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Research Article
Phenotypic Assessment of Genetic Diversity among Twenty Groundnut Genotypes under Well-watered and Water-stressed Conditions Using Multivariate Analysis

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Abstract

Background and Objective: Assessment of the genetic diversity in the available germplasm has high importance for its effective utilization in breeding programs. The aims of the present investigation were to determine the extent of genetic diversity in 20 groundnut genotypes, under well-watered and water-stressed conditions and assess interrelationships between pod yield and yield-related traits under both environments. **Materials and Method:** Two experiments were conducted in two seasons; the first under well-watered and the 2nd under water-stressed conditions. A randomized complete block design with three replications was used. Principle Component Analysis (PCA) and GT-Biplot technique were used. **Results:** Groundnut genotypes recorded significant differences ($p \leq 0.01$) for all studied characteristics under each environment. The promising genotype(s) for each trait were identified. Results of GT biplot indicated that the traits, pods/plant, pod yield/plant seeds/plant and seed yield/plant were strongly correlated with pod yield/ha, had high estimates of heritability and genetic advance from selection and thus could be considered as secondary traits for high pod yield either under well-watered or water-stressed conditions. The clustering analysis assigned the 20 groundnut genotypes into four groups. The highest genetic dissimilarity Euclidean coefficients were recorded between IL34 and each of G13, IL8, IL41 and IL13; they are the most unrelated genotypes, but the lowest dissimilarity was between IL 18 and IL 35, they are the most related genotypes. **Conclusion:** The identified promising genotypes and secondary traits could be offered to groundnut breeders for use in future breeding programs to improve drought tolerance.

Key words: Peanut genotypes, correlations, heritability, PCA, GT-biplot, cluster analysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) or peanut is an allotetraploid (2n = 4x = 40). It is used for human consumption and oil production as it has a valuable commercial oil 40-60%, in addition to high protein content (16-28%), it is considered as the most popular oilseed in the world, following soybean, cotton and canola[1].

Groundnut is grown in Egypt, in the sandy soils of low water holding capacity, which imposes negative effects on its productivity. Moreover, the predicted future shortage of irrigation water will cause severe negative effects on all crops in Egypt, including groundnut. The level of damage caused by water stress is determined by plant growth stage, intensity and duration of the stress[2]. Water deficit during owering and seed development has a severe effect on the pod and seed yield as compared with other growth stages[3-5]. Therefore, peanut tolerance to drought is a very desired trait in breeding programs[6,7]. The main objective of groundnut breeders is to enhance seed yield or oil yield, under drought stress as well as to improve quality of end-use products.

For starting a breeding program for improving drought tolerance of groundnut, available germplasm should be evaluated for genetic diversity under drought stress conditions to identify the best genotypes and the best traits that could be used directly or indirectly for developing high yielding varieties under drought. Studies of genetic diversity on groundnut are well reported by several investigators, therefore providing a rationale on the importance of such studies[8,9]. Dao *et al.*[10] reported that genetic diversity in different genotypes strengthens the adaptability to a range of environments. Genetic advance in traits of economic importance in breeding programs is highly dependent on the extent of genetic variability of the breeding material[11-13]. Thus, selection of the improved breeding population depends on the extent of available genetic diversity[14].

The first step for the description, assessment and classification of germplasm collections to strengthen their use in peanut breeding is phenotypic characterization[15]. Morphological descriptors are easy to register, not expensive and are dependable for estimating heritability[12-13,16-18]. The phenotypic assessment has proven efficient for diversity analysis in grain legume and oil crops including groundnut[4,14,19-21].

Multivariate analysis is the most popular method for estimating genetic variability to study the components of variation and their genetic relationships between germplasm collections[12-13,22-23]. The principal component analyses are preferred for phenotypic characterization of collected germplasm and their clustering on similarity basis based on this method[24]. Multivariate analyses have been used in many studies on peanut[4,14,21,25].

Although investigators studied several traits in different environments, they usually faced problems in evaluations of these traits. Genotype main effect plus genotype × environment interaction (GGE) biplot model is one of the best methods for dependable assessments in experiments of multi-environment[26]. Assessments are generally performed across PC1 and PC2 axes calculated from the data of columns and rows of a two dimensional array generated by the combination between genotypes and environments in datasets of multiple environments[2]. The method is also utilized for visual estimations of correlations among studied traits through Genotype × Trait (GT)-biplot graph[27]. Knowledge of the interrelationships between yield and its attributes is desirable for suggesting appropriate breeding procedures, especially under stressed conditions. Pod yield of groundnut, a quantitative trait, is affected by its various related traits directly and indirectly through other traits, which bring about a complex situation before a breeder for practicing selection[28]. As it is known, polygenic traits are significantly influenced by the environment, so several researchers[29-30] thought of practicing selection of some secondary traits which are strongly correlated with the yield but are not affected by the environment and have high heritability.

Selection effectiveness is dependent upon the extent of genetic variability present in the available germplasm for the trait of interest and its heritability value[31]. It also depends on the direction and magnitude of interrelationships among the traits to be improved[32]. The objectives of the present study were to determine the extent of genetic diversity in 20 groundnut genotypes, under well-watered and water-stressed conditions, using morphological data based on PCA and assess interrelationships between pod yield and yield-related traits under both conditions using GT-biplot analysis, to identify the secondary traits for selection for improved yield under such conditions.

MATERIALS AND METHODS

Plant materials: Twenty groundnuts (*Arachis hypogaea* L.) genotypes were used in this study. Six of them (IL25, IL27r, IL28, IL26, IL27 and IL18) originated from Israel, four (IL35, IL34, IL41 and IL43) from China, three (IL9, IL10 and IL8) from Malawi, three (IL3, IL4 and IL6) from Brazil, one (IL50) from Mexico, one (IL13) from Zambia, one (G13) from Egypt and one (NC) from USA.

Experimental site: This study was carried out at Toshka Station of Desert Research Center, Toshka (Latitude of 22.47047°N, the longitude of 31.53953° E and an elevation of 201 m above sea level), Aswan Governorate, Egypt, in 2017 and 2018 seasons.

Water regimes: Irrigation was applied by drip irrigation system. Field evaluation experiments were carried out using two water regimes; namely normal (well-watered) conditions (100% of field capacity; i.e. 8330 m^3 ha^{-1}) and the drought conditions (67% of field capacity; i.e. 5580 m^3 ha^{-1}).

