26	55.232	22.067
27	59.208	20.262
28	58.260	28.971
29	45.700	17.174

each. Cluster I and II possessing six and five genotypes, respectively. Two genotypes (UG 3 and DhS 102) remained in isolated clusters. The clustering pattern of genotypes demonstrated that the genotypes of different origins were clubbed into one cluster where as the genotypes belonging to same state or origin were grouped into different clusters indicating that the geographic distribution was not the only measure of genetic diversity. Vijay (2015) also observed the same clustering pattern and stated that the pattern of distribution of genotypes within different clusters was random and independent of geographical origin or region of adaptation.Murthy and Arunachalam (1966) also stated that the genetic drift and selection in different environments could be the source of greater diversity than geographic distance. Further the free exchange of genetic materials among the different regions consequently causes characters assemblage because of the human intervention and materials may loose its distinctiveness. Inter cluster distances values were higher than the Intra cluster distance indicating the genotypes included within the cluster tended to diverse less from each other.

Lowest intra-cluster distance was observed between cluster IV (24.933) (table 3). Highest intra-cluster distance was observed within cluster VI followed by cluster V. Inter cluster distance is the foremost criterion for selection of genotypes. Maximum inter-cluster distance was observed between cluster VI and cluster VIII followed by cluster VI and cluster VII. In this contest, the genotypes from cluster VI and VIII or cluster VI and VII could be selected as parents for hybridization. Cluster VIII exhibited high value of days to 50 % flowering, number of branches per plant and haulm yield per plant (table 4).

Cluster VII exhibited high value of plant height, number of pods per plant, number of kernels per plant, harvest index percentage and pod yield per plant. Cluster IV exhibited high value of hundred kernel weight. Cluster V exhibited high value of sound mature kernel percent. Cluster I exhibited high value of shelling percent. Cluster VI and IV exhibited high value of kernel yield per plant. Thus the advance breeding line ALG 234 from cluster I and GPBD 5 from cluster IV, exhibiting highest genetic diversity of 640.258 may be selected as parents in the future hybridization programme to give maximum high yielding segregates with desirable features.

The two canonical roots accounted for 83.6% of the total variability, thus qualifying for graphic presentation (Table 5). For plotting a two dimensional dispersion complex, the values of the first two canonical vectors Z_1 and Z_2 were used as coordinates. The grouping obtained through D^2 analysis are also imposed on the two dimensional representation of the genotypes by canonical analysis (fig.1). The scattered points on the Z_1 –Z_2 graph were broadly in agreement with the extent of divergence measured by D^2 statistic, thus very well validating the grouping by Tocher' s method.

The coefficients of the first two canonical vectors ($Z_1$and Z_2) presented in Table 5 reflects relative importance of the characters contributing towards divergence. The canonical analysis revealed that values in both vectors (Vector I and II) for plant height, 100 kernel weight, kernel yield per plant and harvest index were positive. It was observed that the important characters responsible for genetic divergence were shelling percentage and kernel yield per plant in the first axis and harvest index and kernel yield per plant in the second axis. Such results indicated that these characters contributed maximum towards total divergence of the genotypes. Similar finding was confirmed by Reddy and Reddy (1993). Hossain *et al.* (2003) also observed the similar results in groundnut where the harvest index contributed maximum towards the total divergence of the genotypes. Zaman *et al.* (2010) also recorded the similar results in groundnut. In his finding, the canonical analysis revealed that values in both vectors (Vector I and II) for days to flowering and days to maturity were positive, values in one vectors for branches per plant, mature nuts per plant and 100 kernel weight were positive. Such results indicated that these characters contributed maximum towards total divergence of the genotypes and it was also suggested that attention should be given for these five characters for yield improvement of groundnut. Venkateswarlu *et al*. (2011) also observed the similar results and stated that the characters 100-kernel weight, shelling percentage and harvest index contributed maximum towards genetic divergence in both D^2 analysis and canonical root analysis.

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