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Anjali Kachhadia

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Sneha Macwana

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Sushil Kumar

Department of Biotechnology, Centre of Excellence in Biotechnology, Anand Agricultural University, Anand, Gujarat, India

Bhavya Desai

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Ashita Patel

Department of Genetics and Plant Breeding, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

Corresponding Author:

Anjali Kachhadia

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Morphological and molecular characterization studies in groundnut (*Arachis hypogaea* L.)

Anjali Kachhadia, Sneha Macwana, Sushil Kumar, Bhavya Desai and Ashita Patel

Abstract

Among the oilseeds, groundnut is the most important annual unpredictable legume. The present investigation was undertaken using 83 genotypes of Spanish bunch groundnut to evaluate variability, genetic parameters, genetic divergence, character association and path analysis. The high values of genotypic and phenotypic coefficient of variation were observed for mature pods per plant, immature pods per plant, total number of pods per plant, pod yield per plant, kernel yield per plant, fodder yield per plant, biological yield per plant and harvest index% indicating that sufficient variability existed in the experimental material for these traits. High heritability coupled with high genetic advance as percent of mean was observed for morphological characters viz., mature pods per plant, immature pods per plant, total number of pods per plant, pod yield per plant, 100 pod weight, kernel yield per plant, 100 kernel weight, fodder yield per plant, biological yield per plant, harvest index%. These characters were governed by additive gene action. Hence, direct selection could be made from these traits for improvement of the crop.

Keywords: Variability, heritability, path coefficient, correlation

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important annual unpredictable legumes, both in subsistence and commercial agriculture in arid and semi-arid regions of the world. It is one of the principle economic crops of the world. It is an important food, feed and oilseed crop grown in around 100 countries across the world (Zaman *et al.*, 2010) [27]. Groundnut is self-pollinated, tetraploid with chromosome number $2n = 40$. The genus *Arachis* is a member of family Fabaceae (Synonym: Leguminosae), subfamily Papilionoideae, tribe Aeschynomeneae and sub-tribe stylosanthinae. It belongs to the section *Arachis* and series amphiploidies. (Krapovickas & Gregory, 1980) [7].

Groundnut is valued as a rich source of energy contributed by oil (48–50%) and protein (25–28%) in the kernels. It provides 564 kcal of energy from 100 g of kernels (Jambunathan, 1991) [9]. In addition, the groundnut kernels contain many health enhancing nutrients such as minerals, antioxidants and vitamins and are rich in mono-unsaturated fatty acids. They contain antioxidants like *p*-coumaric acid and resveratrol, Vitamin E and many important B-complex groups of thiamin, pantothenic acid, vitamin B-6, folates and niacin.

Groundnut oil is an excellent cooking medium because of its high smoking point (Singh and Diwakar, 1993) [24]. Groundnut oil contains 46 and 32 percent of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively (Saini *et al.*, 2020) [22].

The genetic variability in groundnut is low due to origin of the crop through a single hybridization event between two diploid species followed by a chromosome doubling and crossing barriers with wild diploid species (Due of ploidy differences). The low genetic variability for the traits of importance and polyploidy nature are a bottleneck to the groundnut improvement (Pasupuleti *et al.*, 2013) [15].

The divergence analysis has a definite role to play in efficient choice for breeding divergent parents. Mahalanobis D^2 statistics is an effective tool in multivariate analysis for estimating relative contribution of each character towards genetic divergence and to identify best parents for hybridization to exploit maximum heterosis for improving the yield.

Information of diversity patterns allows breeders to comprehend the evolutionary associations among genotypes in a better way to collect genotypes in a more organized manner and to make plan to incorporate valuable material in their germplasm (Bretting and Wildrlechner, 1995) [5]. Therefore, in present study, attempts have been made to study phenotypic, genotypic and

genetic divergence in groundnut and detection of genetic relationships among genotypes using a collective approach of morphological variation and genetic variation to accelerate the future crop improvement program of groundnut.

Materials and Methods

Plant materials and field evaluation

A total of 83 accessions were raised at Genetics and plant breeding farm, Anand Agricultural University, Anand in three replications. The average rainfall of the zone is around 750 mm. The soil of the experiment farm is sandy loam in texture and poor in organic carbon. The experiment was laid out in a randomized complete block design with twenty plants per variety in each replication. Rows were spaced 0.30 m apart with 0.10 m plant to plant distance. The standard package of agronomical practices, were followed to raise a healthy experimental crop. The experimental material was procured from Main Oilseed Research Station, Junagadh Agricultural University, Junagadh and Regional Rice Research Station, Navsari Agricultural University, Vyara.

During the course of this study, data on the 18 characters were recorded from randomly selected five competitive plants in each accession in each replication except for days to 50% flowering and days to maturity which were recorded on the plot basis. Seed samples were drawn randomly from the bulk of kernels on plot basis for estimation of oil and protein content by Near Infrared Spectroscopy (NIR) using methodology suggested by Misra *et al.* (2000) [10].

Morphological characterization and statistical analysis

During the study, data were recorded from the five plants of each genotype in each replication. For 16 morphological traits and 2 biochemical parameters, data were analysed for analysis of variance (ANOVA) and LSD test was performed to identify genotypes that were significantly different from each other. R procedures and programmes were used for these calculations. Genetic parameters were estimated to identify genetic variability among accessions and determine genetic and environmental effects on various characters. These genotypic and phenotypic components of variance, coefficients of variability, broad sense heritability and genetic advance were estimated by adapting the formulae suggested by Allard (1960) [1] and Singh and Chaudhary (1985) [25].

