

ORIGINAL RESEARCH ARTICLE

Screening and genotyping of groundnut (*Arachis hypogaea* L.) inbred lines and landraces in the North Central Nigeria

Olalekan Joseph Olasan^{1*}, Celestine Uzoma Aguoru¹, Lucky Osabuohien Omoigui^{2,3}, Iwala Johnson¹, Dughdugh Paul¹, Solomon Soom Terseer⁴

¹ Plant Biotechnology and Molecular Genetics Unit, Department of Botany, Federal University of Agriculture Makurdi, Makurdi 970212, Benue State, Nigeria. E-mail: olasan.olalekan@uam.edu.ng

² Department of Plant Breeding and Seed Science, Federal University of Agriculture Makurdi, Makurdi 970212, Benue State, Nigeria.

³ International Institute of Tropical Agriculture, Ibadan 200001, Oyo State, Nigeria.

⁴ Department of Biological Sciences, Benue State University, Makurdi 102119, Benue State, Nigeria.

ABSTRACT

The study evaluated 33 accessions of groundnut in the field, consisting of 23 landraces from Nasarawa communities in Nigeria and 10 inbred lines. Assessment entailed the determination of plant survivorship, yield related parameters and pathological indices while genetic diversity study was undertaken using SSR and RAPD molecular markers. Data analysis was done on the Minitab 17.0 software. Significant variability was noted in all traits except in pod sizes, seed sizes and % infected seeds. About 33.3% of the accessions had a survival rate of $\geq 70.0\%$ where 9/10 Inbred lines were found with overall yield (kg/ha) ranging from 4.0 ± 1.6 in Akwashiki-Doma to 516.8 ± 46.9 kg/ha in Samnut 24 \times ICGV-91328. Five accessions (15.5%) had pathological indices of zero indicating no traces of any disease of any type and they included one landrace called Agric-Dazhogwa and four Inbred lines: Samnut 25 \times ICGV-91317, Samnut 26 \times ICGV-19324, Samnut 26 \times ICGV-91328 and Samnut 26 \times ICGV-91319. Coefficients of yield determination R^2 by survivorship and pathological index were 50.6% and 15%, respectively. A fit model was established (Yield in kg/ha = $-146 - 7.94 \times \text{Pi} + 5.88 \times S$). Susceptibility to diseases depends on the type of variety ($\chi^2_{(32)} = 127.67, P = 0.00$). Yield was significantly affected by BNR@30 ($F = 5.47, P = 0.025, P < 0.05$) and DSV@60*RUST@60 interaction effect ($F = 4.39, P = 0.044, P < 0.05$). The similarity coefficient ranged from 28.57 to 100 in plant morphology. Four varieties had no amplified bands with SSR primers whereas amplified bands were present only in four landraces accessions using the RAPD primer. The dendrogram generated by molecular data gave three groups where genetic similarity ranged from 41.4 to 100.0. The Inbred lines were noted for their high survivorship, good yield and disease resistance. Samnut 24 \times ICGV-91328, an inbred line, had the highest yield but was susceptible to diseases. Among the landraces, Agric-Musha, Bomboyi-Dugu and Agric-Dazhogwa were selected for high survivorship and disease resistance. The selected inbred lines and landraces are valuable genetic resources that may harbour useful traits for breeding and they should be presented to the growers based on their unique agronomic values. The highest yielding inbred lines should be improved for resistance to late leaf spot diseases while the outstanding landraces should be improved for yield.

Keywords: Groundnut; Inbred Lines; Landraces; Genetic Resources; Improvement

ARTICLE INFO

Received: 7 August 2023

Accepted: 30 August 2023

Available online: 21 September 2023

COPYRIGHT

Copyright © 2023 by author(s).

Trends in Horticulture is published by En-Press Publisher, LLC. This work is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

<https://creativecommons.org/licenses/by-nc/4.0/>

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important monoecious annual legume in the world mainly grown for the oilseed, food and animal feed^[1]. Groundnut seeds are a rich source of oil (35%–56%), protein (25%–30%), carbohydrates (9.5%–19.0%), minerals (P, Ca, Mg and K) and vitamins (E, K and B)^[2]. Apart from food, groundnuts are used as an important source of income since are sold in the local market as boiled and shelled roasted nuts while some are sold in the confectionery trade^[3]. The haulms are used as livestock feed and in compost making.

