World Journal of Agricultural Sciences 6 (5): 547-554, 2010
ISSN 1817-3047

Estimation of Genetic Diversity of New Advanced Breeding Lines of Groundnut (*Arachis hypogaea* L.)

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Abstract: Sixty four groundnut genotypes collected from different sources and evaluated during late rainy season. The plant data recorded on fourteen characters were subjected to multivariant analysis to study the variability within the genotypes and to determine the efficiency of the methods in classifying genotypes. The first three axes both of factor analysis and principal component analyses (PCA) captured 59.52% of the total variability and jointly identified final pod yield per plant, kernel yield per plant and oil yield per plant as characters contributing most to total variation. The first three axes of the canonical and discriminant analyses accounted for 99 and 95% of the total variation respectively and identified in addition to the above characters oil content and branches per plant as important. Ward's clustering method has grouped the genotypes in to three different distinct clusters. The effect of genetic divergence on the choice of parental stock and its improvement for breeding programme was discussed.

Key words: Groundnut · Factor analysis · Principal component analysis · Canonical analysis · Discriminant analysis · Cluster

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the chief protein rich vegetable oil seed crop of the world belonging to the family Fabaceae, which ranks thirteenth in its importance among the world food crops, fourth as most important source of edible oil and third as most important source of vegetable protein. It is an annual legume, native to South America (Brazil), being grown throughout the tropical, sub-tropical and warm temperate regions of the world [1]. The groundnut seed is valued both for its oil and protein content. The seeds contain about 40-45 per cent oil, 25 per cent protein and 18 per cent carbohydrates in addition to minerals and vitamins. Groundnut oil contains a higher proportion of unsaturated fatty acids, including essential fatty acids like linolenic and linoleic acids [2]. Multivariate statistical methods and numerical taxonomy has been used extensively in summarizing and describing variation and its pattern in a population of crop genotypes [3-9]. In common, the Mahalanobis D^2 statistic has been used to quantify the degree of divergence in different crops [8, 10-13]. The technique gave insight into the most genetically divergent parents that could be used for heterotic hybridization purpose. Geographical diversity was not always related to genetic diversity and therefore not an adequate index of genetic diversity [10, 11]. Genotypes within clusters often showed great geographical diversity.

Successful establishment of germplasm collections and plant introduction for crop improvement as well as for germplasm conservation require studies in genetic variability within plant populations. Genetic variability and heterozygosity within populations existed in both natural and cultivated populations [14]. It was also emphasized that the maintenance of this variability depended on complex interactions among a number of genetic and environmental factors [15]. The progress in breeding for economic characters often depends on the availability of a large germplasm representing a diverse genetic variation. For a long term improvement programme, a large and diverse germplasm collection is an invaluable source of parental strains for hybridization and subsequent development of improved varieties. Accurate cultivar evaluations and ability to differentiate between cultivars in respect of genetic parameters associated with adaptedness in cultivated plants and their wild progenitors are critical to any plant breeding programme [16-18]. The objectives of this study therefore,

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are to evaluate and determine the variation pattern in collection of groundnut germplasm, identify the characters that differentiate the genotypes into different groups, suggest potential genotypes that could be used in improvement programme and appraise the suitability of the various multivariate techniques for classification of variation in groundnut.

MATERIALS AND METHODS

Sixty four genotypes of groundnut species used in this study were comprised (Table 1) 34 new germplasm accessions collected from International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, 24 advanced breeding lines collected from research centers within India and six check varieties. Planting was done during late rainy 2006 (August) in the field unit of All India Coordinated Research Project (AICRP) on Groundnut, Agricultural Research Station (13°24^{1} N, 70°4^{1} E), Chintamani, representing Southern Karnataka, India. The field experiment was laid out in an 8 x 8 simple lattice design with two replications as per Cochran and Cox [19]. Each replicate consisted of eight sub-blocks with eight genotypes in each sub block. Entries and sub blocks were randomized. Each genotype was grown in one row of two meter length. A spacing of 45 cm between row and 15 cm between plants was maintained.