Results and Discussion

Determination of mean, minimum and maximum values and coefficient of variations

A perusal of mean values of yield attributing traits revealed that, mature pods per plant and kernel yield per plant was highest and significantly differing from rest of genotypes for JB 402; moreover, JB 402 recorded highest total number of pods per plant and pod yield per plant making JB 402 an elite genotype for choice of breeding programs. Other genotypes namely GJG 31, JB 772, ICGV 971, NIAC 1727, GG 34, ICGV 96174 and ICGV 91677 was at par and had recorded high value of trait pod yield per plant.

JB 772 noted to have highest mean value for 100 pod weight which was at par with NCAC 990, JB 512 and GG 34. Further the genotype JB 512 reported highest mean value for 100 kernel weight being at par with ICGV 00380. The estimate of 100 pod weight gives an idea about boldness of grains, so higher mean performance is desirable for yield improvement. Post-harvest processing practices accounts for wide

possibility of improvement in economic yield of the plant. Highest mean value for shelling out turn (%) of pods was observed for JB 772 while, lowest mean value was observed for NCAC 761.

Additionally, ICGV 96211 reported highest biological yield per plant followed by ICGV 9174; while, least mean value was reported for TG 73. Harvest index referred as “coefficient of effectiveness” in many crops. Groundnut has peculiar habit of profuse vegetative growth in relation to pod yield making harvest index one of important quality trait. Harvest Index was recorded highest in TG 26.

Quality attributing trait holds significant prospect of improvement in term of nutritional security and high value produce. Highest mean value for oil content was observed in JB 649 (52.74%). Furthermore, TG 26 (28.49%) recorded the highest mean value for crude protein.

ANOVA and analysis of phenotypic and genotypic variances

Analysis of variance revealed significant differences among the genotypes for all the 17 traits studied which suggested the existence of sufficient variability in the experimental material (Table 1). Phenotypic and genotypic coefficients of variation (PCV and GCV), broad sense heritability, and genetic advance were calculated for all the characters and are given in Table 1. The PCV was invariably higher than their corresponding GCV indicating that the apparent variability in these characters may not only be due to the genotypes but also environmental factors were influential in expressing these characters.

In the present research GCV ranged from 1.09 to 49.91% and PCV ranged from 2.18 to 51.65%. High genotypic coefficient of variation (GCV %) and phenotypic coefficient of variation (PCV %) were observed for mature pods per plant, immature pods per plant, total number of pods per plant, pod yield per plant, kernel yield per plant, fodder yield per plant, biological yield per plant and harvest index%. High GCV values with marginally high PCV values indicated that between the genotypes variations were high and that the expression of these characters was less influenced by the environment. Selection for these traits would be rewarding as depicted in figure 1.

Low genotypic coefficient of variation and moderate phenotypic coefficient of variation were observed for plant height (9.18% and 14.87%) and shelling out turn % (6.53% and 10.70%). Low genotypic coefficient of variation and phenotypic coefficient of variation was observed for days to 50% flowering (8.21 and 9.25), days to maturity (1.74 and 2.18), sound mature kernel % (1.09 and 2.67), oil content % (4.53 and 4.93) and crude protein % (4.76 and 5.73). Low PCV values with marginally low GCV values in these characters indicated less variability for these traits in the genotypes studied.

Knowledge on the heritability is important to a plant breeder since it indicates the possibility and extent to which improvement is possible through selection. The relative amount of heritable portion was assessed in the present study with the help of estimates of broad sense heritability. Coefficient of variation together with heritability estimates and GAM would give the best picture of the amount of advance to be expected from selection as depicted in figure 2. High heritability coupled with high genetic advance as percent of mean was observed for morphological characters

viz., mature pods per plant (83.17%), immature pods per plant (93.40), total number of pods per plant(85.55%), pod yield per plant(72.59%), 100 pod weight(61.61%), kernel yield per plant (66.62%), 100 kernel weight (62.09%), fodder yield per plant(87.42%), biological yield per plant(86.83%), harvest index% (78.28%). Moderate heritability coupled with high genetic advancement as percent of mean was observed for primary branches per plant (57.62% and 21.71%). It indicated that these traits were most likely to be controlled by additive

gene action and selection for these traits would be rewarding. High heritability coupled with low genetic advance as percent of mean was observed for characters viz., days to maturity(63.36% and 2.85%), oil content%(84.53% and 8.59) and crude protein%(69.16% and 8.16) which indicated non-additive gene action. The high heritability is being exhibited due to favorable influence of environment rather than accession and selection for such traits may not be rewarding.

Table 1: The estimate of variance components and other genetic parameters for different characters in groundnut

Sr. No.	Characters	Variance components		GCV (%)	PCV (%)	H ² _b (%)	GAM
		σ^2_g	σ^2_p				
1	Days to 50% flowering	10.65	13.50	8.21	9.25	78.91	15.04
2	Days to maturity	4.69	7.40	1.74	2.18	63.36	2.85
3	Plant height	38.34	100.62	9.18	14.87	38.11	11.67
4	Primary branches per plant	0.99	1.72	13.88	18.29	57.62	21.71
5	Mature pods per plant	27.22	32.72	27.00	29.61	83.17	50.72
6	Immature pods per plant	5.87	6.29	49.91	51.65	93.40	99.38
7	Total no. of pods per plant	38.06	44.49	25.52	27.59	85.55	48.62
8	Pod yield per plant	24.29	33.47	21.55	25.29	72.59	37.82
9	100 pod weight	285.68	463.71	17.52	22.32	61.61	28.34
10	Kernel yield per plant	12.44	18.68	23.58	28.89	66.62	39.65
11	100 kernel weight	111.12	178.95	18.42	23.38	62.09	29.91
12	Shelling out turn	18.17	48.79	6.53	10.70	37.24	8.21
13	Sound mature kernels	1.06	6.41	1.09	2.67	16.61	0.91
14	Fodder yield per plant	535.26	612.26	37.21	39.79	87.42	71.66
15	Biological yield per plant	613.86	706.94	29.13	31.26	86.83	55.91
16	Harvest index	61.20	78.18	27.21	30.76	78.28	49.60
17	Oil content	4.49	5.32	4.53	4.93	84.53	8.59
18	Crude protein	1.48	2.14	4.76	5.73	69.16	8.16