As a legume, groundnut helps to improve soil fertility in farming systems by fixing atmospheric nitrogen^[3]. The crop is cultivated in more than 100 countries under different agro-climatic conditions on about 26.5 million hectares with a total production of 43.9 metric tons and productivity of 1,654 kg/ha^[4]. India is the second largest producer of groundnut and its oil after China followed by USA and Nigeria. It is cultivated on about 3.7 million hectares with the production of 6.7 metric tons and 1,810 kg/ha, respectively, during 2015–2016^[5].

In Nigeria, the land area grown to groundnut annually from 2000 to 2009 increased by 2.6% but the yield declined by 3.3% over the same period resulting in a stagnation of production at 2.9 million tons^[5]. Groundnut is the most important food legume in Nigeria in terms of consumption and area under production^[6] and is featured prominently in the cropping systems of the Savanna and Forest-Savanna transitional agro-ecological zones. Its production in Nigeria has nearly tripled in the last decade (168,200 to 420,000 metric tons in 2005) primarily due to an increase in the area under cultivation which increased from 184,400 ha in 1995 to 450,00 ha in 2005^[4]. Average yields however continue to remain below 1.0 metric tons/hectare which is far below the potential yield of 2–3 metric tons/hectares. In West Africa, Nigeria is the largest producer of groundnuts with a production of 3.07 million tons on about 2.4 million hectares^[4].

Despite groundnut being an important oil crop in Nigeria. However, groundnut production is constrained by a lack of enough improved groundnut varieties, biotic and abiotic stresses. These are the major constraint of groundnut production in Nigeria. The low yield of groundnuts affects small-scale farmers' livelihoods due to a reduction in household income. The use of host resistant varieties is the most effective, economical and sustainable way to control the disease^[7]. Unfortunately, these resistance sources are from late maturing varieties and poor yielding varieties^[8]. Identification of varieties with combined agronomic qualities such as yield and disease resistance to satisfy farmer's demands and value chains for food security and regional and local markets is a big challenge. The present study was designed to address this gap by

evaluating some accessions consisting of groundnut inbred lines and landraces collected from Nasarawa State. The outcome would help determine the level of diversity among the accessions and select quality accessions that may be useful to growers and breeders in the quest to achieve high productivity and food security in line with the UN goal. The aim of the study was to evaluate groundnut landraces and inbred lines for their agronomic values (yield and disease resistance) and assess the level of their diversity using molecular markers. The specific objectives were to evaluate the plants for survivorship and yield related performances; undertake pathological assessment; select best performing lines and determine the extent of genetic diversity among them using RAPD and SSR molecular markers.

2. Materials and methods

2.1 Study area

This research work was carried out at the Agronomy Teaching and Research Farm, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria. The research farm is situated along Gbajimba Road, just after the School Clinic. Makurdi Local Government Area has a landmass of about 16 km in radius^[9]. It lies between latitude 7°43'50' N 8°32'10' E and longitude 7.73056° N 8.53611° E. It has a population of 300,377^[10]. The mean annual temperature of the area ranges between 22.5 °C and 40 °C but the temperature is high throughout the year while precipitation is about 1,173 mm. The rainfall pattern is from March to November with variation. The vegetation type in Makurdi is the Guinea Savannah. Makurdi Local Government is endowed with great investment potentials both in agro-allied and mineral resources. The major occupation of the inhabitants is farming.

2.2 Sample collection

A total of 10 inbred lines were sourced from the Institute for Agricultural Research (IAR) Samaru, Zaria, Nigeria. Twenty-three (23) landraces were collected from local farmers in different communities within Nasarawa State, Nigeria. Altogether, there were 33 accessions of groundnut used in this study (Table 1).

