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Research Article

Diversity assessment of groundnut genotypes for pod and kernel traits through multivariate analysis

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Abstract
Fifty-five groundnut genotypes from various sources were studied for their diversity. Eleven traits of pod and kernel *viz.,* 100-pod weight, pod length, pod width, 100-kernel weight, kernel length, kernel width, oil content, oleic acid content, linoleic acid content, palmitic and stearic acid content were recorded. The coefficient of variation ranged from 22.40% for linoleic acid content to 6.38% for oil content. The principal component analysis was done to examine the variation. The first four principal components explained about 78.37 % of the total variability. Cluster analysis grouped 55 genotypes into nine clusters. The cluster distance among genotypes ranged from 11.18 to 1.16. All these genotypes were also profiled for allele specific marker for ahFAD2A and ahFAD2B mutation for high oleic acid trait. The molecular analysis revealed that genotypes Girnar 4, NRCGHFS72, ICGV191020, ICGV191027 and ICGV191035 were found to have both ahFAD2A and ahFAD2B mutation with high oleic acid content.

Keywords: Groundnut, Principal Component Analysis, Cluster analysis, high oleic acid, Allele-specific marker.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) also called peanut, is the "King of Oilseeds", and belongs to the family Leguminosae. It is called as the wonder legume for its flowering, pegging, and pod formation pattern. Cultivated peanut (*Arachis hypogaea*) (AABB, 2n= 4x = 40) is an allotetraploid probably originated from two diploid species viz., *Arachis duranensis* (A genome) and *Arachis ipaensis* (B genome) donor followed by doubling of the chromosome (Husted, 1936; Kochert *et al.,* 1991; Halward *et al.,* 1992; Kochert *et al.,* 1996; Cuc *et al.,* 2008; Moretzsohn *et al.,* 2013)

The world production of groundnut is about 48 million tonnes out of which Asia contributes about 64% of the production (FAOSTAT, 2019). China, India, Nigeria and the United States of America are the leading producers with ~70% contribution to global groundnut production. In India, groundnut is one of the major cash crops with an area of about 4.8 million ha and production of 9.9 million tonnes (INDIASTAT, 2020). In China and India, the majority of groundnut are crushed for oil extraction and the preference is for high oil content. However, it is mostly used for confectionary and other food uses in the USA and European countries (Birthal *et al*., 2010). Groundnut is also nutritionally rich crop with 44 – 56% protein, oil 22 – 30%, vitamins and minerals, hence it is named as poor-man's almond (Mukri *et al.,* 2014). The success of any crop improvement program is to effectively identify and incorporate the genetic diversity from various resources such as the germplasm lines, released varieties, newly developed cultures, landraces, farmer's varieties and mutant lines. Hybridization between diverse lines provides an opportunity for the evolution of improved lines with desirable traits (Swarup *et al.,* 2021). A large number of variables are handled by the plant breeders, some of which do not have sufficient power to discriminate in

the case of the evaluation of germplasm lines. Principal Component Analysis (PCA) is a dimension-reduction technique. The PCA helps to assess the magnitude of variation in the population (Das *et al.,* 2017). Cluster analysis is a useful tool for the determination of genetic relationships among genotypes. High oleic acid content trait is due to the mutation of the wild alleles of ahFAD2A and ahFAD2B. The information on the presence of these mutations among groundnut genotypes helps in the development of high oleic groundnut varieties. With this background, the present study was aimed to assess the diversity among genotypes for pod and kernel traits of 55 groundnut genotypes.

