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GROUNDNUT GENOTYPES' DIVERSITY ASSESSMENT FOR YIELD AND OIL QUALITY TRAITS THROUGH MULTIVARIATE ANALYSIS

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SUMMARY

Genetic variability is essential in plant breeding for crop adaptation in a specific environment, enhancing yield potential, creating resistance to biotic and abiotic stresses, improving quality attributes, and most importantly, selecting desirable and better parents for hybridization programs. The study was designed to ascertain the genotypic diversity of 54 accessions of groundnut (*Arachis hypogaea*). The study evaluated these accessions/genotypes for 13 different traits (morphological, yield, and oil quality) under the rainfed climate of Pakistan. Significant differences were observed for all studied traits. Likewise, significant difference in the percent coefficient of variability (CV%) was also found for these traits. The research included classifying the genotypes further into six different clusters using the Ward method. Principal component analysis was performed that showed variability in components for different traits. The first five principal components (PCs) showed an eigenvalue of more than one that contributed about 71.83% of the total observed variation. Major characters accounted for by PC1 included pod weight per plant, grain weight per plant, and the number of pods per plant. PC2 positively contributed to oleic acid and shelling percentage, PC3 contributed positively to dry pod yield, plant height, and days to flower initiation, and PC4 contributed for days to 50% flowering, the number of pods per plant, and dry pod yield. These data on genotypic diversity for studied traits in the recent investigation will help breed new groundnut lines to strengthen germplasm sources for cultivar development in rainfed areas of Pakistan.

Keywords: *Arachis hypogaea,* cluster analysis, divergence, groundnut, oleic acid, PCA

Key findings: Fifty-four groundnut genotypes were studied for the presence of genetic variability through multivariate statistics, divided into six hierarchical clusters based on 13 observed morphological traits. Results revealed a distinct and high amount of genetic variability in groundnut cultivars that can be utilized in designing future crop improvement programs.

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INTRODUCTION

Peanut or groundnut (*Arachis hypogaea* L.) is one of the important oil crops of the *Kharif* season (Abd-El-Saber *et al.,* 2020). It is widely grown in subtropical and tropical regions of the world (Abbas *et al.,* 2020). It belongs to the genus *Arachis* and the family Leguminosae (Krapovickas and Gregory, 1994). The cultivated type of peanut is a self-pollinated plant having genome size of about 2891 Mbp, which is concentrated on 40 chromosomes exhibiting its tetraploid nature. This genus is divided into nine taxonomic sections based on geographical distribution, cross compatibility, and plant morphology. The genus *Arachis* comprises about 80 species (Valls and Simpson, 2005), which include diploids and tetraploids.

Peanut (groundnut) is a rich source for numerous essential food ingredients like vegetable oil and protein consumed in various ways worldwide (Machado *et al.,* 2017). The peanut grain (kernel) contains about 20%–25% high-quality protein and up to 45% refined quality oil (Silveira *et al.,* 2011). It is an annual leguminous cum oilseed crop cultivated in both summer and winter worldwide (Aninbon *et al.,* 2017). Most peanuts are consumed as raw or roasted snacks (Chibarabada *et al.,* 2017). Ahmed *et al.* (2016) observed that peanut grain contains about 40%–60% oil, 20%–40% protein, and 10%–20% carbohydrates. About 100 g of peanut kernels provide around 567 kcal of energy (USDA nutrient database); it contains lipids (fats) of around 49.66 g, fiber (8.0 g), and water (1.55 g) (Settaluri *et al.,* 2012). It also provides a good source of antioxidants, i.e., p-coumaric acid and resveratrol, vitamin E, and many important vitamin B-complex groups of thiamin, pantothenic acid, vitamin B-6, and foliates, niacin and biologically active polyphenols, flavonoids, and isoflavones. As they are highly nutritious, groundnut and groundnut-based products are promoted as nutritional foods to combat energy, protein, and micronutrient malnutrition.

Peanut skins are rich in phenolic compounds and these polyphenols act as antioxidants (Christman *et al.,* 2018) and its butter is also widely used in cookies improving its nutritional quality (Timbadiya *et al.,* 2017). Oil quality is also a vital attribute along with crop yield as the peanut is being consumed both as raw and as an oilseed. A peanut seed (kernel) contains carbohydrates of about 9.5% to 19.0% and is also a great source of essential minerals (Ca, Mg, P, and K) and vitamins (B, K, and E) (Gulluoglu *et al.,* 2016). Globally, 41% of groundnut production serves food purposes and 49% to extract oil. The oilcake meal after oil extraction is used as industrial raw material and as a protein supplement in livestock feed rations (Janila *et al*., 2016; Rahmah *et al*., 2020). Groundnut is ranked fifth among oilseed crops globally after oil palm, soybean, rapeseed, and sunflower (FAO, 2013).