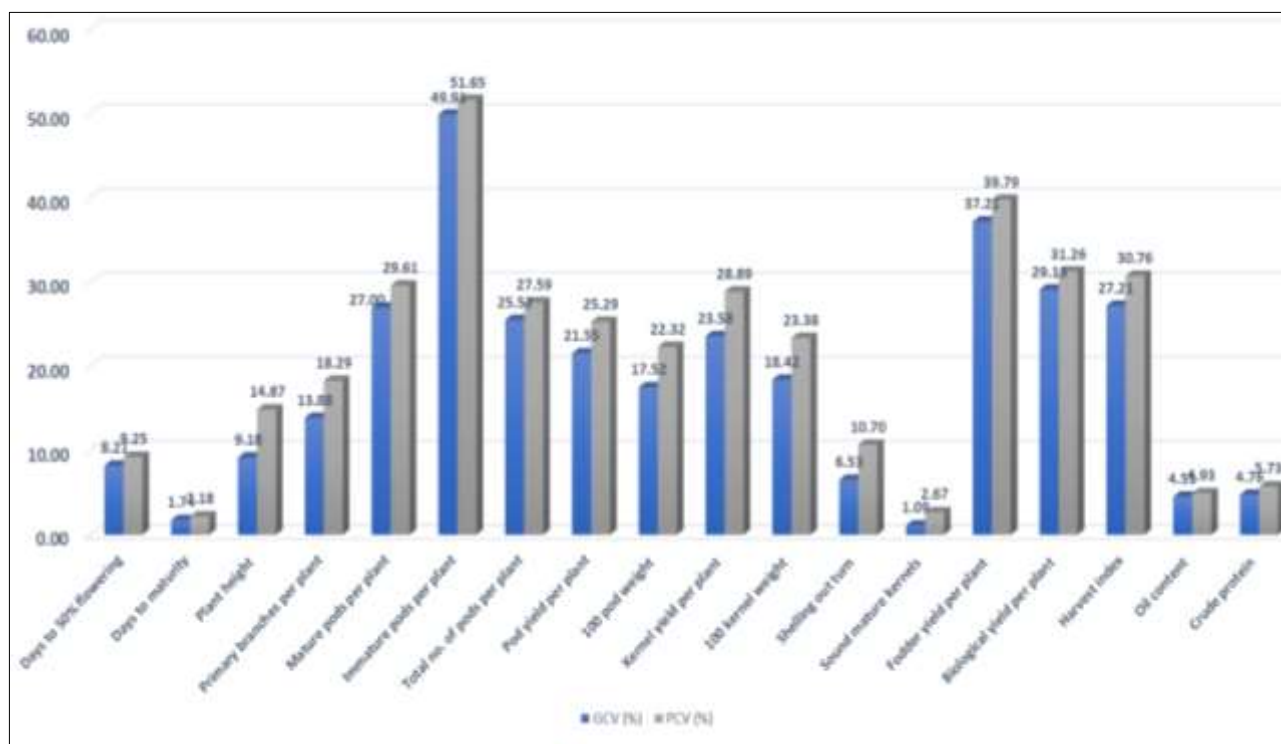


Fig 1: Graphical representation of genotypic coefficient of variation (%) and phenotypic coefficient of variation (%)

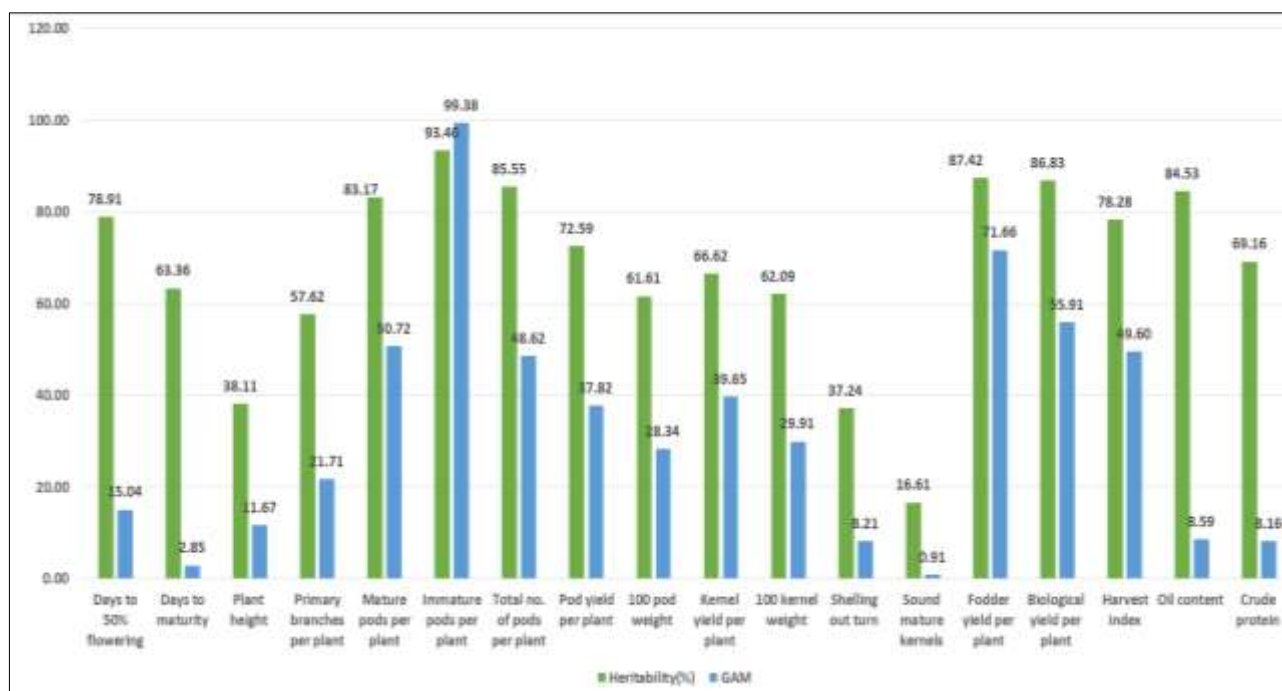


Fig 2: Graphical representation of broad sense heritability and genetic advance as a percent of mean