Experimental design: The experimental design was a randomized complete block design with three replications. Each experiment contained 20 groundnut genotypes. Each genotype was allotted to two rows plot of 10 m long and 60 cm apart with 30 cm between hills (one plant per hill).

Agricultural practices: Planting was done in the two summer seasons at 16th and 19th of April in 2017 and 2018, respectively. All other cultural practices were done according to the standard recommendations for sowing peanut at the Toshka region.

Soil analysis: Physical and chemical soil analyses of the field experiments were performed at laboratories of Soil and Water Research Institute of ARC, Egypt. The soil type is sandy. As an average of the two seasons, the soil consists of 92.48% sand, 2.24% silt, 5.28% clay and 0.27% organic matter. The pH was 7.61 and EC was 2.95 dS m^{-1}.

Meteorological data: The required weather data for the experimental site through the two growing seasons were obtained from Central Lab for Agricultural Climate, Agricultural Research Center at Giza, Governorate, Egypt. The average temperature in April, May, June, July, August and September was 27.89, 32.02, 32.93, 33.80, 35.11 and 34.77°C in 2017 season and 26.86, 33.62, 34.42, 33.74, 34.52 and 33.79°C in 2018 season.

Data recorded: After maturity, a random sample of ten plants from each unit was taken to determine pod yield/plant, seed yield/plant, number of pods/plant, number of seeds/plant, number of seeds/pod, shelling percentage and 100 seed weight. To determine seed yield or pod yield/unit area, each experimental unit was harvested and weighted and converted to ton ha^{-1}. Water Use Efficiency (WUE) in g seed/1 m^3 irrigation water was calculated by the following formula (http://eagri.org/eagri50/AGRO103/lec09.pdf):

WUE = (Seed yield ha^{-1} in kg) / (quantity of irrigation water ha^{-1} in m^3 given during the whole season)

Biometrical analysis: Data were analyzed by SAS software package. A separate analysis of variance of Randomized Complete Block Design (RCBD) was carried out for each water stress level and each year. A combined analysis of variance of RCBD across the two years for each water stress level and across the two levels was also performed if the homogeneity test was non-significant. LSD values were calculated to test the significance of differences between means according to Steel *et al.*[33].

Morphological evaluations: The best use of the information contained in the data for morphological characterization is an important issue in plant breeding. To display the genetic variability among groundnut genotypes, a Genotype×Trait biplot (GT biplot) of standardized data was applied for each of the two environments drought stressed environment (WS) and well watering (WW) as well as combined analysis (Com.). To generate a GT biplot[34], the genotype by trait two-way table of data was first trait-standardized. The standardization is necessary to remove the units, because different traits use different units. The trait-standardized table (data standardized) was then decomposed into Principal Components (PC). The first two PC's (PC1 and PC2) were used to generate a GT biplot. PC1 and PC2 were scaled so that values are symmetrically distributed between the genotype scores and trait scores. A genotype by trait biplot is constructed by plotting the PC1 scores against the PC2 scores for each genotype and each trait. The biplot technique provides a powerful tool for data analysis of genotype×trait data in individual environments and can be used to visualize the genetic correlations among traits and evaluation of the genotype on the basis of multiple traits[35]. The GT biplot software XLSTAT Addinsoft, 2014[36] was used for all calculations.

RESULTS

Phenotypic identification and variation: Analysis of variance of RCBD across the two water environments (well-watered and water-stressed) and across two years indicated that mean squares due to genotype (G), were significant ($p \leq 0.01$) for all studied traits, suggesting significant differences among the 20 groundnut genotypes for all studied 10 traits (Table 1). Coefficient of Variation (CV) was generally very low ($\leq$5%) for all studied traits under both and across environments. The coefficient of determination (R^2) for all traits under both and across environments was very high (0.88-1.00).

Table 1: Summary statistics across two seasons for 10 phenotypic attributes of 20 peanut genotypes evaluated in the field under well-watered, water-stressed conditions and combined across them