To have a result-oriented cultivar breeding strategy, one has to explore the diversity among the genotypes under investigation. Genetic variability study is one of the main objectives for scientists to understand population diversity for desired traits to be focused on and incorporated into desired combinations and produce ideal plant type for growers and consumers (Fatimah *et al*., 2018, 2021; Desmae *et al*., 2019; Memon *et al.,* 2021). Diversity profiling in groundnut is mainly performed through evaluation and analysis of plant morphological characteristics, as they are easy to calculate, handle and analyze. At the same time, it is labor intensive and environment dependent. The study objectives aimed to identify genotypes with better quality traits and great potential for economic yield. The research assessed groundnut genotypes based on agro-morphological and quality traits, especially oleic acid, which is one of the essential components of oil quality parameters in premium oil crops.

MATERIALS AND METHODS

Plant materials and experiment location

The experiment was carried out at the Barani Agricultural Research Institute (BARI), Chakwal, Pakistan, during the cropping season of 2019 under rainfed conditions. The location coordinates for the experimental site include 32°55'44.9"N latitude, 72°43' 31.9"E longitude, and 575 m altitude. Fifty-two diverse groundnut genotypes from different sources were tested for agro-morphological and quality traits. These genotypes were planted in augmented design, using two cultivated varieties as local checks (BARI-11 and BARI-16). Blocks of two rows at 4 m long for each genotype with 45 cm row spacing and 15 cm plant spacing and repeating checks after 13 genotypes were prepared. The study performed all recommended crop management practices accordingly. These included seedbed preparation, timely sowing, timely weeding and

Table 1. Rainfall, mean temperature (minimum and maximum), and humidity for groundnut cropping season 2019.

Month	Rainfall (mm) / month	Minimum temp. (°C)	Maximum temp. (°C)	Humidity (%)
Jan	64.6	1.9	15.8	75.4
Feb	61.4	4.8	17.2	82.0
Mar	40.3	8.4	22.6	69.9
Apr	80.8	15.1	31.3	59.7
May	32.5	18.3	35.4	44.6
Jun	7.5	21.9	39.4	41.5
Jul	4.8	24.4	36.1	69.6
Aug	134.1	23.7	34.3	77.0
Sep	113.7	23.0	33.6	77.1
Oct	14.0	15.4	29.9	63.6
Nov	19.0	9.2	22.9	76.5
Dec	7.6	1.1	18.2	81.6

hoeing, and fertilizers application. Table 1 shows data regarding monthly rainfall, mean monthly temperature (minimum and maximum), and humidity.

Data collection

The study collected and recorded data on the following for each of the 54 studied groundnut genotypes: germination percentage (number of seeds germinated after sowing), days to flower initiation (number of days from sowing till 10% of plants started flowering), days to 50% flowering (number of days from sowing till 50% of plants started flowering), plant height (cm) (main stem height), dry pod yield (kg/plot), 20 pod length, pods per plant, pod weight per plant (g), and grain weight per plant (g).

Biochemical analysis

The non-destructive method was performed for fatty acids like oleic acid, stearic acid, and palmitic acid estimation with the help of FTNIR, Bruker, Germany (Raigar *et al.*, 2021).

Statistical analysis

Statistical analyses of the data collected were through univariate statistics, i.e., ANOVA, mean, range, standard deviation, and multivariate statistics, i.e., hierarchical clustering, and principal component analysis. These were performed by SAS-JMPPro16 (SAS Inc., Cary, NC, US).

RESULTS

Simple statistics for quantitative and oil quality traits

Fifty-four groundnut genotypes were tested for morphological yield and some oil quality-related attributes. Simple statistical parameters, i.e., mean, standard deviation and range (minimum to maximum) are given in Table 2. Range in minimum and maximum values showed a great diversity for these studied traits in the germplasm samples.