Association among the traits (Correlation)

Genetic and phenotypic correlations were calculated among 18 characters followed by path coefficient analysis to partition the correlation coefficients of traits with seed yield per plant into direct effects.

The association between two characters can directly be observed as phenotypic correlation, while genotypic correlation expresses the extent to which two traits are genetically associated. Both genotypic and phenotypic correlations among and between pairs of characters provide scope for indirect selection in a crop improvement program (Pavan *et al.*, 2011) [17].

When genotypic correlations were higher than their phenotypic correlation coefficients, it indicates the reason of association was genetic and heritable.

Values of genotypic correlation coefficients were higher than those of their respective phenotypic correlation coefficients in most of the cases, suggesting that there was a strong and inherent association between pair of characters. The results of correlation association between characters revealed that many of the characters in present study were positively and significantly correlated with each other. Two characters; kernel yield and mature pods per plant have been identified as economically important and selection criteria for improvement of pod yield. The appropriate knowledge of interrelationships of yield components can significantly improve the efficiency of breeding program by determining the appropriate traits to be used for yield improvement.

Perusal of above results from character association analysis revealed that the characters *viz.*, mature pods per plant, immature pods per plant, total number of pods per plant, 100 pod weight, kernel yield per plant 100 kernel weight, biological yield per plant and harvest index had significant positive association with pod yield per plant. Hence, improvement of these characters simultaneously increases pod yield per plant.

In contrast, days to 50% flowering had significant negative association with pod yield per plant which is desirable in that

direction. Therefore, it could be suggested that selection of genotypes with earliness is desirable.

The estimates of path analysis revealed that kernel yield per plant exhibited high positive direct effect on pod yield per plant followed by mature pods per plant. This direct contribution represented true correlation indicating that there is a higher scope for selection through these traits to improve pod yield per plant.

However, the genotypic correlation coefficient of total number of pods per plant was found positive but their direct effect was negative. Positive indirect effect of total number of pods per plant was high *via* kernel yield per plant (0.868) and mature pods per plant (0.317). So, the significant positive correlation with yield was found due to indirect path coefficient *via* kernel yield per plant and mature pods per plant. Therefore, the indirect causal factors are to be considered for selection.

By and large to conclude, path analysis estimates in the present investigation revealed that kernel yield per plant had true relationship with pod yield per plant by establishing significant positive association and high positive direct effect on pod yield per plant. Therefore, the character kernel yield per plant should be given due weightage during selection program for enhancing pod yield per plant in groundnut.

Path analysis

Since yield components are interrelated and develop sequentially at different growth stages, correlations may not provide a clear picture of the importance of each component in determining pod yield, *i.e.*, correlation coefficient analysis measures mutual relationship between pair of characters, while path coefficient analysis is a method of partitioning the correlation coefficient into direct and indirect effect. It also reveals whether the association of characters with yield is due to their direct effect on yield or is a consequence of their indirect effects through other component traits and thereby, permitting a critical examination of specific forces acting to produce a given correlation by measuring relative importance

of each causal factor.

Based on genotypic correlation coefficient of high magnitude, path coefficient analysis for pod yield per plant was carried out with 17 different characters excluding biological yield per plant as it was a derived data being summation of fodder yield and pod yield. The results of direct and indirect effects of different characters on pod yield presented in the Table 2 and figure 3.

The estimates of path analysis revealed that kernel yield per plant exhibited high positive direct effect on pod yield per plant followed by mature pods per plant. This direct contribution represented true correlation indicating that there is a higher scope for selection through these traits to improve pod yield per plant.

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Pods per plant was high via kernel yield per plant (0.868) and mature pods per plant (0.317) as shown in table 3.

High positive direct effect of kernel yield per plant on pod yield per plant was observed by Bhargavi (2016) [3], Rao (2016) [20] and Hampannavar *et al.* (2018) [8]. So, the significant positive correlation with yield was found due to indirect path coefficient via kernel yield per plant and mature pods per plant. Therefore, the indirect causal factors are to be considered for selection.

By and large to conclude, path analysis estimates in the present investigation revealed that kernel yield per plant had true relationship with pod yield per plant by establishing significant positive association and high positive direct effect on pod yield per plant. Therefore, the character kernel yield per plant should be given due weightage during selection program for enhancing pod yield per plant in groundnut.