MATERIALS AND METHODS

Fifty five groundnut genotypes from various sources were used for diversity analysis (**Table 1**). These genotypes were raised at the Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam. Eleven pod and kernel traits *viz*., 100-pod weight (g), pod length (cm), pod width (cm), 100-kernel weight (g), kernel length (cm), kernel width (cm), oil content (%), oleic acid content (%), linoleic acid content (%), palmitic and stearic acid content (%) were measured. The data obtained were analyzed with DARwin (ver. 6.0.21) (Perrier and Jacquemoud-Collet, 2006) for cluster analysis and with STAR (ver 2.0.1) (IRRI, 2014) for descriptive statistics and Principal Component Analysis (PCA). The groundnut genotypes were screened with allele-specific markers suggested by Yu *et al.* (2013). Among the four reaction primers proposed by Yu *et al.* (2013), Reaction I, Reaction II, Reaction III, and Reaction IV primers were designed to detect the ah*FAD2A* wild allele (Ol_1), ah*FAD2A* mutant allele (ol_1), ah*FAD2B* wild allele (Ol_2), and ah*FAD2B* mutant allele (ol_2), respectively. The details of the primers are mentioned in **Table 2**. For this assay, the PCR mixture with a total volume of 10 µL includes 0.6 µL of template DNA at a concentration of 10 ng/µL, 0.4 µL of 10 pmol/ µL of each specific forward primers, 0.1 µL of 10 pmol/ µL of the reverse primer, 5 µL of 2X smart Prime PCR Master Mix, and 3.5 µL of sterile water. The amplification of these reaction primers was carried out using a PCR protocol, which involved an initial denaturation step at 94 °C for 1 minute. This was followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 30 seconds, extension at 72 °C for 90 seconds, and a concluding final extension step at 72 °C for 5 minutes. Oil content and fatty acid composition were estimated with Near-Infrared Spectrometer (NIR) (Make: M/s ZEUTEC, Germany; Model: SPA 1.0).

RESULTS AND DISCUSSION

Variability for pod and kernel traits:The descriptive statistical measures such as the mean, standard deviation (SD), coefficient of variation (CV%), and minimum and maximum values are mentioned in **Table 3**. High CV was observed for the linoleic acid content (22.40%). Studies by Shukla and Rai (2014) with 30 groundnut genotypes, showed that the coefficient of variation was high for oleic acid. Moderate CV were observed for 100-pod weight, pod length, 100-kernel weight, kernel length, oleic acid, palmitic and stearic acid content. Studies by Patidar and Nadaf (2017) in the backcross derived high oleic advanced breeding lines showed moderate to high variability for 100-kernel weight. Low CV was observed for pod length, kernel width and oil content. Similar studies by Patidar and Nadaf (2017) in the backcross derived high oleic advanced breeding lines showed that shelling per cent, protein content and oil content showed low variability indicating the narrow range of variability and restricting the scope of selection. These results were also similar to studies by Yusuf *et al.* (2019) as they recorded low CV for oil content.

Mean performance for pod and kernel traits: TMV7 had the minimum value for 100-pod weight and 100-kernel weight. Variety AK 303 had the highest value for 100-pod weight, pod width, 100-kernel weight, kernel length and kernel width. Gangapuri had highest value for pod length and lowest values for 100-kernel weight and kernel width. NRCGHFS 72 had highest value for oleic acid and lowest values for linoleic and palmitic acid content. Among the genotypes, ICGV191035 had the lowest oil content of 42.92 % while genotype VG19817 had the highest oil content of 56.27%. Lowest oleic acid content was recorded in TAG 24 (37.53 %). Highest linoleic acid content and palmitic acid content were recorded in VRI 4 and GJG 33 respectively. The stearic acid was high (4.72 %) in genotype K1812 and lowest (2.58 %) in VRI 8. (**Table 3**)

Principal Component Analysis: The scree plot (**Fig. 1**) displays the eigenvalue associated with each component. The principal component with an eigenvalue more than 1 *i.e.,* PC1 (4.1024), PC2 (2.254), PC3 (1.183), and PC4 (1.080) contributes maximum to the variability. The components with eigenvalue less than 1 *i.e.,* PC5 to PC11 are less informative and account for lesser variance and hence not retained. The results from the PCA indicated that 78.37 % of the total variance is contributed by the first four principal components. The first principal component (PC1) with an eigenvalue of 4.102 explained 37.29 % of the total variability studied in the groundnut genotypes. PC1 gave high positive weight to palmitic acid content (0.3341), linoleic acid (0.3071), stearic acid (0.0953), and oil content (0.0576). PC1 is negatively correlated with 100 pod weight, pod length, pod width, 100 kernel weight, kernel length, kernel width, and oleic acid content. The second principal component (PC2) with an eigenvalue of 2.254 contributed 20.49% of the total variability. PC2 is positively correlated only with oleic acid content (0.4643) and is negatively correlated with all other traits. The third principal component (PC3) with an eigenvalue of 1.183 is responsible for 10.76% of the total variability. Kernel width (0.6270), 100-kernel weight (0.2561), oil content (0.0848), linoleic acid content (0.0709), 100-pod weight (0.0364) and stearic acid content (0.0246) contributed