Cluster and principal component analyses

Cluster analysis

Cluster analysis is one of the important and effective methods to identify and establish the structured relationship between different accessions. It also establishes a hierarchical classification between these accessions/genotypes. The Ward method was used for cluster analysis of 54 groundnut genotypes with diverse backgrounds. These studied genotypes were classified on morphological, seed yield, and some oil quality characteristics, such as, palmitic acid (%), stearic acid (%), and oleic acid (%). Figure 1 illustrates the results in the cluster heat map dendrogram.

The genotypes were grouped in six different clusters (Table 3, Figure 1). These comprised four major clusters (Cluster-I, Cluster-II, Cluster-IV, and Cluster-V) and two

Table 2. Simple statistics of 54 groundnut genotypes for 13 traits.

Characters	N	d.f.	Mean	Std. Dev.	Range
Germination %	54	53.00	84.444	13.818	29.00 -100.00
Days to flower initiation	54	53.00	28.370	0.938	27.00 - 29.00
Days to 50% flowering	54	53.00	46.537	1.881	44.00 - 49.00
Plant height (cm)	54	53.00	36.111	8.011	23.00 - 60.00
20 Pod length (cm)	54	53.00	55.759	8.098	40.00 - 78.00
Pods/plant	54	53.00	33.926	14.625	15.00 - 80.00
Pod weight/plant (g)	54	53.00	32.778	15.738	9.00 - 86.00
Grain weight/plant (g)	54	53.00	19.148	9.874	4.00 - 48.00
Dry pod yield (kg/plot)	54	53.00	152.85	55.525	46.00 - 302.00
Shelling %age	54	53.00	57.726	9.415	28.57 - 72.41
Palmitic acid (%)	54	53.00	8.944	1.393	7.00 - 11.00
Stearic acid (%)	54	53.00	2.989	0.136	2.07 - 3.09
Oleic acid (%)	54	53.00	60.518	4.197	52.00 - 67.00

N = Number of entries/genotypes, d.f. = Degree of freedom, Std. Dev. = Standard deviation