Table 1. List of groundnut accessions (landraces and inbred lines) used for the study

Accession number	Accession name	Accession code
V1	Chika buhu-Doma	CBD
V2	Agric-Musha	AMU
V3	Agric-Agyaragu	AAG
V4	Agric-Alwaza	AAL
V5	Chika buhu-Lafia	CBL
V6	Agric-Kadorko	AKA
V7	Nada	NAD-1
V8	Chika buhu	CB-1
V9	Akwashiki-Obi	AKO
V10	Nada-Isgugu	NAD-IS
V11	Kpoklo-Gude	KPG
V12	Agric-Dazhogwa	ADA
V13	Samnut 25 × ICGV-91317	INBRED LINE-1
V14	Samnut 26 × ICGV-19324	INBRED LINE-2
V15	Samnut 26 × ICGV-91328	INBRED LINE-3
V16	Samnut 22 × ICGV-91324	INBRED LINE-4
V17	Samnut 23 × ICGV-91324	INBRED LINE-5
V18	Samnut 22 × ICGV-91328	INBRED LINE-6
V19	Samnut 26 × ICGV-91319	INBRED LINE-7
V20	Samnut 24 × ICGV-91317	INBRED LINE-8
V21	Samnut 25 × ICGV-91328	INBRED LINE-9
V22	Samnut 24 × ICGV-91328	INBRED LINE-10
V23	Bomboyi-Dugu	BOD
V24	Agric-Gidiye	AGG
V25	Kwaya biyu-Kpangwa	KWK
V26	Agric-Obi	AGO
V27	Akwashiki-Nene	AKN
V28	Agric-Dedere	ADE
V29	Nada-Doma	NAD-D
V30	Akwashiki-Doma	AKD
V31	Agric-Duglu	AGD
V32	Agric-Igbavo	AGI
V33	Barnada-Zaki-Biam	BAZ

2.3 Experimental design and planting

After land clearing, the field layout was a randomized complete block design (RCBD) with two replicates and two blocks. Three seeds of each of the 33 accessions were sown as an experimental unit, replicated twice per block. There were 66 experimental units per block. A total of 132 experimental units were evaluated. Post planting activities comprised weeding, fertilizer application, monitoring and characterization.

2.4 Field evaluation

Standard field procedures, guidelines and descriptors as given by Jambunathan^[11] and the International Crops Research Institute for the Semi-Arid Tropics^[12] were used in the evaluation of the 33 accessions of groundnut. Published charts/pictures were used in the identification of groundnut

diseases^[11]. Disease incidences were calculated using standard methods^[13]. Characters were assessed using a standard groundnut descriptor guide^[14]. They include survival rate, pod per plant, pod sizes, seeds per plant, seed sizes, % diseased seeds, 100 seed weight, yield per plot (g), yield (kg/ha), DSV@30/60 (incidence of DSV infection at day 30 and 60), ELS@30/60 (incidence of early leaf spot disease), GNR@30/60 (incidence of groundnut rosette disease), LLS@60 (incidence of late leaf spot disease at day 60), TLS@60 (incidence of Tikka leaf spot at day 60) and RUST@60 (incidence of groundnut rust disease at day 60). Pathological index (Pi) was calculated as the average of all incidences per accession.

2.5 SSR molecular studies

Twelve SSR primers linked to aflatoxin resistance in groundnut^[13] and other reagents such as

PRC pre-mix were procured from Genomics Training Center and Laboratory Limited, Uyo Akwa Ibom State, Nigeria. They were stored in the freezer at $-20\text{ }^{\circ}\text{C}$ at the Molecular Biology Laboratory of the Department of Plant Breeding, Joseph Sarwuan Tarka University Makurdi, Nigeria where the molecular aspect of this study was carried out. The forward sequences of selected primers are given as MP32 (F-AGTGTGTGTGTGAAAGTGG), PM36 (F-ACTCGCCATAGCCAACAAC) and PM42 (F-ACGGGCAAGTCAAGTGAT). Two RAPD primers selected from the optimization process were employed in diversity studies. They were OPA-07 (F-GTCAGTGCGG) and OPA-10 (F-GGTCACCTCA).