Table 1. List of genotypes used in this study

S. No.	Genotype	Source	S. No.	Genotype	Source
1	AK303	PDKV, Akola	29	R2001-2	UAS, Dharwad
2	ALR1	Coconut Research Station, Aliyarnagar	30	R2001-3	UAS, Dharwad
3	ALR2	Coconut Research Station, Aliyarnagar	31	RKM	RRS, Vridhachalam
4	CO 2	TNAU, Coimbatore	32	TAG24	BARC, Mumbai
5	CO 6	TNAU, Coimbatore	33	TG86	BARC, Mumbai
6	CO7	TNAU, Coimbatore	34	TMV12	ORS, Tindivanam
7	Gangapuri	JNKVV, Khargone	35	TMV14	ORS, Tindivanam
8	GG-7	JAU, Junagadh	36	TMV7	ORS, Tindivanam
9	GG-16	JAU, Junagadh	37	TMVGn13	ORS, Tindivanam
10	GG-20	JAU, Junagadh	38	TVG17204	ORS, Tindivanam
11	GG-22	JAU, Junagadh	39	VG12	RRS, Vridhachalam
12	Girnar4	DGR, Junagadh	40	VG13110	RRS, Vridhachalam
13	GJG18	JAU, Junagadh	41	VG13113	RRS, Vridhachalam
14	GJG19	JAU, Junagadh	42	VG13127	RRS, Vridhachalam
15	GJG33	JAU, Junagadh	43	VG19809	RRS, Vridhachalam
16	Haridandra	ANGRAU, Hyderabad	44	VG19812	RRS, Vridhachalam
17	ICGV191019	ICRISAT, Hyderabad	45	VG19814	RRS, Vridhachalam
18	ICGV191020	ICRISAT, Hyderabad	46	VG19815	RRS, Vridhachalam
19	ICGV191027	ICRISAT, Hyderabad	47	VG19817	RRS, Vridhachalam
20	ICGV191035	ICRISAT, Hyderabad	48	VG34	RRS, Vridhachalam
21	ICGV00348	TNAU, Coimbatore	49	VRI 2	RRS, Vridhachalam
22	ICGV05182	ICRISAT, Hyderabad	50	VRI 4	RRS, Vridhachalam
23	K1812	ANGRAU, Kadiri	51	VRI(Gn) 6	RRS, Vridhachalam
24	K6	ANGRAU, Kadiri	52	VRI(Gn)7	RRS, Vridhachalam
25	K7	ANGRAU, Kadiri	53	VRI8	RRS, Vridhachalam
26	KDG123	MPKV, Rahuri	54	VRI 9	RRS, Vridhachalam
27	KDG128	MPKV, Rahuri	55	VRI 10	RRS, Vridhachalam
28	NRCGHFS72	NRCG, Junagadh			

Table 2. Primer combinations to identify the mutant type of ahFAD2A and ahFAD2B (Yu *et al.,* 2013)

Primer	Linkage group	Sequence (5' to 3')	Annealing temperature
FAD2A-F		GATTACTGATTATTGACTTGCTTTG	53
FAD2A-G	A09	GTTTTGGGACAAACACTTCTTC	53
FAD2A-A		GTTTTGGGACAAACACTTCTTT	53
FAD2B-F		CAGAACCATTAGCTTTGTAGTAGTG	53
FAD2B-C	B09	AACACTTCGTCGCGGTTG	53
FAD2B-A		AACACTTCGTCGCGGTTT	53
FAD2-R		CTCTGACTATGCATCAGAACTTGT	-

Reaction I: FAD2A-F +FAD2A-G+FAD2-R (557 bp)
Reaction II: FAD2A-F +FAD2A-A+FAD2-R (550 bp)
Reaction III: FAD2B-F +FAD2B-C+FAD2-R (539 bp)
Reaction IV: FAD2B-F +FAD2B-A+FAD2-R (550 bp)
Note: Within parenthesis, product size is given in base pair