Observations were recorded on ten randomly selected plants in each genotype within the replication for fourteen characters, *viz*., plant height, branches per plant, days to 50% flowering, days to maturity, matured pods per plant, pod yield per plant, kernel yield per plant, shelling percentage, 100-kernel weight, sound mature kernel percent, harvest index, oil content, oil yield per plant and specific leaf area. The percentages of oil content were determined using nuclear magnetic resonance spectrometer (NMR) unit at Dept. of oil seeds, TNAU, Coimbatore. The specific leaf area (SLA) was recorded at 70 DAS utilizing the 3rd or 4th leaf from the top on the main axis. Leaf area of each leaf was measured by an automatic leaf area meter. Dry weight of leaves was recorded after oven drying at 80°C for 48 hrs and SLA was calculated using the formula SLA (cm^2/g) = Leaf area (cm^2) / Oven dry weight of leaf (g).

The PCA analysis reduces the dimensions of a multivariate data to a few principal axes, generates an Eigen vector for each axis and produces component scores for the characters [20]. Factor analysis uses the covariance matrix of characters to generate factor loadings

Table 1: List of genotypes of groundnut accessions and advanced breeding lines and their source

Sl.No	Accession name	Origin / source
1	ICGV-88145	ICRISAT, India
2	ICGV-89104	ICRISAT, India
3	ICGV-01337	ICRISAT, India
4	ICGV-89322	ICRISAT, India
5	ICGV-00350	ICRISAT, India
6	ICGV-01354	ICRISAT, India
7	ICGV-87846	ICRISAT, India
8	ICGV-02322	ICRISAT, India
9	ICGV-99210	ICRISAT, India
10	ICGV-05089	ICRISAT, India
11	ICGV-05090	ICRISAT, India
12	ICGV-05094	ICRISAT, India
13	ICGV-05099	ICRISAT, India
14	ICGV-05100	ICRISAT, India
15	ICGV-05103	ICRISAT, India
16	ICGV-04071	ICRISAT, India
17	ICGV-02409	ICRISAT, India
18	ICGV-04096	ICRISAT, India
19	ICGV-02099	ICRISAT, India
20	ICGV-02063	ICRISAT, India
21	ICGV-05049	ICRISAT, India
22	ICGV-03042	ICRISAT, India
23	ICGV-05033	ICRISAT, India
24	ICGV-05052	ICRISAT, India
25	ICGV-03037	ICRISAT, India
26	ICGV-03016	ICRISAT, India
27	ICGV-03010	ICRISAT, India
28	ICGV-03157	ICRISAT, India
29	JL - 24	Maharashtra
30	ICGV-92267	ICRISAT, India
31	ICGV-86031	ICRISAT, India
32	TMV-2	Tamilnadu, India
33	Narayani	Tirupathi, India
34	ICGV-91114	ICRISAT, India
35	CTMG-1	ARS, Chintamani, India
36	VRI-2	Tamilnadu, India
37	GPBD-4	Dharwad, India
38	PAFRGVT-60	ICRISAT, India
39	PAFRGVT-58	ICRISAT, India
40	ICGX-020063-F2-B1-SSD-P12-B1	ICRISAT, India
41	ICGX-020063-F2-B1-SSD-P11-B1	ICRISAT, India
42	ICGX-020063-F2-B1-SSD-P18-B1	ICRISAT, India
43	ICGX-020063-F2-B1-SSD-P16-B1	ICRISAT, India
44	ICGX-040038-F2-SSD	ICRISAT, India
45	ICGX-030043-F2-SSD-SSD-P2	ICRISAT, India
46	TAG-24 X ICGS-76	Junagadh, Gujarat, India
47	PBS-111039 X NRCG 4839	Junagadh, Gujarat, India
48	GG-20 X ICGV-91114	Junagadh, Gujarat, India
49	TKG-19A X K3	Junagadh, Gujarat, India
50	ICGV-86031 X TAG 24	Junagadh, Gujarat, India
51	ICGX-020063-F2-B1-SSD-P20-B1	ICRISAT, India
52	ICGX-020063-F2-B1-SSD-P18-B2	ICRISAT, India
53	ICGX-020063-F2-B1-SSD-P15-B1	ICRISAT, India
54	ICGX-020058-F2-SSD-SSD-P7-B1	ICRISAT, India
55	CO-3 X JAL-31	ARS, Chintamani, India
56	PBS-111039 X TAG-24	ARS, Chintamani, India
57	ICGV-86031 X TAG-24 X CSMG 84-1	ARS, Chintamani, India
58	ICGX-020063-F2-B1-SSD-P19-B1	ICRISAT, India
59	ICGX-020063-F2-B1-SSD-P18-B3	ICRISAT, India
60	ICGX-020063-F2-B1-SSD-P13-B1	ICRISAT, India
61	ICGX-020055-F2-SSD-SSD-P37-B1	ICRISAT, India
62	JAL-18 X ALR-2	Junagadh, Gujarat, India
63	GG-2 X ICGV-91114	Junagadh, Gujarat, India
64	JAL-31 X CO-3	Junagadh, Gujarat, India