Traits	Minimum	Maximum	Mean	LSD_{05} (IL)	MS (IL)	R^2	CV (%)	PCV (%)	GCV (%)	H^2 (%)	GA (%)
Well-watered											
Pods/plant	37.25 (IL34)	103.25 (IL8)	65.86	3.29	**	0.99	4.35	38.5	33.3	74.7	586.8
Seeds/plant	54.78 (IL34)	149.13 (IL13)	90.83	3.96	**	0.99	3.79	34.7	28.9	69.3	509.3
Seeds/pod	1.14 (IL8)	1.52 (IL28)	1.39	0.08	**	0.81	4.84	8.2	7.2	76.9	126.8
100-Seed weight(g)	48.92 (IL27r)	78.50 (IL34)	64	1.7	**	0.98	2.3	17.0	16.4	92.8	289
Pod yield/plant (g)	66.5 (IL25)	170.88 (IL8)	106.97	4.39	**	0.99	3.57	40.5	35.0	74.7	617
Seed yield /plant (g)	31.18 (IL50)	90.43 (IL13)	58.17	2.45	**	0.99	3.66	40.2	33.5	69.5	590.6
Shelling (%)	44.54 (IL50)	64.81 (IL25)	54.63	1.93	**	0.93	3.08	10.8	9.4	76.1	166.6
Pod yield/ ha(ton)	3.70 (IL25)	9.45 (IL8)	5.93	0.24	**	0.99	3.55	40.1	35.2	77.1	620.4
Seed yield/ha(ton)	1.73 (IL50)	4.96 (G13)	3.23	0.14	**	0.99	3.65	39.7	33.7	71.8	593.4
Water use efficiency (g m^{-3})	208.47 (IL50)	601.21 (IL13)	376.23	16.25	**	0.99	3.65	40.90	34.70	72.0	611.0
Water stressed											
Pods/plant	27.25 (IL34)	68.00 (IL8)	44.94	2.24	**	0.99	4.33	32.4	27.7	73.1	488.3
Seeds/plant	40.13 (IL34)	110.13 (IL13)	69.63	3.71	**	0.99	4.64	34.0	29.0	72.9	511.3
Seeds/pod	1.32 (IL8)	1.72 (IL13)	1.54	0.09	**	0.86	4.86	8.5	7.9	85.7	139.9
100-Seed weight(g)	38.77 (IL50)	64.26 (IL34)	50.65	1.77	**	0.96	3.04	14.3	13.8	93.8	244.2
Pod yield/plant (g)	41.00 (IL25)	107.13 (IL13)	68.05	3.24	**	0.99	4.14	37.6	32.6	75.1	573.6
Seed yield/plant (g)	21.08 (IL50)	53.10 (IL13)	34.98	1.73	**	0.99	4.3	33.9	28.4	70.1	499.7
Shelling (%)	43.03 (IL50)	63.06 (IL25)	52.1	2.71	**	0.90	4.53	13.1	12.3	88.1	217.2
Pod yield/ ha(ton)	2.28 (IL25)	5.93 (IL13)	3.77	0.18	**	0.99	4.15	37.2	32.7	77.4	576.5
Seed yield/ha(ton)	1.17 (IL50)	2.94 (IL13)	1.94	0.1	**	0.99	4.35	33.5	28.4	72.1	501.1
Water use efficiency (g m^{-3})	209.7 (IL50)	526.5 (IL13)	347.5	17.37	**	0.99	4.35	33.6	28.6	72.4	502.0
Combined											
Pods/plant	32.25 (IL34)	85.63 (IL8)	55.4	1.75	**	0.99	4.42	6.4	5.9	84.4	104.2
Seeds/plant	47.45 (IL34)	129.63 (IL13)	80.23	2.05	**	0.99	4.16	4.3	4.2	95.8	74.7
Seeds/pod	1.24 (IL8)	1.61 (IL28)	1.46	0.02	**	0.88	4.86	174.3	148.9	73.0	2621
100-Seed weight(g)	43.91 (IL34)	71.38 (IL50)	57.32	0.78	**	0.99	2.63	6.2	5.6	81.1	98.5
Pod yield/plant (g)	53.75 (IL25)	137.75 (IL13)	87.51	2.46	**	1.00	3.83	3.9	3.6	86.7	64.4
Seed yield /plant (g)	26.13 (IL50)	71.76 (IL13)	46.58	0.9	**	1.00	3.95	6.9	6.1	79.2	108.7
Shelling (%)	43.89 (IL50)	63.92 (IL25)	53.36	0.89	**	0.91	3.84	7.7	6.9	80.0	122.2
Pod yield/ ha(ton)	2.99 (IL25)	7.69 (IL13)	4.85	0.13	**	0.99	3.82	72.8	67.8	86.7	1193
Seed yield/ha(ton)	1.45 (IL50)	3.97 (IL13)	2.58	0.05	**	0.99	3.97	128.2	114.2	79.31	2010
Water use efficiency (g m^{-3})	209.1 (IL50)	563.89 (IL13)	367.35	11.8	**	0.99	3.98	48.8	47.2	93.6	830

Values are followed by line (IL) No. in parenthesis, MS: Mean squares from ANOVA, CV: Coefficient of variation, **Indicate significance at 0.01 probability level, PCV: Phenotypic Coefficient of Variation, GCV: Genotypic Coefficient of Variation, H^2: Heritability GA: Genetic advance

Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) were the highest for Water Use Efficiency (WUE) and Pod Yield/Plant (PYPP) under normal conditions, PYPP and Pod Yield/Ha (PYPH) under water-stressed conditions and Seeds/Pod (SPPod) followed by Seed Yield/Ha (SYPH) for combined data across the two irrigation regimes. On the contrary, the lowest PCV and GCV were recorded by the number of Seeds/Pod (SPP) under normal and drought conditions and PYPP for combined data across the two regimes (Table 1).

Hundred seed weight (100-SW) had the highest heritability estimate (92.8 and 93.8%) under well-watered and water-stressed conditions respectively. Across the two irrigation regimes, number of seeds/ plant (SPP) followed by water use efficiency had the highest heritability estimate (95.8 and 93.6%), respectively (Table 1). All other traits under both and across irrigation regimes had moderate estimates of heritability, ranging from 69.3-86.7%. Expected genetic advance from selection (GA) as a percent of the mean was generally very high (>100 %) for all studied traits under both and across irrigation regimes, except for the number of seeds/plant (74.7%), 100-seed weight (98.5%) and seed yield/plant (108.7 %) for combined data across the two irrigation regimes.

Mean pod yield/ha (PYPH) ranged from 3.7 to 9.45 ton with an average of 5.93 ton under well-watered environment, from 2.28 to 5.93 ton with an average of 3.77 ton under water-stressed environment and from 2.99 to 7.69 ton with an average of 4.85 ton for combined data across the two environments (Table 1). The line IL 8 had the highest PYPH (9.45 ton), the highest pods/plant (PPP) (103.25) and the highest PYPP (170.88 g) under well-watered environment. The line IL13 occupied second place in PYPH, PPP, PYPP and the first place in seeds/plant, seed yield/plant, seed yield/ha and WUE under well-watered environment. The line IL 13 had the highest PYPH (5.93 and 7.63 ton) under water-stressed and combined across the two irrigation regimes, respectively, the highest SPP, seeds/pod, PYPP, SYPP, SYPH and WUE under

drought conditions and the highest SPP, PYPP, SYPP, PYPH, SYPH and WUE for combined data across the two irrigation regimes. The line IL8 occupied the first place in pods/plant (68.0 and 85.63), under water-stressed and combined across the two irrigation regimes, respectively. The line IL25 had the highest shelling percentage (64.81, 63.06 and 63.92%) under well watered, water-stressed and combined across the two irrigation regimes, respectively.

It is observed that on average, the mean pod yield/ha was reduced from 5.93 ton under well-watered to 3.77 ton under water-stressed environment with a reduction of 36.42% due to moderately water-stressed conditions.

Mean number of pods/plant ranged between 37.25 and 103.25 with an average of 65.86 for well-watered environment, from 27.25 to 68.00 with an average of 44.94 for water-stressed environment and from 32.25 to 85.63 with an average of 55.4 for combined data across the two irrigation systems. The highest number of pods/plant was achieved by the line IL 18 under normal conditions and IL 8 under drought and combined across the two irrigation regimes.

Hundred seed weight (100-SW) ranged between 48.92 g (IL35) and 78.50g (IL 41) with an average of 64.0g for well-watered environment, from 38.77g (IL50) to 64.26g (IL 34) with an average of 50.65g for drought environment and from 43.91g (IL34) to 71.38g (IL 50) with an average of 57.32g for combined data across the two irrigation regimes.