Table 3. Descriptive statistics of groundnut genotypes for pod and kernel traits

Trait	Mean	SD	CV (%)	Minimum	Maximum
100-pod weight (g)	106.56	17.02	15.98	TMV 7 (77.06)	AK303 (162.6)
Pod length (cm)	2.63	0.28	10.63	ICGV191027 (1.97)	Gangapuri (3.34)
Pod width (cm)	1.23	0.11	8.80	TMV 7 (1.04)	AK303 (1.54)
100 kernel weight (g)	41.47	7.74	18.66	Gangapuri (25.2)	AK303 (62)
Kernel length (cm)	1.32	0.14	10.71	ICGV191027 (0.99)	AK303 (1.73)
Kernel width (cm)	0.81	0.06	6.97	Gangapuri (0.71)	AK303 (1.00)
Oil content (%)	50.37	3.21	6.38	ICGV191035 (42.92)	VG19817 (56.27)
Oleic acid content (%)	47.50	8.44	17.77	TAG 24 (37.53)	NRCGHFS72 (77.92)
Linoleic acid content (%)	30.49	6.83	22.40	NRCGHFS 72 (6.01)	VRI 4 (38.33)
Palmitic acid content (%)	16.97	2.02	11.91	NRCGHFS 72 (12.16)	GJG 33 (21.1)
Stearic acid content (%)	3.92	0.53	13.61	VRI 8 (2.58)	K1812 (4.72)

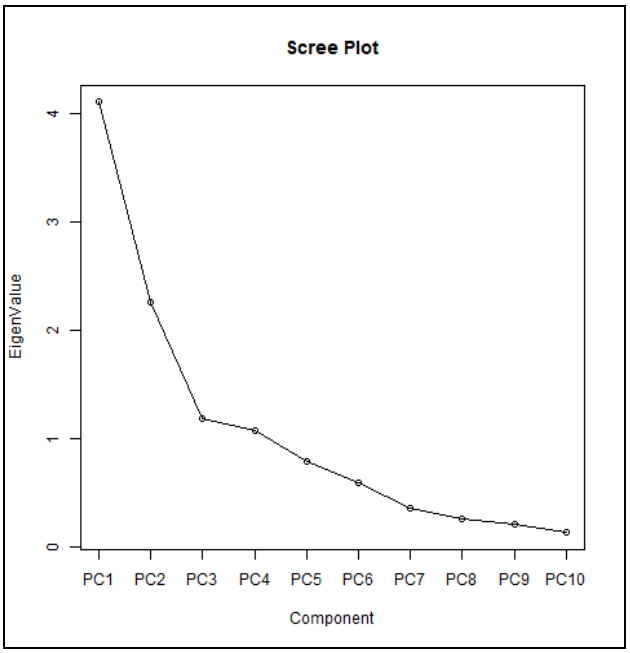


Fig. 1. Scree plot showing Eigen value variation

positively towards PC3. The fourth principal component with an eigenvalue of 1.08 contributed 9.83% of the total variability. The traits linoleic acid content (0.2524), kernel length (0.1281), kernel width (0.0962), and pod width (0.0744) positively contributed to PC4. Traits 100-pod weight, kernel length, and pod width contributed the maximum for the variability in PC1. In PC2, traits such as oleic acid, linoleic acid, and oil content contributed to most of the variability (**Table 4**). Similar PCA study was carried out by Ali *et al.* (2022) in 54 accessions of groundnut for 13 different morphological, yield, and oil quality traits showed that the first five principal components with eigenvalue above one contributed about 71.83% of the total variation. Major character contributions for PC1 were the pod weight per plant, grain weight per plant, and the number of pods per plant. PC2 positively contributed to oleic acid and shelling percentage. The first PC explains the maximum variability in any PCA studies. Studies by Sukrutha *et al.* (2023) showed that first five PCs contributed 73.24