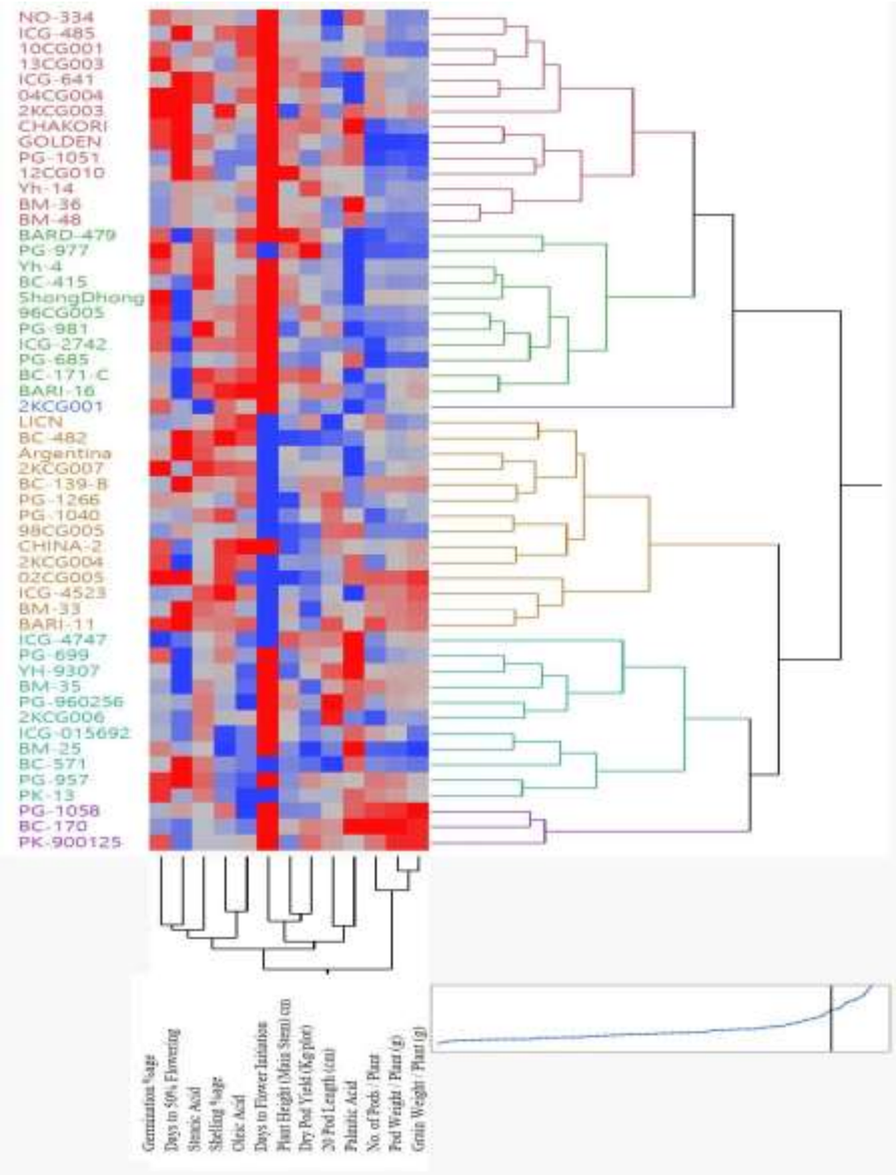

Figure 1. Cluster heat map dendrogram for groundnut genotypes developed by Ward method.

Table 3. Distribution of 54 groundnut genotypes into different clusters using Ward method.

No.	Cluster	Number of genotypes	Genotypes
1	Cluster-I	14	No. 334, ICG-485, 10CG001, 13CG003, ICG-641, 04CG004, 2KCG003, CHAKORI, GOLDEN, PG-1051, 12CG010, YH-14, BM-36 and BM-48
2	Cluster-II	11	BARD-489, PG-977, Yh-4, BC-415, ShongDhong, 96CG005, PG-981, ICG-2742, PG-685, BC-171-C, and BARI-16
3	Cluster-III	1	2KCG001
4	Cluster-IV	14	LICN, BC-482, Argentina, 2KCG007, BC-139-B, PG-1266, PG-1040, 98CG005, CHINA-2, 2KCG004, 02CG005, ICG-4523, BM-33, BARI-11
5	Cluster-V	11	ICG-4747, PG-699, Yh-9307, BM-35, PG-960256, 2KCG006, IC-015692, BM-25, BC-571, PG-957, PK-13
6	Cluster-VI	3	PG-1058, BC-170, PK-900125

minor clusters (Cluster-III and Cluster-VI). Among the six clusters, cluster-I and cluster-IV are major clusters comprising 14 genotypes in each cluster, while cluster-II and cluster-V have 11 genotypes each. Cluster-VI is a comparatively smaller cluster with three genotypes in it. However, cluster-I is the smallest cluster with only one genotype. Genotypes included in one cluster were significantly different from genotypes grouped in another cluster. Observations on genotypes from different backgrounds have been found in the same clusters that indicate genetic drift. Genotype 2KCG001 showed completely different studied features from other genotypes.

Principal component analysis

Principal component analysis (PCA) was performed using 10 morphological and yield traits and three oil quality traits. These studied traits indicated that about 71.83% variability was accounted for by the first five components (PCs) with an eigenvalue ≥1 (Table 4, Figure 2). The first PC (PC1) had an eigenvalue of 3.15 and explained 24.25% of the total variability present in the studied groundnut material. Pod weight per plant, grain weight per plant, and pods per plant had maximum and positive eigenvectors in PC1, while plant height (cm) and oleic acid had negative eigenvectors. The second principal component

Table 4. Eigenvalues for different eigenvectors of studied traits in groundnut genotypes.

No.	Eigenvalue	Percent		Cum Percent	Chi-Square	d.f.	Prob>ChiSq
1	3.15	24.25		24.251	398.573	77.899	<.0001*
2	2.07	15.94		40.195	334.167	70.751	<.0001*
3	1.54	11.82		52.012	295.533	61.830	<.0001*
4	1.33	10.20		62.212	269.283	52.734	<.0001*
5	1.25	9.62		71.834	244.752	44.018	<.0001*
6	0.95	7.29		79.121	214.849	35.873	<.0001*
7	0.74	5.69		84.809	192.271	28.241	<.0001*
8	0.68	5.24		90.046	174.488	21.300	<.0001*
9	0.49	3.77		93.811	151.182	15.297	<.0001*
10	0.42	3.22		97.030	132.966	10.144	<.0001*
11	0.28	2.12		99.149	105.314	5.809	<.0001*
12	0.10	0.80		99.953	65.164	1.961	<.0001*
13	0.01	0.05		100.000	0.000	.	.