Table 2: Genotypic path-coefficient analysis showing direct and indirect effects of different characters on pod yield in groundnut

Characters	DF	DM	PH	BPP	MP	IP	PPP	PW	KY	KW	SO	SMK	FYP	HI	OC	CP	r _g
DF	-0.038	-0.001	0.034	0.020	-0.116	-0.047	0.280	-0.002	-0.407	-0.041	0.063	-0.019	0.000	0.002	-0.005	0.011	-0.269*
DM	0.002	0.021	-0.017	0.010	0.112	0.009	-0.203	0.003	0.115	0.028	0.049	0.008	0.000	-0.003	0.006	-0.012	0.129
PH	0.007	0.002	-0.182	-0.017	-0.050	-0.012	0.104	-0.003	-0.187	-0.009	0.150	0.070	0.008	0.071	-0.010	0.026	-0.031
BPP	-0.012	0.003	0.048	0.063	0.010	0.015	-0.044	0.000	0.026	-0.011	-0.100	-0.047	-0.004	-0.026	0.006	-0.009	-0.082
MP	0.013	0.007	0.026	0.002	0.344	0.028	-0.623	0.004	0.838	0.055	-0.086	0.006	0.002	-0.009	0.000	0.003	0.610**
IP	0.013	0.001	0.015	0.007	0.068	0.144	-0.377	0.002	0.406	0.026	-0.032	0.032	0.001	-0.003	0.004	-0.008	0.298**
PPP	0.016	0.006	0.028	0.004	0.317	0.080	-0.675	0.004	0.868	0.057	-0.085	0.017	0.002	-0.009	0.001	-0.001	0.633**
PW	-0.006	-0.004	-0.032	0.001	-0.101	-0.022	0.208	-0.014	0.443	-0.114	0.028	-0.039	0.000	-0.013	-0.006	0.035	0.362
KY	0.012	0.002	0.026	0.001	0.220	0.045	-0.447	-0.005	1.309	-0.030	-0.113	-0.052	0.002	-0.026	0.000	0.015	0.958**
KW	-0.012	-0.004	-0.013	0.005	-0.141	-0.028	0.285	-0.012	0.293	-0.134	-0.003	-0.061	0.001	-0.003	-0.008	0.056	0.222*
SO	0.008	-0.003	0.088	0.021	0.095	0.015	-0.185	0.001	0.477	-0.001	-0.309	-0.088	-0.003	-0.034	0.008	0.005	0.093
SMK	-0.005	-0.001	0.092	0.022	-0.014	-0.034	0.085	-0.004	0.489	-0.059	-0.196	-0.138	-0.004	-0.044	0.019	0.004	0.213
FYP	0.000	0.001	-0.130	-0.022	0.059	0.012	-0.120	0.000	0.185	-0.013	0.101	0.052	0.011	0.073	-0.005	0.035	0.238
HI	0.001	0.001	0.149	0.019	0.036	0.004	-0.067	-0.002	0.394	-0.005	-0.121	-0.070	-0.009	-0.087	0.006	-0.030	0.219*
OC	0.005	0.003	0.049	0.010	0.000	0.014	-0.026	0.002	0.002	0.029	-0.061	-0.069	-0.001	-0.014	0.039	-0.042	-0.061
CP	0.004	0.003	0.051	0.006	-0.010	0.013	-0.007	0.005	-0.205	0.080	0.017	0.007	-0.004	-0.028	0.017	-0.094	-0.146