and communalities using the method of principal component extraction [21, 22]. PCA and factorial analysis were performed in STATISTICA (Version 9.0) [23]. Canonical analysis also measures the axis along which variation between canonical variables and was performed using the SPSS (Version 10.0) package [24]. The discriminant canonical analysis summarizes the multivariate data in the same way as the canonical correlation. The analysis uses the Wilks' lambda as the statistics for entering or removing new variables and thereby identifies the variables that provide the best discrimination among the entries. Ward' clustering has been used to extract the clusters using STATISTICA (Version 9).

RESULTS

Factor Analysis: The results obtained from the factor analysis of the characters were presented in Table (2). The analysis identified nine factors out of which only four were extracted which together explained 68% of the variance among the entries. The first factor with eigen value of 3.940 accounted for only 28.14% of the variance and is primarily related to pod yield per plant, kernel yield per plant, oil yield per plant, matured pods per plant, branches per plant. The factor that accounted for 18.89% of the total variance is mainly loaded by sound mature kernel percent and days to 50% flowering. The third factor that accounted for 12.49% of variance is mainly described by shelling percentage and harvest index. The fourth is factor is loaded by specific leaf area and days to maturity. And it accounted for 8.92% of the total variance. The communality values ranged from 1 for oil content to 0.293 for days to maturity.

Principal Component Analysis (PCA): Results from PCA presented in Table (3), revealed that only first five of the fourteen principal component analysis with eigen values 0.18, 0.10, -0.43, -0.33, 0.28 respectively, jointly accounted for 76% of the total variance among the genotypes. First principal component laid most weight on pod yield per plant, kernel yield per plant, matured pods per plant and oil yield per plant. Second and third principal components described largely by sound mature kernel percent, days to 50% flowering and harvest index, plant height respectively. Fourth and fifth principal components had more weight on specific leaf area and oil content respectively. The configuration of the sixty four groundnut genotypes along the first four principal component axes are shown in Figure 1. The ordination of the genotypes on all the axes together (Fig. 1) revealed that genotypes 3, 23, 56, 58 and 63 found to be most distinct genotypes for the characters studied.

Discriminant Analysis: The eigen values, variance and pooled within correlation between discriminant variable and the discriminant functions were presented in Table (4). The analysis has revealed four functions which accounted for 100% variance for all the functions together. The first function itself accounted for 84% of total variance within the genotypes whereas the second and third functions explained 13.7 and 2.2% of the total variance respectively. The first discriminant function was highly positively correlated with kernel yield, pod yield and oil content. Shelling percentage and oil content positively and pod yield negatively had correlation with second function. Third function showed high positive correlation with pod yield and high negative correlation with shelling percentage and mature pods per plant.

Table 2: Eigen values, proportion of variance,% cumulative variance, factor scores of the fourteen quantitative characters from factor analysis

Character	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Plant height (cm)	0.361	0.157	-0.565	-0.370	0.505
Branches per plant	0.653	-0.449	0.027	0.023	0.609
Days to 50% flowering	0.182	-0.658	0.282	0.182	0.521
Days to maturity	0.151	-0.441	0.184	0.575	0.293
Matured pods per plant	0.817	-0.066	-0.293	0.142	0.799
Pod yield per plant (g)	0.891	0.126	-0.225	0.044	0.996
Kernel yield per plant (g)	0.864	0.405	0.157	-0.052	0.999
Shelling percentage	0.027	0.497	0.696	-0.177	0.985
100 Kernel weight (g)	-0.222	0.578	-0.377	0.204	0.483
Sound mature kernel%	-0.360	0.769	0.019	0.092	0.692
Harvest index (%)	0.045	0.096	0.649	-0.149	0.511
Oil content (%)	0.373	0.289	0.227	0.310	1.000
Oil yield per plant (g)	0.863	0.443	0.202	0.034	0.998
Specific leaf area (cm2/g)	-0.231	0.381	-0.123	0.723	0.477
Eigen value	3.940	2.645	1.749	1.249	
Proportion of variance	28.144	18.893	12.498	8.921	
Cumulative variance	28.144	47.038	59.536	68.458	