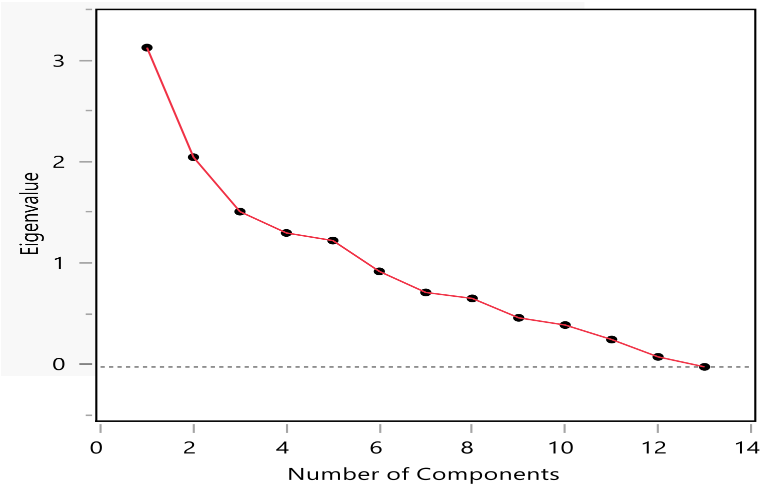

Figure 2. The screen plot shows Eigenvalues in response to respective components for estimated variables of different groundnut genotypes.

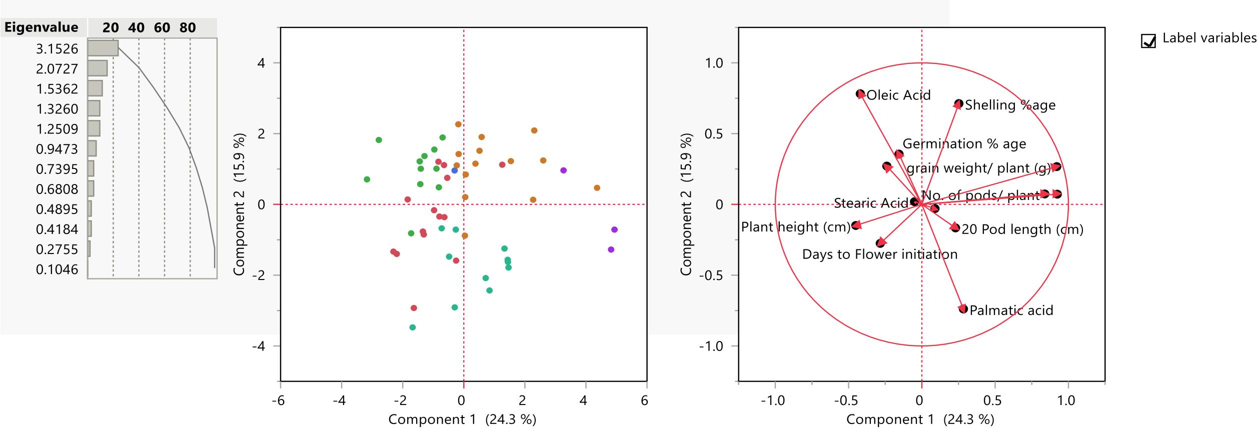

Figure 3. Box plot for principal components 1 and 2 for (a) groundnut genotypes, and (b) various studied traits.

(PC2) explained 15.94% of the total variation with an eigenvalue of 2.07. PC2 was positively correlated with oleic acid and shelling percentage while negatively correlated with palmitic acid. The third PC explained 11.82% variability with an eigenvalue of 1.54. PC3 was positively correlated with dry pod yield, plant height, days to flower initiation, and 20 pod length while negatively correlated with days to 50% flowering. The fourth principal component (PC4), with an eigenvalue of 1.33, explained 10.2% of the total variability. PC4 was positively correlated with days to flower initiation, pods per plant, dry pod yield, and stearic acid, while negatively correlated for 20 pod length. The fifth principal component (PC5), with an eigenvalue of 1.25, explained 9.62% of the total variance. PC5 was positively correlated with days to flower initiation and germination percentage, while negatively correlated with stearic acid.

Box plots of PC1 and PC2 illustrations indicate that most of the studied genotypes scattered on the left side of the figure were positively correlated with the principal component (PC2), as shown in Figure 3a. The maximum of the studied traits is located on the right side of the plot (Figure 3b), indicating that these traits are positively correlated with PC1, while other traits are related to PC2.