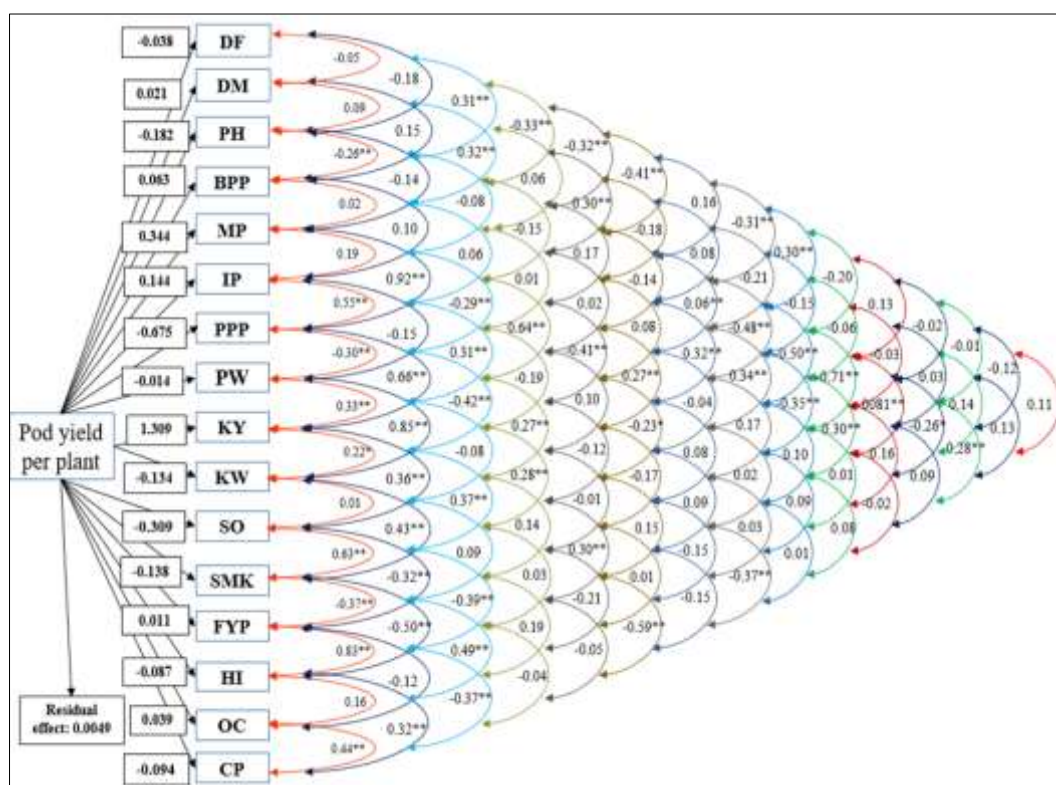


Fig 3: Path diagram showing the effects of various traits on pod yield per plant

Genotypic analysis using SSR markers

A total of 70 SSR primers were screened using two random DNA samples to check amplification and to identify polymorphic primers. Out of 70, 43 (61.43%) primers showed amplification. Out of 43 amplified primers, merely 5 (11.62%) primers were polymorphic. 5 primers generated a total of 23 alleles with band size ranging from 90 (DGR 39) to 297 bp (DGR 27). The number of polymorphic bands ranged from 23 bands with an average of 5.31. The percentage polymorphism was observed to be 100%. In current study, resolving power ranged from 1.47 (DGR 27) to 3.25 (DGR 39) with an average of 2.48. Mean resolving power ranged from 0.29 (DGR 27) to 0.73 (DGR 22) with an average of 0.55. Fraction of polymorphism, multiplex ratio, effective multiplex ratio and marker index for SSR primers was observed to be 1, 4.8, 4.8, and 1.45, respectively as mentioned in table 3. PIC values were reported ≥ 0.5 by Mace *et al.* (2006) ^[12] in groundnut. Varshney *et al.* (2009) ^[26] reported

PIC value ranged from 0.38 to 0.88 with an average of 0.70 in their experiment with groundnut.

Total 70 pair of SSR primers were screened using two random DNA samples to check amplification and to identify polymorphic primers. Out of 70, 43 (61.43%) primers showed amplification. 5 (11.62%) primers out of 43 proved to be polymorphic. 5 primers generated 23 alleles with band size ranging from 90 (DGR39) to 297bp (DGR27). 83 groundnut genotypes were grouped into 8 main clusters using SSR markers. 15 genotypes fall under in cluster I containing genotypes like JB 402 and ICGV 00380. Cluster II contained maximum genotypes being 21 in it. GG 7 was grouped in cluster III. Cluster IV and V contained 20 and 19 genotypes respectively. JB-369 and KISHAN were grouped in cluster VI. JB 693, AK 1341 and ICGV 00387 were grouped in cluster VII. JB 831 and ICGV 00309 were found divergent from the rest of genotypes and fall under cluster VII as depicted in figure 4.

Table 3: Results of SSR Marker Analysis

Sr. no.	Maker locus	Band size	TB	PB	Polymorphism (%)	Average PIC	PIC	RP	Mean RP
1	DGR 22	180-200	4	4	100.00	0.361	0.654	2.94	0.73
2	DGR 27	242-297	5	5	100.00	0.242	0.764	1.47	0.29
3	DGR 30	192-210	3	3	100.00	0.377	0.609	1.69	0.56
4	DGR 31	133-168	6	6	100.00	0.267	0.688	3.06	0.51
5	DGR 39	90-115	5	5	100.00	0.265	0.638	3.25	0.66
	Total		23	23	500	1.512	3.353	12.41	2.75
	Average		4.6	4.60	100	0.302	0.671	2.48	0.55