Table 3: Eigen vector, eigen root and associated variation for different principal components in Groundnut germplasm

	Eigen vectors													
Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Plant height (cm)	0.18	0.10	-0.43	-0.33	0.28	0.38	0.06	0.28	0.17	0.57	0.06	0.09	-0.02	-0.01
Branches per plant	0.33	-0.28	0.02	0.02	0.23	-0.20	-0.17	0.16	0.58	-0.31	0.47	0.11	-0.02	0.00
Days to 50% flowering	0.09	-0.40	0.21	0.16	0.25	0.04	-0.43	-0.49	-0.15	0.45	0.02	0.22	-0.01	-0.01
Days to maturity	0.08	-0.27	0.14	0.51	-0.04	0.43	-0.18	0.60	-0.14	-0.05	-0.15	-0.08	0.00	0.00
Matured pods per plant	0.41	-0.04	-0.22	0.13	-0.21	-0.19	0.20	0.04	-0.11	-0.08	-0.32	0.72	0.01	-0.01
Pod yield per plant (g)	0.45	0.08	-0.17	0.04	-0.28	0.06	-0.13	-0.14	-0.14	0.00	0.17	-0.29	0.67	-0.24
Kernel yield per plant (g)	0.44	0.25	0.12	-0.05	-0.13	-0.08	-0.21	0.00	-0.05	0.07	-0.06	-0.21	-0.19	0.76
Shelling percentage	0.01	0.31	0.53	-0.16	0.23	-0.24	-0.16	0.26	0.23	0.15	-0.39	0.10	0.38	-0.14
100 Kernel weight (g)	-0.11	0.36	-0.28	0.18	0.09	0.38	-0.40	-0.31	0.35	-0.32	-0.31	0.10	0.00	0.00
Sound mature kernel%	-0.18	0.47	0.01	0.08	0.06	-0.04	-0.31	0.19	-0.39	-0.03	0.55	0.38	0.02	0.00
Harvest index (%)	0.02	0.06	0.49	-0.13	-0.47	0.51	0.20	-0.14	0.27	0.07	0.23	0.26	-0.02	0.01
Oil content (%)	0.19	0.18	0.17	0.28	0.59	0.22	0.54	-0.20	-0.14	-0.15	0.09	0.00	0.17	0.15
Oil yield per plant (g)	0.43	0.27	0.15	0.03	0.04	0.00	-0.05	-0.05	-0.09	0.00	-0.03	-0.19	-0.58	-0.57
Specific leaf area (cm2/g)	-0.12	0.23	-0.09	0.65	-0.18	-0.27	0.17	-0.03	0.37	0.46	0.11	-0.08	-0.03	-0.01
Eigen values	0.18	0.10	-0.43	-0.33	0.28	0.38	0.06	0.28	0.17	0.57	0.06	0.09	-0.02	-0.01
Variation (%)	28.145	18.893	12.499	8.922	6.905	6.475	5.112	4.078	3.054	2.709	2.040	1.109	0.056	0.004

Table 4: Eigen values, total variance, cumulative variance and pooled within group correlation between discriminate variables and the canonical discriminant functions

Function	Eigen value	Variance%	Cumulative%	Pooled within group correlations *		
1	15.454	83.8	83.8	Kernal yield (0.699)	Pod yield (0.324)	Oil content (0.138)
2	2.525	13.7	97.5	Shelling percentage (0.318)	Pod yield (-0.239)	Oil content (0.131)
3	0.404	2.2	99.7	Pod yield (0.662)	Shelling percentage (-0.625)	Matured pods/plant (-0.386)
4	0.049	0.3	100	Plant height (0.622)	100-kernal weight (0.586)	Days to 50% flowering (-0.314)

* Largest absolute correlation between each variable and any discriminant function.

Table 5: Eigen values, total variance and correlation between original and canonical variables of sixty four groundnut genotypes

Canonical factors	Eigen values	Variance %	Correlation of canonical factors with		
1	4.53	38.10	Branches/plant (-0.531)	Matured pods/plant (-0.504)	100-kernal weight (0.469)
2	4.26	31.40	Sound mature kernel percent (0.608)	Branches per plant (-0.534)	Plant height (-0.485)
3	2.33	26.30	Pod yield/plant (0.676)	Matured pods/plant (0.550)	Kernal yield (0.469)

High positive correlation of plant height and 100-kernal weight and high negative correlation of days to 50% flowering was seen in the case of fourth function.