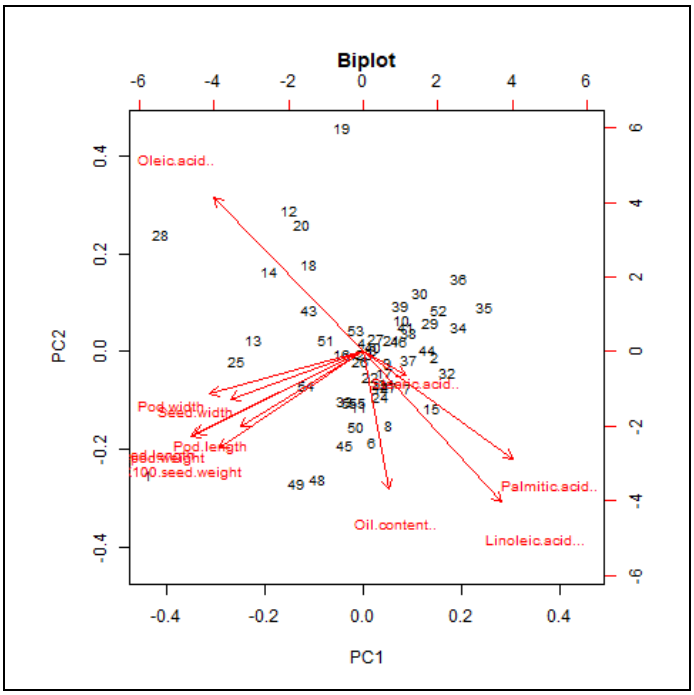


Fig. 2. Distribution of the groundnut genotypes under two major principal component axis

Table 4. Principal Component Analysis (PCA) of four components

	PC1	PC2	PC3	PC4
Eigen value	4.1024	2.2541	1.1833	1.0808
Variability %	37.29	20.49	10.76	9.83
Cumulative %	37.29	57.79	68.54	78.37
Variable		Eigenvector		
100 pod weight (g)	-0.3845	-0.2566	**0.0364**	-0.0926
Pod length (cm)	-0.2731	-0.2269	-0.6401	-0.0403
Pod width (cm)	-0.3415	-0.1286	-0.0062	**0.0744**
100 kernel weight (g)	-0.3218	-0.2898	**0.2561**	-0.1646
Kernel length (cm)	-0.3735	-0.2477	-0.2935	**0.1281**
Kernel width (cm)	-0.2967	-0.1419	**0.6270**	**0.0962**
Oil content (%)	**0.0576**	-0.4138	**0.0848**	-0.1325
Oleic acid content (%)	-0.3339	**0.4643**	-0.0652	-0.1000
Linoleic acid content (%)	**0.3071**	-0.4525	**0.0709**	**0.2524**
Palmitic acid content (%)	**0.3341**	-0.3221	-0.1643	-0.0414
Stearic acid content (%)	**0.0953**	-0.0730	**0.0246**	-0.9157

% variation with eigen value more than 1. In our study, PC1 accounted for a maximum of 37.29% of the total variation than PC2 (20.49%). Similar results were obtained by Khan *et al.* (2021) in which PC1 accounted for 45.88% variability than PC2 (10.68%). Multivariate analysis by Ajay *et al.* (2012) for improving the confectionary traits in groundnut concluded that palmitic acid, oleic acid, linoleic acid, saturated fatty acid, primary branches, and plant height are traits that contribute more to the total variation.