Water Use Efficiency (WUE) ranged between 208.47 g m^{-3} (IL 50) and 601.21 g m^{-3} (IL 13) with an average of 376.23 g m^{-3} for well-watered environment. From 209.7 g m^{-3} (IL 25) to 526.5 g m^{-3} (IL 13) with an average of 347.5 g m^{-3} for drought environment. And from 209.1 g m^{-3} (IL25) to 563.89 g m^{-3} (IL 13) with an average of 367.35 g m^{-3} for combined across the two irrigation regimes.

Principal component analysis: To exhibit the genetic variability among groundnut lines under the two environments (well-watered, water-stressed) and combined across them, a principal component analysis was applied (Table 2). The first two principal components, PC1 (F1) and PC2 (F2) were scaled for symmetrically distribution of values among the scores of genotypes and traits. A genotype × trait biplot (GT-biplot) is drawn by plotting the PC_1 scores for each of the 20 genotypes and each of the 10 traits. The GT-biplot efficiently reveals the correlations among peanut characteristics (Fig. 1, 2 and 3). GTbiplot is used also as a tool for comparison among genotypes usually based on multiple characteristics. The GT-biplot results, explained 85.21, 81.52 and 83.27% of the total variation for well-watered, water-stressed regimes and combined across the two irrigation regimes, respectively (Table 2). Results are a close approximation of the total variation of the standardized data.

Morphological traits, involving principal component analyses combined across well watered and water-stressed environments, are also usually utilized in genetic diversity assessment. Principal component analyses of morphological traits (Table 2) found that the first principal component, which explained 68.89% of the total variability among genotypes,

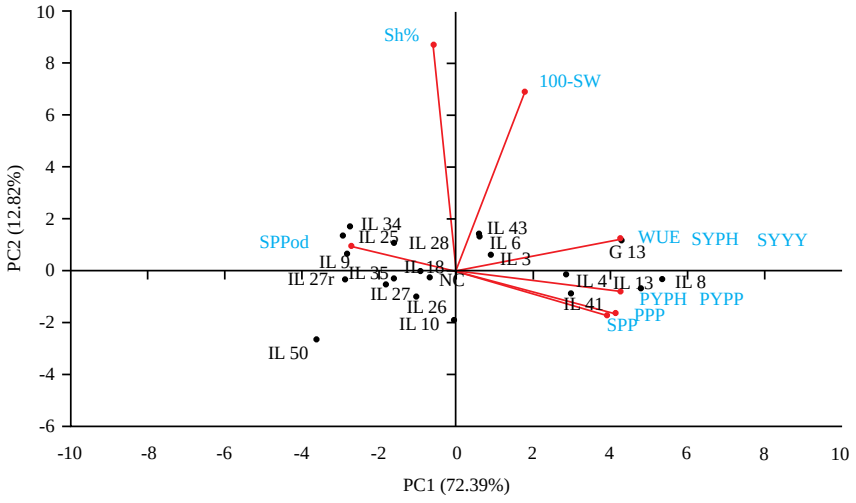

Fig. 1: GT-biplot showing the relationship between PC1 and PC2 for 20 groundnut lines and 10 traits under well-watered conditions

Table 2: Principal component analysis (PCA) for all data across two seasons, under normal and drought conditions and combined across them

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Normal							
Pods/plant	0.36	-0.14	0.15	-0.24	-0.48	-0.16	-0.72
Seeds/plant	0.34	-0.15	0.35	-0.03	-0.37	0.67	0.38
Seeds/pod	-0.23	0.08	0.61	0.72	-0.08	-0.11	-0.16
100-Seed wt (g)	0.15	0.59	-0.53	0.40	-0.35	0.21	-0.10
Pod yield/plant (g)	0.37	-0.07	-0.02	0.14	-0.13	-0.45	0.32
Seed yield/plant (g)	0.37	0.10	0.08	0.07	0.39	0.08	-0.14
Shelling (%)	-0.05	0.75	0.42	-0.46	-0.07	-0.17	0.15
Pod yield ha^{-1}	0.37	-0.07	-0.02	0.14	-0.14	-0.45	0.36
Seed yield/ha(ton)	0.37	0.10	0.08	0.07	0.39	0.09	-0.12
WUE	0.37	0.10	0.08	0.07	0.39	0.09	-0.12
Eigenvalue	7.24	1.28	1.13	0.34	0.01	0.00	0.00
Variability (%)	72.39	12.82	11.27	3.40	0.07	0.04	0.02
Cumulative (%)	72.39	85.21	96.48	99.87	99.95	99.98	100.00
Drought							
Pods/plant	0.36	-0.03	-0.05	-0.38	-0.31	-0.53	-0.58
Seeds/plant	0.37	-0.23	0.01	-0.13	-0.42	-0.22	0.75
Seeds/pod	0.08	-0.64	0.15	0.70	-0.06	-0.18	-0.20
100-Seed wt (g)	0.01	0.68	0.45	0.44	-0.26	-0.29	0.04
Pod yield/plant (g)	0.38	0.11	-0.12	0.10	0.52	-0.23	0.10
Seed yield/plant (g)	0.38	0.03	0.16	0.01	-0.06	0.37	-0.08
Shelling (%)	-0.12	-0.23	0.82	-0.37	0.33	-0.13	0.06
Pod yield ha^{-1}	0.38	0.11	-0.12	0.10	0.51	-0.21	0.08
Seed yield/ha (ton)	0.38	0.03	0.16	0.01	-0.07	0.39	-0.11
WUE	0.38	0.03	0.16	0.01	-0.07	0.39	-0.11
Eigenvalue	6.74	1.41	1.09	0.74	0.01	0.01	0.00
Variability (%)	67.45	14.08	10.92	7.37	0.11	0.06	0.02
Cumulative (%)	67.45	81.52	92.44	99.81	99.92	99.97	100.0
Combined							
Pods/plant	0.37	0.11	-0.07	-0.29	-0.47	0.25	0.69
Seeds/plant	0.35	0.30	-0.02	-0.11	-0.17	0.60	-0.61
Seeds/pod	-0.09	0.67	0.19	0.68	-0.11	0.00	0.17
100-Seed wt (g)	0.10	-0.64	0.43	0.49	-0.25	0.30	0.02
Pod yield/plant (g)	0.38	-0.03	-0.08	0.10	-0.29	-0.46	-0.18
Seed yield/plant (g)	0.38	0.01	0.13	0.03	0.40	-0.03	0.15
Shelling (%)	-0.10	0.18	0.84	-0.42	-0.14	-0.21	-0.09
Pod yield ha^{-1}	0.38	-0.03	-0.08	0.10	-0.29	-0.47	-0.21
Seed yield/ha (ton)	0.38	0.01	0.13	0.03	0.41	-0.02	0.13
WUE	0.38	0.02	0.13	0.04	0.40	0.04	0.02
Eigenvalue	6.89	1.44	1.12	0.54	0.01	0.01	0.00
Variability (%)	68.89	14.37	11.15	5.44	0.06	0.05	0.02
Cumulative (%)	68.89	83.27	94.42	99.86	99.93	99.98	100.0