Canonical Analysis: Canonical variable that described the variation in the characters, Total variances eigen values and correlation between variables are presented in Table (5). The three canonical variables extracted from the analysis contributed 41, 31 and 28% of variance respectively. Branches/plant, Matured pods per plant, 100-kernal weight were important characters in the first canonical variable while second canonical variable comprised of sound mature kernel percent, branches per plant, plant height and Third canonical variable comprised of pod yield per plant, matured pods per plant, kernel yield.

Ward's Cluster Analysis: Ward's hierarchical cluster analysis based on first ten principal component scores (total variation accounted more than 90%) resulted in three clusters (Fig. 2). The first cluster comprised thirty six accessions which include fourteen new germplasm lines and four advanced breeding lines.

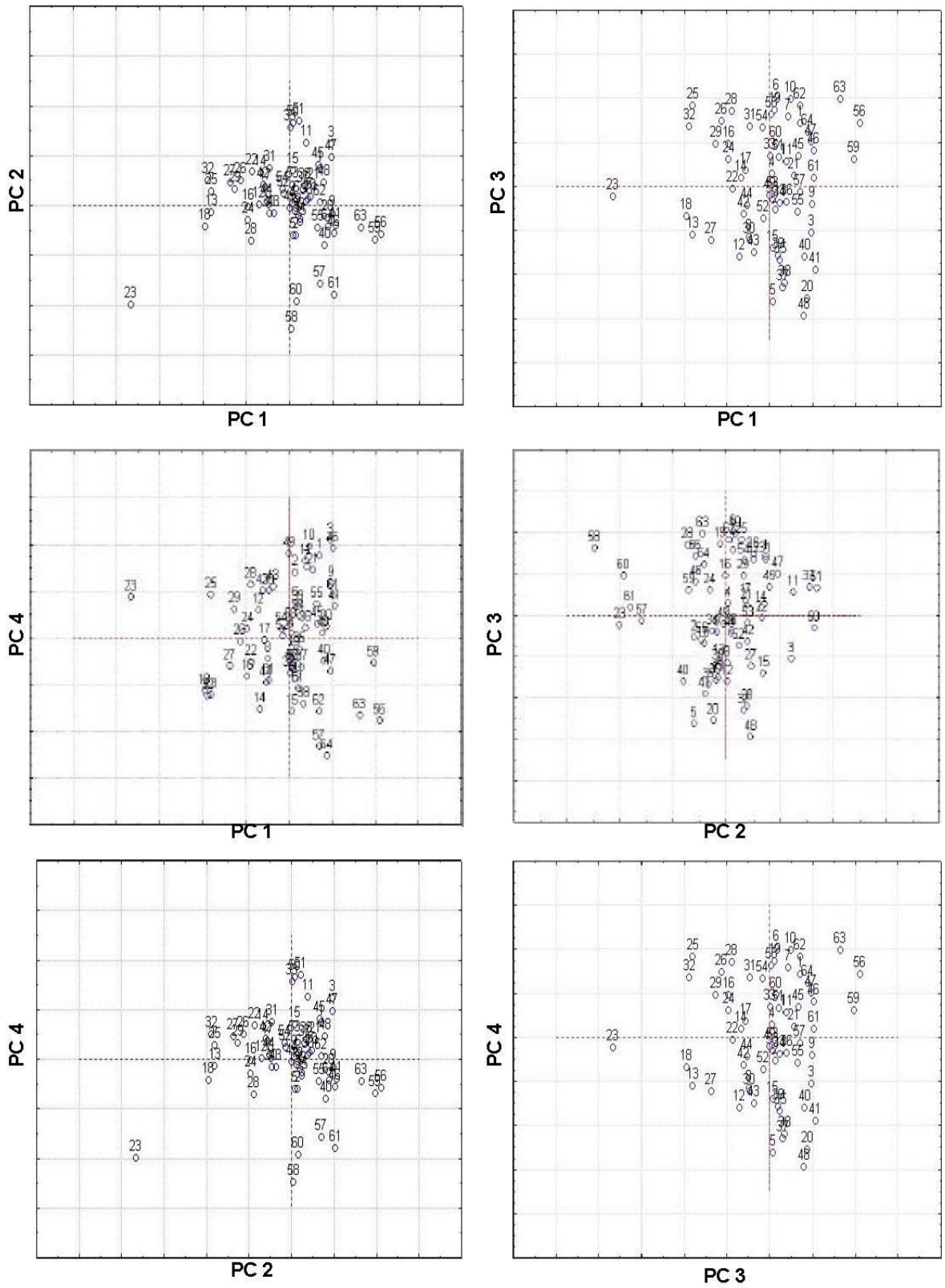

Fig. 1: Configuration of sixty four groundnut genotypes under principal components 1, 2, 3 and 4.

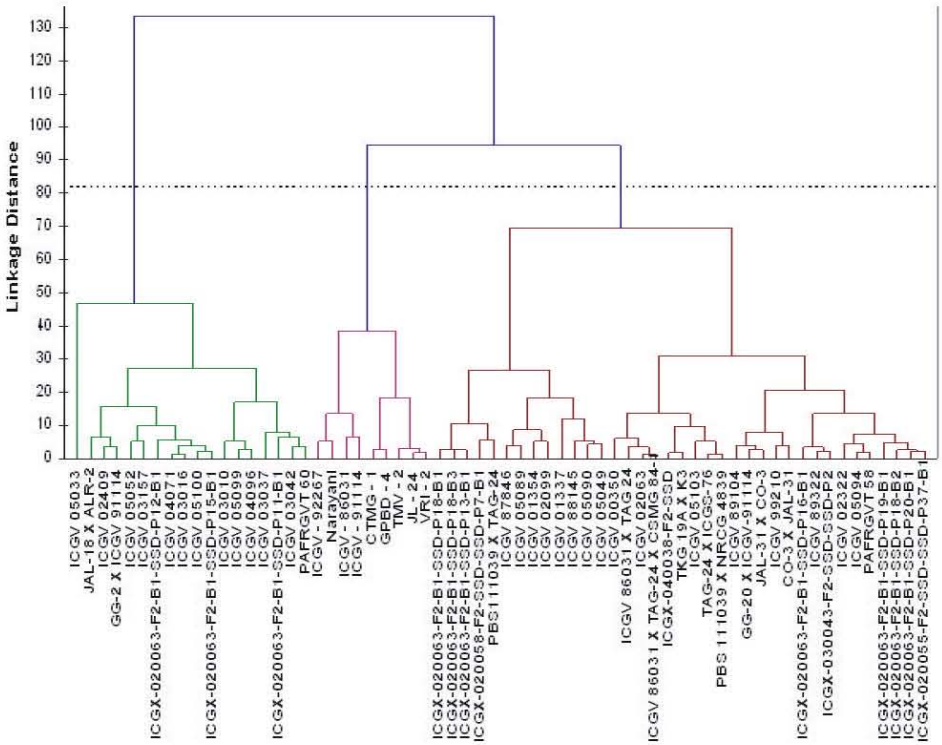

Fig. 2: Dendrogram showing sixty four accessions of groundnut derived from ward's cluster analysis.

Table 6: Range, mean and variance of three clusters of groundnut genotypes from single linkage cluster analysis