DISCUSSION

The genetic diversity was studied by multivariate analysis of 54 genotypes under rainfed conditions. This study explained considerable variation in morphological, yield, and oil quality parameters. The obtained results would be beneficial for selecting elite accessions in groundnut breeding programs as potential parents, as varieties developed eventually lose their potential because of continuous cultivation and genetic erosion, thus leading to decreases in the production capacity of the cultivar (Memon *et al.,* 2021). Considerable variability in germination percentage, days to flower initiation, and days to 50% flowering contributed mainly to genetic diversity due to different responses of genotypes to the available growing conditions. These traits are vital for early maturity duration and yield (Upadhyaya and Nigam, 1994; Yol *et al.,* 2018) and ultimately lead to an early harvest. Ibrar *et al.* (2020) also suggested that grouping patterns observed, based on hierarchical clustering, can be effectively utilized in hybrid/cultivar development. According to past studies, short plant height, minimum days taken to initiate flowering, and accumulation of the maximum number of flowers at an early stage lead to the development of new cultivars with a shorter duration (Nigam and Aruna, 2008; Ahmad et al., 2021). The study observed some genotypes with early flower initiation patterns and short main stem/height from different source populations, which have great potential to breed a variety for early maturity.

Several pods per plant are also a principal attribute that provides an opportunity to improve seed yield potential. The accessions having more pods per plant can improve seed/grain yield in groundnut crops (Nath and Alam, 2002; Awal and Ikeda, 2003; Yol *et al.,* 2018) and ultimately enhance the economic status of the farming community. Similar results explaining a solid correlation between the number of pods per plant and overall seed yield were observed by Swamy *et al.* (2003); Upadhyaya *et al.* (2006), and Manzoor *et al.* (2021). It is a crucial component and one of the main criteria for genotypic selection in groundnut breeding and improvement programs. The study found considerable diversity for the pods per plant. The pods per plant were mainly contributed by PC1, while studies by Makinde and Ariyo (2010) showed that PC4 was a major contributor to this trait. Shelling percentage is also a significant trait indicative of grain/seed ratio over pod (Dapaah *et al.,* 2014). Groundnut breeders generally consider this trait while making germplasm selections in breeding and population development programs (Anothai *et al.,* 2008). In the recent investigation, the shelling percentage positively contributed to PC2 and exhibited considerable variation among genotypes from different source populations. Some studies observed that shelling percentage is mainly affected by genotype × environment interaction (Upadhyaya, 2003; Abd-El-Saber *et al*., 2020).

Seed weight is another essential character to be studied for breeding high-yielding and early maturing groundnut cultivars. The study showed that PC1 positively correlated with and was the major contributor to the number of pods per plant, pod weight per plant, and grain/seed weight per plant. Similar results were concluded by Makinde and Ariyo (2010) and Abd-El-Saber *et al.* (2020). These are primary characteristics to be studied for improvement in the yield of the groundnut crop. The evaluation of studied accessions by PC analysis would aids in genotype selection. Seed yield improvement is an important challenge for plant scientists. The study noted a high variation in seed yield per plant. The principal component analysis demonstrated that pods per plant and grain weight are positively correlated, having a high value in PC1. Similar findings were obtained by previous studies (Kumar *et al.,* 2010; Yol *et al.,* 2018), in which they observed wide variability between collected accessions of groundnut for seed yield. The selection of genotypes for seed yield traits from different sources and taxonomic groups is imperative for groundnut improvement and breeding.

Different fatty acid proportion in groundnut oil determines the oil quality (Mondal *et al.,* 2011), as some monounsaturated fatty acids are essential to health. The study found a great diversity for oleic acid percentage. Mondal *et al.* (2011) also observed variability for different fatty acid contents in studied groundnut mutants. Studies by Konuskan *et al.* (2019) showed that peanut oil has a high percentage of oleic acid that is important and an acceptable level of these acids for consumption. The oleic acid percentage was observed from 45.2% to 53.8%. The study recorded the oleic acid percentage as 52% to 67%. These results were also supported by the studies of Hassan and Ahmed (2012) and Mora-Escobedo *et al.* (2015).

CONCLUSIONS

This study provides comprehensive information on morphological, yield, and yield-related components, as well as, some oil quality characteristics of groundnut. This kind of study can help future breeding and population improvement programs. Genotypes from different backgrounds and clusters showed better performance for some vital and desirable traits for population development and yield improvement of the groundnut crop. Characteristics like pod yield, grain yield, number of pods per plant, and oleic acid percentage are relevant to producers' and consumers' interests.

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