contrasted seeds/pod, shelling percentage and 100- seed weight with the pod yield/plant, seed yield/plant, WUE, pod yield/plant, seed yield/plant, pods/plant and seeds/plant. The result implies that genotypes characterized by lower seeds/pod, shelling percentage and 100- seed weight were higher in pod yield/plant, seed yield/plant, WUE, pod yield/plant, seed yield/plant, pods/plant and seeds/plant. Moreover, the 1st principal component indicated the joint importance of in discriminating peanut genotypes. The 2nd principal component explained 14.37% of the total variability and suggested the joint importance of seeds/pod, 100-seed weight and seeds/plant, in the discrimination of peanut genotypes (Table 2).

Biplot in the PCA characterizes variables that are super imposed on a plot as vectors where relative length of vectors characterizes the relative proportion of variability in each variable characterized on biplot. Based on PC1 and PC2, shelling percentage followed by 100-seed weight under well-watered conditions, seeds/pod followed by 100-seed weight under water-stressed environment and for combined data across the two environments had relatively long vectors, indicating that there was relatively large variation among genotypes. In other words, they show large variation among the 20 studied genotypes, indicating that they are the most discriminator of the phenotypic data under the respective environments. PYPH, SYPH, WUE, PYPP, SYPP, SPP and PPP

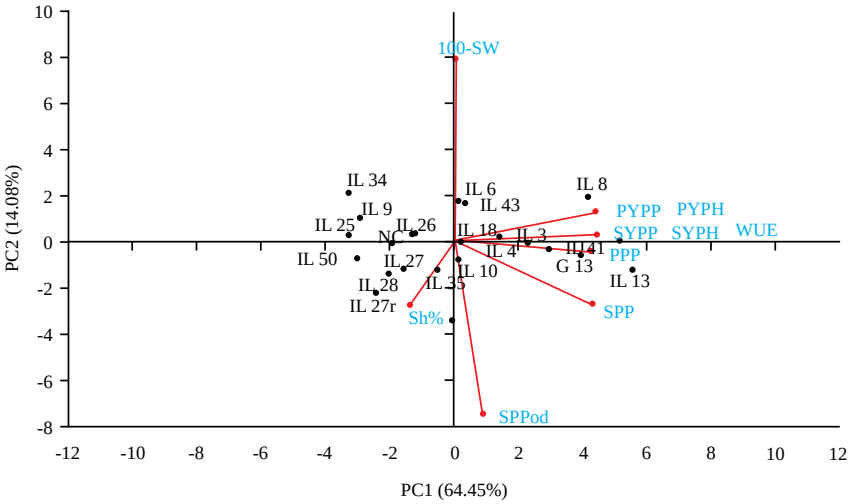

Fig. 2: GT-biplot showing the relationship between PC1 and PC2 for 20 groundnut lines and 10 traits under water-stressed conditions

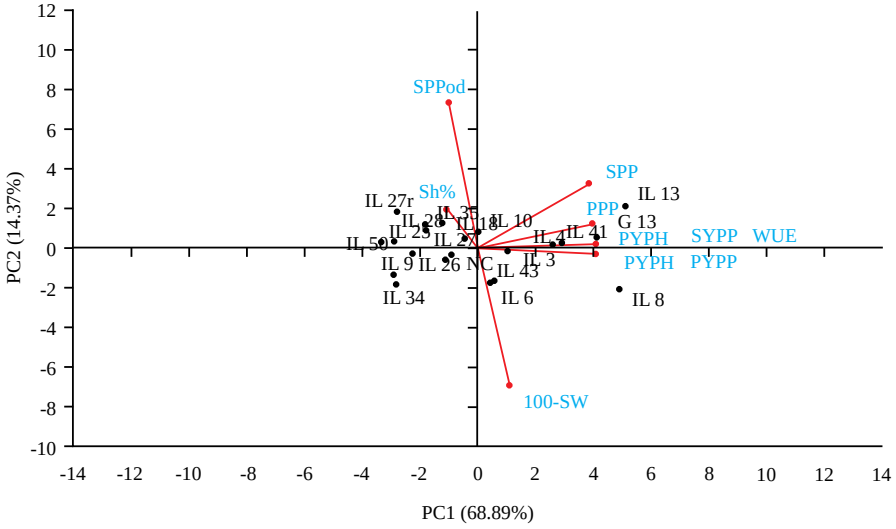

Fig. 3: GT-biplot showing the relationship between PC1 and PC2 for 20 groundnut lines and 10 traits combined across well watered and water-stressed conditions

under well-watered, water-stressed and combined across the two irrigation regimes based on PC1 only, 100-SW and shelling percentage under well-watered, 100-SW under water-stressed and SPP and SPPod across the two irrigation regimes, based on PC2 only are the most discriminator of the phenotypic studied traits. On the contrary, seeds/pod under well-watered, shelling percentage and pods/plant under water-stressed and combined across the two irrigation regimes were the least discriminators, based on both PC1 and PC2 (Table 2 and Fig. 1 to 3).

The cosine of the angle between the vectors of two traits measures the correlation or similarity between them relative to their variation among genotypes. Therefore, an angle of zero suggests a correlation of +1, an angle <90° suggests a positive correlation, an angle of 90° suggests no (zero) correlation, indicating independence, an angle >90° suggests a negative correlation and an angle of 180° indicates a correlation of -1.