	Range			Mean			Variance		
Character	Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	Cluster 3
Plant height (cm)	19.20-34.15	20.00-34.00	20.15-34.80	26.28	28.23	26.40	0.14	0.13	0.17
Branches / plant	4.90-9.50	6.10-9.30	4.10-8.00	7.37	8.19	5.50	0.18	0.13	0.22
Days to 50% flowering	33.50-40.50	36.50-39.50	30.50-39.50	38.08	37.97	35.10	0.05	0.02	0.08
Days to maturity	100.50-121.00	102.50-121.50	104.50-108.00	111.89	112.36	110.35	0.05	0.04	0.04
Matured pods / plant	12.50-23.00	14.00-23.00	13.50-16.50	15.72	18.50	14.80	0.17	0.13	0.06
Pod yield / plant (g)	12.00-20.90	12.50-24.50	11.75-18.10	14.37	16.73	13.68	0.13	0.20	0.13
Kernel yield / plant (g)	7.15-14.10	9.05-19.85	7.65-13.30	10.28	12.00	9.78	0.15	0.19	0.18
Shelling percentage	58.24-81.70	59.14-81.05	59.21-80.57	71.76	72.09	71.49	0.11	0.08	0.11
100 Kernel weight (g)	19.45-37.10	20.20-34.45	26.05-38.00	27.48	27.13	34.41	0.16	0.14	0.10
Sound mature kernel%	57.00-79.00	59.50-75.50	75.00-87.50	68.92	67.22	79.70	0.08	0.06	0.05
Harvest index (%)	22.05-42.20	21.02-41.50	25.50-34.00	32.59	32.81	30.06	0.17	0.14	0.09
Oil content (%)	36.00-49.15	42.85-50.00	40.05-46.60	44.37	45.81	44.48	0.06	0.05	0.05
Oil yield / plant (g)	3.29-6.83	4.08-9.69	3.19-5.94	4.57	5.51	4.36	0.17	0.22	0.20
Specific leaf area (cm^2/g)	83.85-147.95	93.70-134.95	120.35-187.75	115.19	116.50	148.15	0.11	0.10	0.14