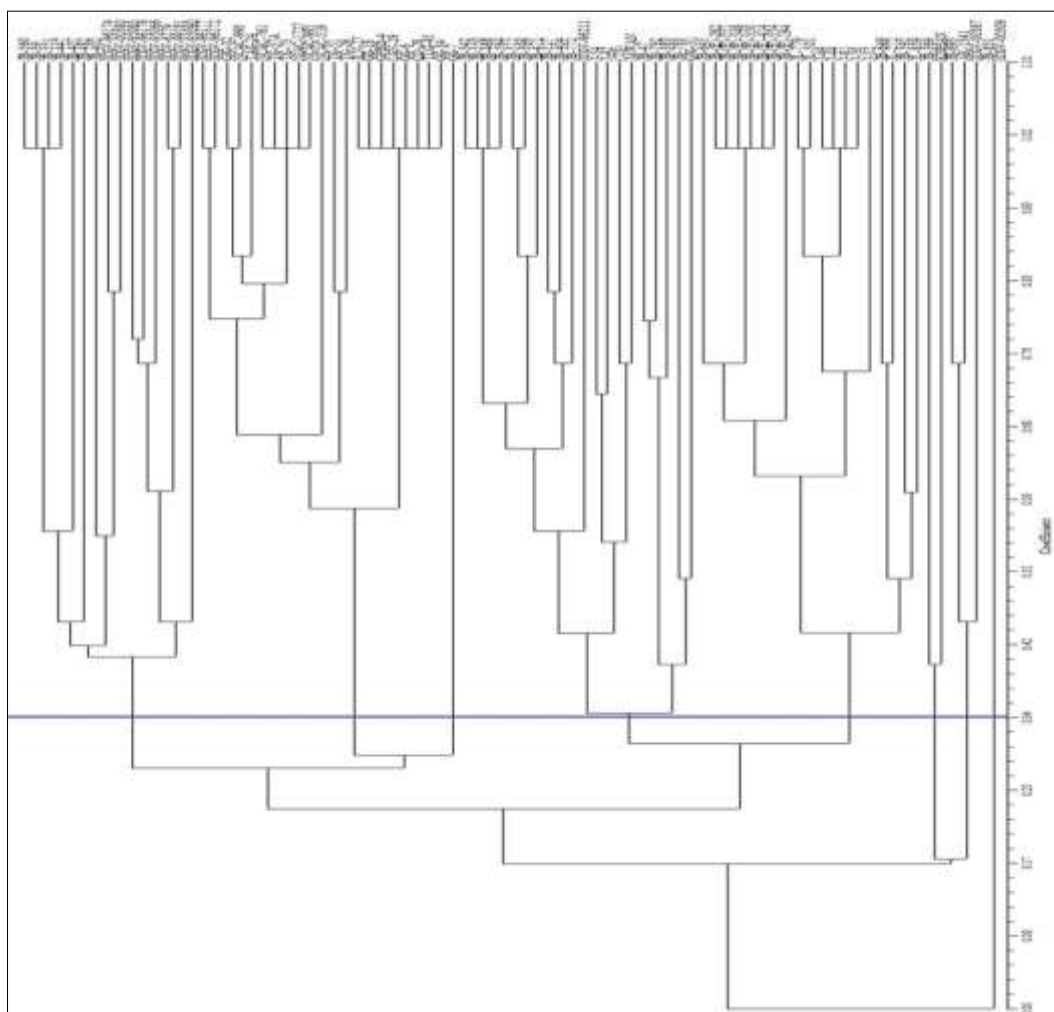


Fig 4: Dendrogram based on genetic coefficient of similarity among the genotypes

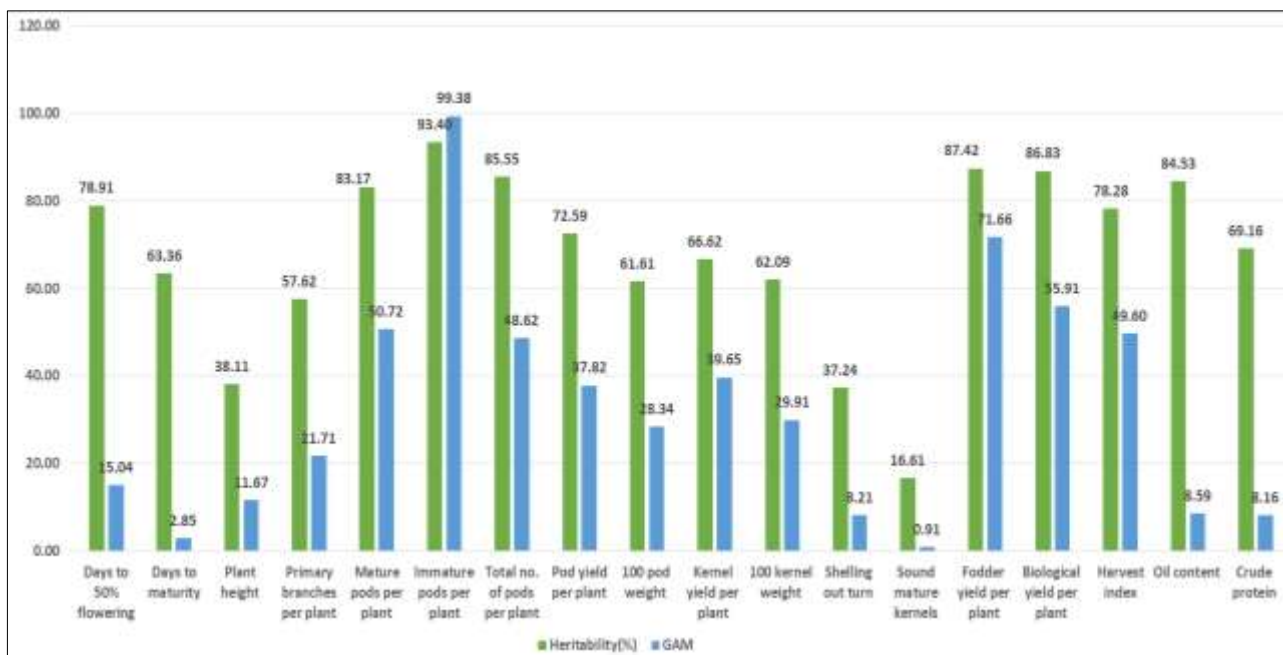


Fig 5: Graphical representation of genotypic coefficient of variation (%) and phenotypic coefficient of variation (%)