Each of Fig. 1, 2 and 3 showed three groups of traits versus four groups of genotypes; each group is characterized

by high values in the respective traits. Under well-watered environment (Fig. 1), PYPH, PYPP, PPP and SPP for the genotypes IL4, IL13, IL8 and IL41 in the first group, WUE, SYPH, SYPP and 100-SW for the genotypes G13, IL3, IL6 and IL43, in the second group, shelling percentage and seeds/pod for the genotypes IL34, IL25, IL28, IL9 and IL28 in the third group. Under water-stressed environment (Fig. 2), pods/plant, seeds/plant and seeds/pod for the genotypes IL13, G13, IL41, IL4 and IL10 in the first group, seed yield/plant, seed yield/ha, WUE, PYPP, PYPH and 100-SW for the genotypes IL3, IL18, IL43, IL6 and IL8 in the second group, shelling percentage for the genotypes IL35, IL27r, IL28, IL27 and IL50 in the third group. For combined data across the two irrigation regimes (Fig. 3), 100-SW, PYPH and PYPP for the genotypes IL6, IL8, IL43 and IL3 in the first group, SYPH, SYPP, WUE, PPP and SPP for the genotypes IL4, IL41, IL10, IL13 and G13 in the second group, shelling percentage and seeds/pod for the genotypes IL35, IL27, IL18, IL28, IL25, IL50, IL27 and IL27r in the third group.

The pairs of traits (PYPP vs. PYPH), (WUE vs SYPH), (WUE vs. SYPP) and (SYPP vs. SYPH) had an angle of zero under the three studied cases (well-watered, water-stressed and combined across the two irrigation regimes) as illustrated in Fig. 1, 2 and 3, indicating a perfect correlation of +1. The pairs of traits among SPP, (PPP, PYPH), (WUE, SYPH, SYPP) group of traits and between 100-SW and shelling percentage under well-watered conditions, among PPP, (PYPP, PUPH), (WUE, SYPH, SYPP) group of traits and among SPP, seeds/pod and shelling percentage under water-stressed conditions and among SPP, PPP, (SYPH, SYPP, WUE), (PYPH, PYPP) and between seeds/pod and shelling percentage for combined data across the two irrigation regimes had acute angles (<90°) angles between them, suggested positive correlation and that their variation were similar, so each characteristic inside a specific group can be measured instead of the other characteristic in the same group. The traits of each group are inter-correlated.

The pairs of traits shelling vs SYPH, shelling vs SYPP, shelling vs WUE under well-watered, shelling vs PPP under water-stressed and across the two irrigation regimes had a near-right angle, suggested that variation of one characteristic was more or less independent of the other characteristic (near-zero correlation), implying independence. Seeds/plant and pods/plant vs shelling percentage and seeds/pod under well-watered, seeds/plant, seeds/pod and shelling percentage vs. 100-SW under water-stressed and seeds/pod, shelling percentage vs. 100-SW for combined data across the two irrigation regimes had obtuse angle (>90-180), suggested that their variation was in opposite directions (negative correlation). Pairs of Traits seeds/pod vs. pods/plant and seeds/pod under well-watered, seeds/pod vs. 100-SW under water-stressed and for combined data across the two irrigation regimes had an angle of 180° indicating a perfect negative correlation of -1.

Genotype by trait biplot Figures indicated that pod yield/ha is positively correlated with PYPP, PPP, SPP, WUE, SYPP and SYPH and negatively correlated with seeds/pod and shelling percentage under well-watered environment (Fig. 1), positively correlated with PYPP, SYPP, SYPH, WUE, PPP and SPP and negatively correlated with shelling percentage under water-stressed environment (Fig. 2) and positively correlated with PYPP, SYPP, SYPH, WUE, PPP and SPP and negatively correlated with seeds/pod and shelling percentage for combined across the two irrigation regimes (Fig. 3). These traits could be considered selection criteria for pod yield of peanut under respective environment if the heritability and genetic advance from selection of these traits are high; the common selection criteria are high values of PPP, SYPP, SPP, WUE, PYPP and SYPP and low values of seeds/pod and shelling under all environments.

Dissimilarity Euclidean coefficients based on phenotypic traits: The dissimilarity Euclidean coefficients (Table 3) among the 20 peanut lines, based on morphological data across the two irrigation regimes ranged from 0.05 to 0.69 with an average of 0.3169 (Table 3).

Dissimilarity Euclidean distances showed that the genotype IL34 was the most dissimilar with each of IL13 (0.69), IL8 (0.63), IL41 (0.61) and G13 (0.60) genotypes, since IL34 exhibited the highest dissimilarity Euclidean coefficients with these lines; so these pairs of lines are the most unrelated genotypes.

In contrast, dissimilarity Euclidean coefficients indicated that the most closely related lines genotypes based on phenotypic data; i.e. those showed the lowest dissimilarity Euclidean coefficients, were the pair of lines IL 18 and IL 35 (0.05), followed by the pair of lines IL 6 and IL 43 (0.05), the pair of genotypes L 26 and NC (0.06) and the pair of lines G13 and IL 41 (0.09); they are the most related lines in this experiment.

Agglomerative Hierarchical Clustering (AHC) analysis: The dendrogram of the peanut lines generated from the standardized phenotypic data across well-watered and water stressed environments using complete linkage method is illustrated in Fig. 4. The analysis classified the 20 lines into

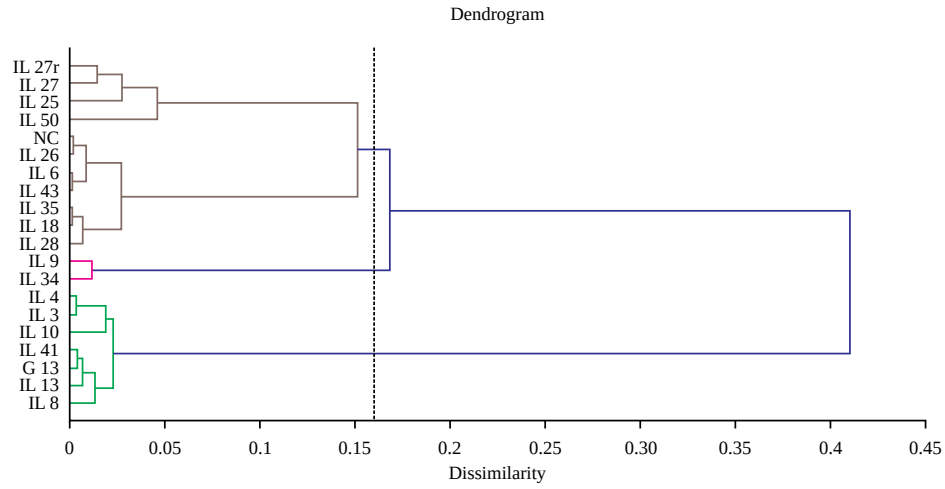

Fig. 4: Dendrogram of 20 peanut genotypes based on 10 traits measured across well-watered and water-stressed environments using the average method of clustering