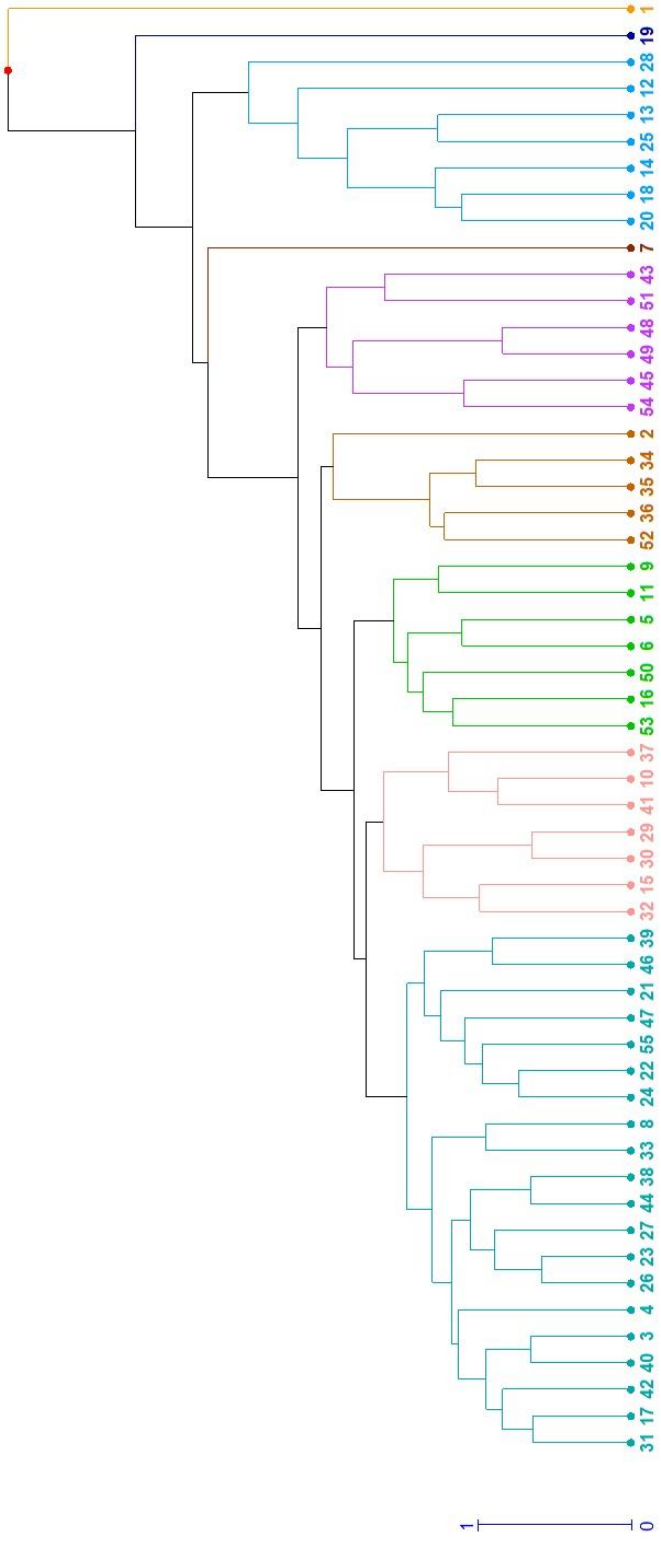


Fig. 3. Dendrogram based on cluster analysis for the quantitative and qualitative traits of the groundnut genotypes

The angle between the vectors shows the correlation of the characteristics with each other. When the two vectors are close, forming a small angle, the variables are positively correlated. The variables pod width, kernel (seed) width, pod length, seed length, 100-pod weight, and 100-kernel weight are positively correlated. Similarly, the variables linoleic acid, palmitic acid, and stearic acid are positively correlated. When the angle between the vectors is at 90°, the variables are not correlated. The traits linoleic acid, palmitic acid, and stearic acid are not correlated with pod width, pod length, kernel width, kernel length, 100-pod weight, and 100- kernel weight. When the vectors are very diverged at a larger angle, thevariables are negatively correlated. The variables palmitic acid, linoleic acid, stearic acid, and oil content are negatively correlated with oleic acid content. Genotype 28 (NRCGHFS72) is highly correlated with the trait oleic acid content.

Cluster Analysis: Cluster analysis was done using DARwin following the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of hierarchical clustering of the groundnut genotypes. The 55 genotypes fall under 9 different clusters (**Table 5**). The ninth cluster is divided into two sub-clusters having 12 and 8 genotypes each. The genotype AK303 and TMV 14 are located at the maximum genetic distance (11.1829) whereas the genotypes K1812 and KDG123 are located at a minimum genetic distance (1.16924). The cluster IX is the largest cluster with 20 genotypes and the cluster I, II and IV are the smallest clusters with one genotype in each cluster. Studies by Khan *et al.* (2021), grouped the 15 groundnut accessions into five major clusters with 27 quantitative and qualitative traits using UPGMA method of clustering.