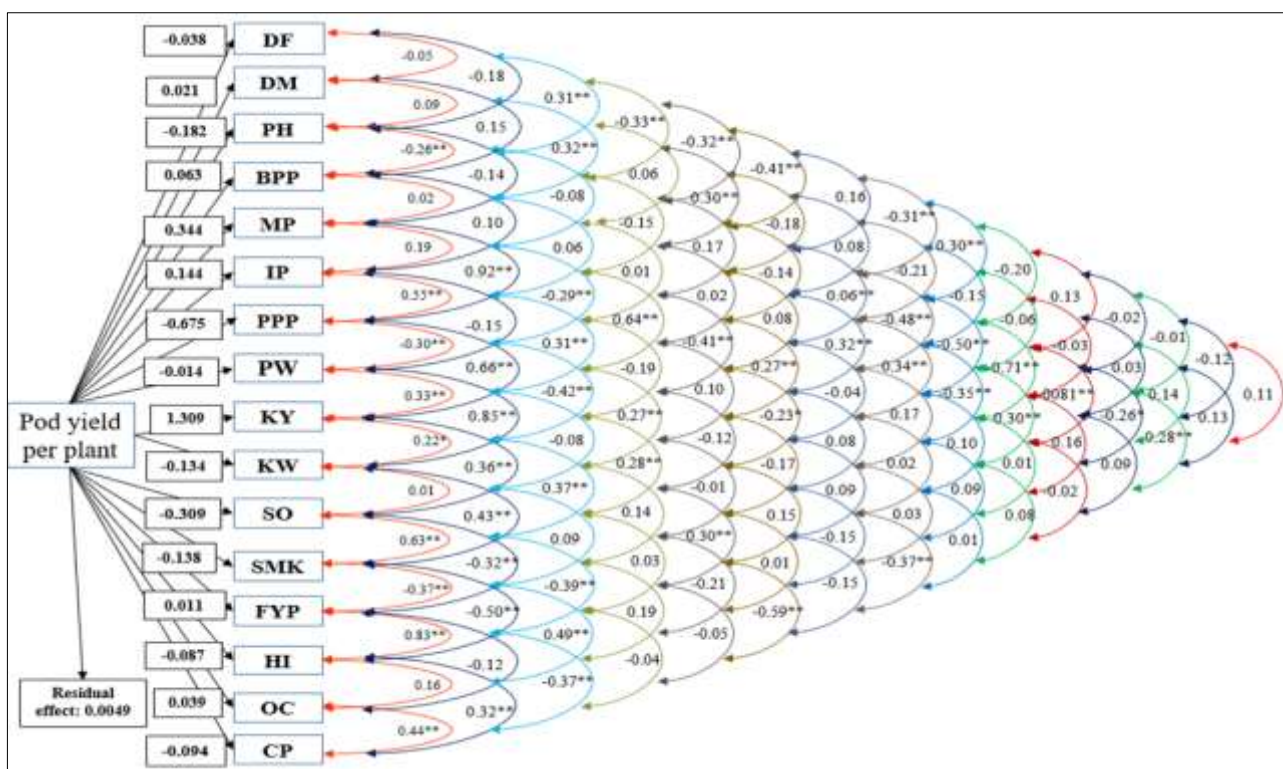


Fig 6: Path diagram showing the effects of various traits on pod yield per plant

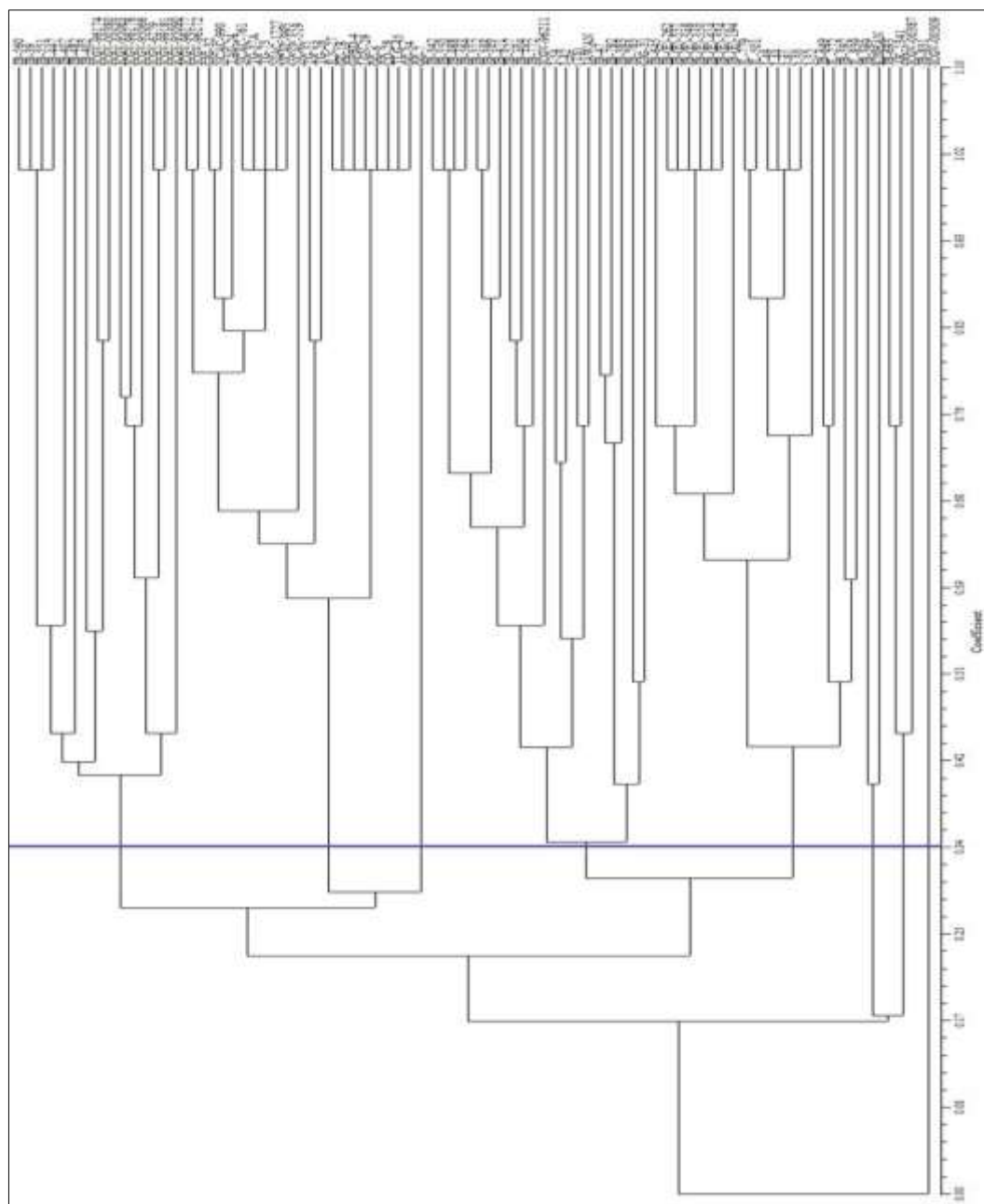


Fig 7: Dendrogram based on genetic coefficient of similarity among the genotypes

Conclusion

The present investigation entitled “Morphological and molecular characterization studies in groundnut (*Arachis hypogaea* L.)” was undertaken using 83 genotypes of Spanish bunch groundnut to evaluate variability, genetic parameters, character association, genetic divergence, and path analysis along with molecular characterization. Morphological characters *viz.*, mature pods per plant, immature pods per plant, total number of pods per plant, pod yield per plant, kernel yield per plant, fodder yield per plant, biological yield per plant and harvest index expressed sufficient variability and manifested high genetic advance as percent of mean coupled with high heritability. These traits were governed by additive gene action and will respond to phenotypic selection. It was evident from correlation studies that characters like kernel yield per plant and mature pod per plant were found most important characters to improve pod yield per plant. Amalgamation of phenotypic selection with molecular selection will lead to more pronounced judgement in

identifying major yield attributing characters in groundnut improvement.

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