The second cluster comprised eighteen accessions of which four were advanced breeding lines rest of them were new germplasm lines. Third cluster comprised ten accessions including six control varieties (JL 24, TMV 2, Narayani, CTMG 1, VRI 2, GPBD 4) and four new germplasm lines.

DISCUSSION

When dissimilarity between a pair of variety is defined on a multivariate criterion, it is useful to be able to determine the plant characters which cause the dissimilarity to arise and the relative contributions that

the various characters make to the total variability in the germplasm. Factor analysis and principal component analysis identified some similar characters as the most important for classifying the variation within and among groundnut genotypes. These included, pod yield, kernel yield, sound mature kernel percent, 100-kernal weight and days to 50% flowering. The similarity between the two techniques had been reported earlier in rice [25] and in groundnut [7]. Although the two techniques produced similar results, their underlying principles are substantially different from each other. While PCA does not rely on any statistical model and assumptions, factorial analysis does. It is also imperative to note that factor analysis suffers from other drawbacks, such as absence of 'error' structure and the dependence upon scale used to measure the variable [4]. The discriminant analysis considered the kernel yield as the important discriminatory trait among the entries. Pod yield and oil content are the other important characters identified by the discriminate analysis. The canonical analysis gave different picture of the relative importance of the various characters within the entries when compared to principal component and factorial analysis. This analysis in present study considered branches per plant as the character that best discriminated the groundnut genotypes. Other important variable included matured pods per plant and 100-kernel weight. Interestingly, factor analysis and PCA captured equal amount of variation by the axes. Difference in results of multivariate techniques, with respect to characters which best summarized within population variance. The similar results were reported earlier in rice and okra [3, 12]. As compared to other techniques, discriminant analysis explained a high figure of 83.8% of the within entries variance in the same number of axes.

In the present study involving germplasm from different geographical regions, no distinct relationship was observed between geographical origin and cluster formation. The random pattern of distribution of genotypes into various clusters from different eco-geographic regions suggests that forces other than geographic influence such as exchange of breeding material, genetic drift, natural and artificial selections are responsible for diversity as reported earlier [26]. The absence of correlation between genetic diversity and geographic diversity has also been supported in a few studies [27]. Further, it is note worthy to mention that the genetic divergence would be possible with the inclusion of more breeding stock from different sources and with evaluation of other qualitative characters. Mean values of characters were more or less continuous across clusters hence no, sharp distinction between clusters was observed. This was an indication that clusters were under polygenic control. Cluster three was found to have higher mean values for 100-kernel weight, sound mature kernel percent and specific leaf area while cluster two was found to have high mean values for the remaining characters under study. Clusters one and two were found to have wide range for all the characters under study. Hybridization between genotypes of different clusters with high cluster mean will result into transgressive segregates with high yield potential [28]. Therefore the genotypes from clusters one and two which showed high range and mean for important characters like pod yield, kernel yield, sound mature kernel percent, oil content can be used in the future breeding programme to recombine these traits.

CONCLUSION

In the present study principal component analysis was captured most of the variation within the germplasm in higher number of axes compared to other techniques used in the study. However, the techniques were not showed considerable differences in the characters considered most important other than canonical analysis. Thus, a combination of PCA and any of the Factorial, discriminant or canonical analyses would be appropriate for describing the variation either from germplasm or advanced breeding lines in any crops especially in groundnut germplasm, which is an autopolyploid showing tremendous diversity between and among the cultivated species.

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