Screening of Groundnut genotypes for high oleic acid content : Yu *et al.* (2013) suggested an accurate allele-specific PCR assay to identify the mutant allele in ahFAD2A (substitution) and ahFAD2B (insertion) genes. This allele specific PCR (AS-PCR) assay consists of primers under four reaction combinations. The Reaction I, Reaction II, Reaction III, and Reaction IV primers were designed to detect the ah*FAD2A* wild allele (Ol_1), ah*FAD2A* mutant allele (ol_1), ah*FAD2B* wild allele (Ol_2), and ah*FAD2B* mutant allele (ol_2), respectively. Screening of 55 genotypes using R2 (**Fig. 4**) and R4 (**Fig. 5**) reaction primers indicated the presence of mutant alleles in the ahFAD2A and ahFAD2B genes respectively. The internal control band at 1200 bp indicates the success or failure of Polymerase chain reaction. The target band appears at 550 bp. The genotypes Girnar 4, NRCGHFS72, ICGV191020, ICGV191027, and ICGV191035 have both ahFAD2A (substitution) and ahFAD2B (insertion) mutation. (**Table 6**). Phenotypically the genotypes Girnar 4, NRCGHFS72, ICGV191020, ICGV191027, and ICGV191035 showed more than 60 % of oleic acid.

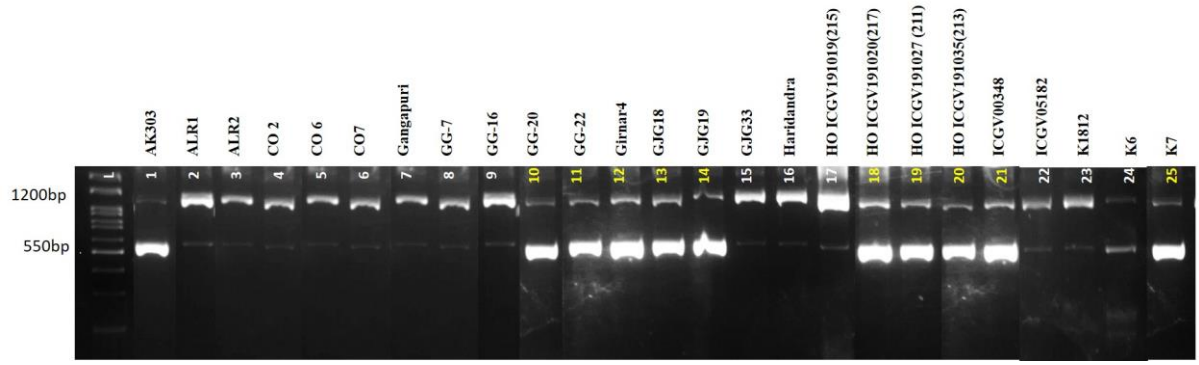
Based on the foregoing discussion, it can be concluded that the variability analysis for pod and kernel traits of 55 genotypes indicated that the trait high CV was observed for linoleic acid content and low CV for pod length, kernel width and oil content. Highest oleic and linoleic was observed in NRCGHFS 72 and GJG 33 respectively. In PCA analysis, the first four principal components explained about 78.37 % of the total variability. Cluster analysis grouped 55 genotypes into nine clusters. All these genotypes were also profiled for allele specific marker for ahFAD2A and ahFAD2B mutation for high oleic

Table 5. Clustering of the genotypes and the size of each cluster

Cluster	Size	% of lines	Genotypes	Prominent trait
I	1	1.81	AK303	Maximum 100 pod weight, 100 kernel weight, pod width, kernel length, kernel width
II	1	1.81	ICGV191027	
III	7	12.7	Girnar4, GJG18, GJG19, ICGV191020, ICGV191035 K7, NRCGHFS72	Mid (>50%) to high (>60%) in oleic acid content
IV	1	1.81	Gangapuri	
V	6	10.9	VG19809, VG19814, VG34, VRI2, VRI (Gn)6, VRI9	
VI	5	9.09	ALR1, TMV12, TMV14, TMV7, VRI (Gn)7	
VII	7	12.7	CO 6, CO7, GG-16, GG-22, Haridandra, VRI4, VRI8	
VIII	7	12.7	GG-20, GJG33, R2001-2, R2001-3, TAG24, TMVGn13, VG13113	
IX	12	21.8	RKM, HO ICGV191019(215), VG13127, VG13110, ALR2, CO 2, KDG123, K1812, KDG128, VG19812, TVG17204, TG86	
	8	14.5	GG-7, K6, ICGV05182, VRI10, VG19817, ICGV00348, VG19815, VG12	