Table 3: Dissimilarity Euclidean coefficients based on yield and the analysis of its components among 20 peanut genotypes across well-watered and water-stressed conditions

	G 13	IL 10	IL 13	IL 18	IL 25	IL 26	IL 27	IL 27r	IL 28	IL 3	IL 34	IL 35	IL 4	IL 41	IL 43	IL 50	IL 6	IL 8	IL 9
IL 10	0.20																		
IL 13	0.12	0.19																	
IL 18	0.20	0.21	0.29																
IL 25	0.48	0.45	0.57	0.30															
IL 26	0.28	0.24	0.36	0.11	0.31														
IL 27	0.29	0.21	0.35	0.16	0.25	0.21													
IL 27r	0.43	0.37	0.50	0.30	0.19	0.34	0.17												
IL 28	0.31	0.29	0.40	0.12	0.20	0.13	0.16	0.25											
IL 3	0.15	0.18	0.23	0.13	0.42	0.15	0.26	0.41	0.23										
IL 34	0.60	0.57	0.69	0.42	0.38	0.34	0.48	0.53	0.35	0.47									
IL 35	0.23	0.20	0.31	0.05	0.27	0.12	0.12	0.26	0.10	0.16	0.42								
IL 4	0.10	0.16	0.15	0.19	0.49	0.23	0.30	0.45	0.30	0.09	0.55	0.22							
IL 41	0.09	0.13	0.10	0.22	0.50	0.28	0.28	0.43	0.33	0.16	0.61	0.24	0.10						
IL 43	0.22	0.25	0.32	0.10	0.35	0.10	0.25	0.39	0.17	0.10	0.39	0.15	0.18	0.24					
IL 50	0.45	0.29	0.48	0.31	0.33	0.28	0.20	0.27	0.29	0.37	0.48	0.27	0.41	0.40	0.37				
IL 6	0.24	0.26	0.33	0.14	0.38	0.10	0.28	0.42	0.20	0.10	0.37	0.18	0.18	0.25	0.05	0.37			
IL 8	0.16	0.20	0.15	0.29	0.57	0.31	0.37	0.53	0.40	0.19	0.63	0.32	0.12	0.12	0.26	0.46	0.26		
IL 9	0.50	0.45	0.59	0.31	0.25	0.23	0.34	0.38	0.22	0.38	0.15	0.30	0.46	0.50	0.30	0.34	0.29	0.54	
NC	0.26	0.20	0.32	0.13	0.36	0.06	0.22	0.37	0.17	0.12	0.38	0.13	0.19	0.24	0.11	0.28	0.10	0.27	0.28

IL: Inbred line, G: Genotype

four groups. Group 1 included seven lines; namely IL8, IL13, G13, IL41 in one subgroup and IL10, IL3 and IL4 in another subgroup. The genotypes IL3 and IL4 are very closely related. Genotypes of each subgroup are closely interrelated.

The second group included two genotypes, namely IL9 and IL34. The 1st group is widely distant from the 2nd group. The 3rd group included seven lines, namely IL28, IL18 and IL35, in one subgroup and IL43, IL6, IL26 and NC, in another subgroup. Lines of each subgroup are closely interrelated, the first subgroup; especially IL18 and IL35 in the first subgroup and IL26 and NC in the 2nd group, which were the most related genotypes. The 4th group included four lines located in two sub-groups; the first sub-group included the IL50; the 2nd subgroup included two classes. The 1st class included IL50 and the 2nd class included two lines, namely IL27 and IL27r.

DISCUSSION

The present study investigated 20 groundnut lines by 10 phenotypic traits. The analysis of variance revealed significant ($p \leq 0.01$) differences among the lines for all 10 characteristics,

suggesting presence of sufficient variability in the germplasm studies. The existence of genetic variability among the lines for pod yield and its related traits under water-stressed and well-watered environments suggested that significant genetic advance could be made in selecting for improved pod yield and other traits under water stressed conditions. Although phenotypic analysis for assessment of genetic diversity presents many limitations as low polymorphism and influence of environment on morphological expression[37], phenotypic data are helpful as a preliminary assessment of groundnut genetic diversity and provided practical and helpful information required to assess available germplasm[28]. Morphological traits are very important for grouping peanut genetic resources and also are essential and useful for plant breeders seeking to improve existing germplasm by introducing novel genetic variation for certain traits into the breeding populations[8,28,38-39]. They reported the presence of genetic diversity in different populations of groundnut germplasm[41].

In general, coefficient of variation (CV) was very low (<5%) for all studied traits, suggesting good precision of the experiment. The coefficient of determination (R^2) for all traits under both and across environments was very high (0.88-1.00), indicating that the variables explained a high amount (at least 88%) of the variability in the characteristic performance. Similar results were observed in groundnut by several researchers[4,6,40]. It is observed that the magnitude of PCV was little higher than GCV for all studied traits, suggesting the minimum influence of the environment on the expression of these traits. These results are in accordance with the findings of other studies[8,28,38-39]. The high variability existed among the lines indicated that the studied germplasm was adapted to a wide range of environmental conditions and could provide valuable genes for groundnut breeding[41].

The tendency of some of the yield attributes that recorded high estimates of heritability as found in the present study to be used as the selection criteria is in agreement with the reports of other legume crop investigators[42]. High estimate of heritability coupled with high estimate of genetic advance for a given trait suggests that this trait is controlled mainly by additive genetic variance . Selection is effective only when the additive genetic variance is large and environmental effect is relatively low. The traits that had high estimates of both heritability and genetic advance are 100-seed weight under both well-watered and water-stressed conditions and WUE for combined data across the two irrigation regimes. The results of the present study are in agreement with those reported by previous investigators[43].

The data obtained will guide parental selection for groundnut improvement and broadening of the genetic base of breeding populations[21,44]. The existence of genetic diversity among the lines for pod yield and other traits under water-stressed, well-watered or for combined data of the two irrigation regimes suggested that significant genetic advance could be made in selecting for improved pod yield and other traits under such conditions[28-30].