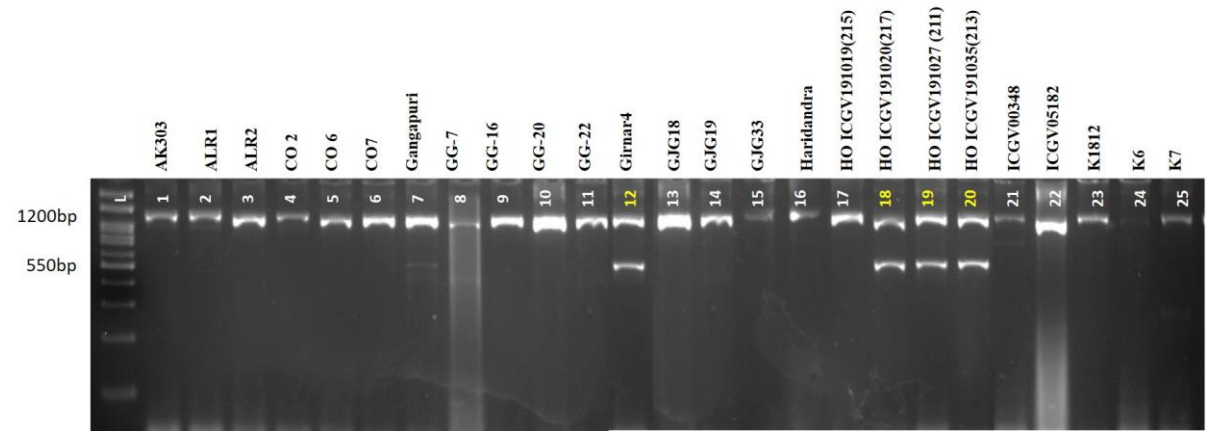
Table 6. Profiling of the genotypes for high oleic acid trait using allele-specific primers (Yu *et al.,* 2013)

Genotypes	Mutant allele of		Genotypes	Mutant allele of	
	ahFAD2A	ahFAD2B		ahFAD2A	ahFAD2B
AK303	+	-	RKM	-	-
ALR1	-	-	TAG24	-	-
ALR2	-	-	TG86	+	-
CO 2	-	-	TMV12	-	-
CO 6	-	-	TMV14	-	-
CO7	-	-	TMV7	-	-
Gangapuri	-	-	TMVGn13	-	-
GG-7	-	-	TVG17204	-	-
GG-16	-	-	VG12	+	-
GG-20	+	-	VG13110	-	-
GG-22	+	-	VG13113	-	-
Girnar4	+	+	VG13127	+	-
GJG18	+	-	VG19809	-	-
GJG19	+	-	VG19812	+	-
GJG33	-	-	VG19814	-	-
Haridandra	-	-	VG19815	-	-
ICGV191019	-	-	VG19817	-	-
ICGV191020	+	+	VG34	-	-
ICGV191027	+	+	VRI2	-	-
ICGV191035	+	+	VRI4	-	-
ICGV00348	+	-	VRI (Gn)6	+	-
ICGV05182	-	-	VRI (Gn)7	-	-
K1812	-	-	VRI8	+	-
K6	-	-	VRI9	-	-
K7	+	-	VRI10	-	-
KDG123	-	-			
KDG128	-	-			
NRCGHFS72	+	+			
R2001-2	+	-			
R2001-3	+	-			



L- 100 bp Ladder

Fig. 4. Screening of groundnut genotypes for ahFAD2A mutant allele



L- 100 bp Ladder

Fig. 5. Screening of groundnut genotypes for ahFAD2B mutant allele

acid trait. The molecular analysis revealed that genotypes Girnar4, NRCGHFS72, ICGV191020, ICGV191027, ICGV191035 were found to have both ahFAD2A and ahFAD2B mutation with high oleic acid content.

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