The highest pod yield and its related traits under the water-stressed environment, were shown by the genotype IL13 followed by IL8, while the highest shelling percentage under both stressed and non-stressed environments were recorded by the line IL25. Genotypes IL13 and IL8 had also high WUE under water-stressed conditions. The highest number of pods/plant was achieved by the line IL 18 under normal conditions and IL 8 under drought and combined across the two irrigation regimes. The highest 100-SW was recorded by IL41, IL34 and IL50 under well-watered, water-stressed and combined across the two environments, respectively. These lines might possess favorable alleles that could be utilized to improve groundnut for the traits of interest under the water-stressed and non-stressed environments. These promising groundnut genotypes could be potentially utilized for the introgression of adaptive traits, which may be found in extreme environments[8-9,14,21,29-30].

Several studies have used multivariate statistical analysis such as Principal Component Analysis (PCA) to assess the extent of genetic diversity among the crop germplasm[14,25,43] and to decrease a large number of observed characteristics into a smaller number of characteristics that have the maximum contribution in discriminating the genotypes. The PCA was performed to classify the peanut genotypes on the basis of the most discriminating traits. Our results exhibited two main components accounting for 85.21, 81.52 and 83.27% of the total variation for well-watered, water-stressed regimes and combined across the two irrigation regimes, respectively. Amarasinghe *et al.*[14] revealed that the principal axis 1 to 10 accounted for more than 98% of the total variability observed among the groundnut lines evaluated. According to Makinde and Ariyo[25,43] previous studies on groundnut germplasm, found that first five axes together explain more than 70% of the total variation among the genotypes.

Biplot in the PCA characterizes variables that are super imposed on a plot as vectors where relative length of vectors characterizes the relative proportion of variability in each variable characterized on biplot[45]. The GT biplot analysis showed that among the characteristics analyzed, shelling percentage followed by 100-seed weight under well-watered

conditions, seeds/pod followed by 100-seed weight under water-stressed environment and for combined data across the two environments showed relatively long vectors, indicating that there was relatively large variation among groundnut lines. In other words, they exhibit large variation among the 20 lines studied, indicating that they are the most discriminator of the phenotypic data under the respective environments.

Higher yield performance under water stress conditions is an important and dependable index of drought tolerance[46]. The information of interrelationships between pod yield and its attributed components will enhance the effectiveness of breeding programs via the use of appropriate indices of selection[47]. The small acute angle observed between pod yield/ha and each of PYPP, PPP, SPP, WUE, SYPP and SYPH (Fig. 1) indicated the presence of very strong positive correlations between pod yield and such traits and that improving such traits would contribute to significant genetic advance in pod yield under water-stressed and well-watered conditions. These results indicate that the combination of some favorable characteristics may result in successful end products after proper groundnut breeding. Girdthaia *et al.*[48] reported a significant and positive correlation between pod yield and seed yield and there is the possibility to simultaneously improve a number of pods per plant, seed size and pod yield in peanut and that seed number is generally correlated with seed yield rather than weight of individual seeds. Makinde and Ariyo[25] stated that number of pods per plant showed significant positive correlation with yield per plant. Other researchers[8,31] also reported similar findings.

The results of the present study suggest that selecting groundnut lines in one of the research environments would also be effective in the other. However, it would be easier and cost effective to select under well-watered conditions rather than under drought environments. Since the results of the present study revealed that pod yield/plant, number of pods/plant, number of seeds/plant and seed yield/plant were the most dependable traits under both drought and well-watered conditions, improvement of pod yield under well-watered conditions would indirectly result in improved pod yield in drought environments. These results are in accordance with those reported previously[49].

Cluster analysis is a method used in grouping a set of characteristics into clusters. Genotypic clustering makes use of a procedure called Agglomerative Hierarchical Clustering (AHC) using Unweighted Pair Group Method with Arithmetic mean (UPGMA)[12-13,24]. The analysis classified the 20 groundnut lines four groups. The clusters represent uncorrelated groups which may be useful for future breeding programs as their trait performance may be governed by different sets of alleles. The highest genetic distance was found between IL34 and each of G13, IL8, IL41 and IL13, since they showed the highest dissimilarity Euclidean coefficients. Such dissimilarity indicates that IL34 might be crossed with each of IL13, IL8, IL41 or IL13 followed by selection in the segregating generations of transgressive segregation of higher pod yield than both of its parents. On the contrary, the lowest genetic distance was exhibited between the pair of genotypes IL 18 and IL 35, followed by the pair of genotypes IL 6 and IL 43, the pair of genotypes L 26 and NC and the pair of genotypes G13 and IL 41, since they showed the lowest dissimilarity Euclidean coefficients. These results indicate that each pair of these genotypes might have a common ancestor.

CONCLUSION

The principal component analysis (PCA) of phenotypic data was able to evaluate the extent of genetic diversity, characterize and classify a set of 20 groundnut lines. The genetic variation observed in this work suggests the possibility of genetic improvement of groundnut to meet the agronomic and morphological requirements for increased productivity and adaptation to stressed conditions. The Genotype×Trait (GT) biplot model allowed easy and better evaluation of associations among the traits and identification of the dependable secondary traits for enhancement of pod yield under water stressed environments. The Agglomerative Hierarchical Clustering (AHC) based on morphological data was able to identify the most unrelated genotypes to be used as parents when crossed would show maximum heterosis in the F_1 or wide genetic variability in the F_2 generation, useful for successful selection of higher-yielding genotypes under water-stressed conditions. Further research should be done on genetic diversity and characterization of new collected groundnut genotypes.

SIGNIFICANCE STATEMENT

This study discovered that Principal Component Analysis (PCA) and GT-Biplot technique can be beneficial for assessment of genetic diversity among peanut genotypes based on phenotypic traits in an easy way and giving helpful information for plant breeding programs. This study will help the researchers to uncover the critical areas of some limitations in using the morphological analysis for genetic diversity assessment, as low polymorphism and influence of

environment on phenotypic expression that many researchers were not able to explore. Thus a new theory on the assessment based on phenotypic traits, using multivariate analysis is helpful as a preliminary evaluation of groundnut genetic diversity and provide practical and critical information required for characterizing genetic resources and classifying germplasm collections to enhance their use in groundnut breeding may be arrived at.

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