2.6 DNA extraction, amplification, and separation

DNA extraction was done on 14-day-old seedlings using the CTAB method^[15]. The pellet was suspended in 100 mL of molecular grade water/RNase water. The quality was checked using 0.8% Agarose gel. Polymerase Chain Reaction (PCR) was carried out in a Bio-Rad Thermal cycler under the following thermal cycler conditions for PCR reaction, such as denaturation ($95\text{ }^{\circ}\text{C}$) in 30 sec, annealing ($55\text{--}60\text{ }^{\circ}\text{C}$) in 30 sec, and extension ($72\text{ }^{\circ}\text{C}$). PCR products were made to run on 2.0% agarose gel electrophoresis stained with ethidium bromide for 40 min. A photographic record was obtained under the UV-illumination using a Benchtop Trans illuminator with the aid of a digital camera that captured all gel images.

2.7 Data analysis

Descriptive analysis was carried out using the Minitab 17.0 application. Two-way ANOVA (analysis of variance) was used. The Fisher LSD method was used to separate means at a 95% confidence limit. The model for groundnut yield was given by simple linear and surface response regression methods. A test of dependence was done using the Chi-square method. DNA bands were scored and converted to binary matrices for both SSR and RAPD gel images in a separate analysis.

Dendrograms were constructed by performing cluster analysis using the average linkage method.

3. Results

3.1 Description of groundnut accessions

A quantitative description of groundnut accessions planted in the field is presented in **Table 2**. Accessions varied in their characters. Coefficients of variation (CV) in plant counts at seedling and harvesting stages were 35.1% and 38.6%, respectively. At both stages, the plant count ranged from 2 to 12 plants. Some accessions failed to produce pods while maximum pods of 32.5 pods were recorded in some accessions giving an average of 10.40 ± 1.09 pods per plant that measured 3.27 ± 0.383 cm. Number of seeds per plant varied from 0.2 to 63 seeds. The maximum percentage of infected seeds was 18% (18 seeds out of 100) while 100 seed weight was 51.5 g per 100 seeds. Seed sizes had the least CV (12%) while pod sizes had the highest CV (95.25). Significant variation was recorded in mean stand counts at seedling for accessions ($F = 2.48, P < 0.05$) where inbred line-5 had the highest mean count while the block factor was also significant ($F = 10.97, P < 0.05$). The same trend was observed in stand count at harvest. The mean number of pods per plant was significantly different at the accession level only ($F = 11.4, P < 0.05$) where inbred line-1 had the highest number of pods. The main effect plot showed five major and minor peaks above the threshold that accounted for the variability in pod production among the accessions (**Figure 1**). Significant differences were recorded in the number of seeds produced per plant ($F = 2.45, P < 0.05$) where inbred line-1 had the highest value. The main effect plot showed six major and minor peaks above the threshold that accounted for the variability in seed production among the accessions (**Figure 2**). The 100 seed weight also varied significantly ($F = 3.17, P < 0.05$) where inbred lines-1 and 10 were distinct. Pod sizes, seed sizes and % infected seeds had no significant differences among the accessions.

Table 2. Description and variability assessment of characters

Characters	Mean \pm S.E	CV%	Min	Max	F-variety	P-value	Pointer
Stand count at harvest	8.60 \pm 0.409	38.61	2.00	12.00	3.10	0.001	V17
Pod per plant	10.40 \pm 1.09	85.46	0.10	32.50	11.14	0.000	V13
Pod sizes (cm)	3.27 \pm 0.383	95.19	1.833	28.10	1.03	0.468	NIL
Seeds per plant	15.05 \pm 1.57	84.54	0.20	63.00	2.45	0.007	V13
Seed sizes (cm)	1.22 \pm 0.018	12.03	1.080	2.190	0.90	0.621	NIL
% Diseased seeds	6.167 \pm 0.385	50.77	1.00	18.000	0.82	0.706	NIL
100 seed weight (g)	25.93 \pm 1.81	56.57	0.50	51.50	3.17	0.001	V13, V22

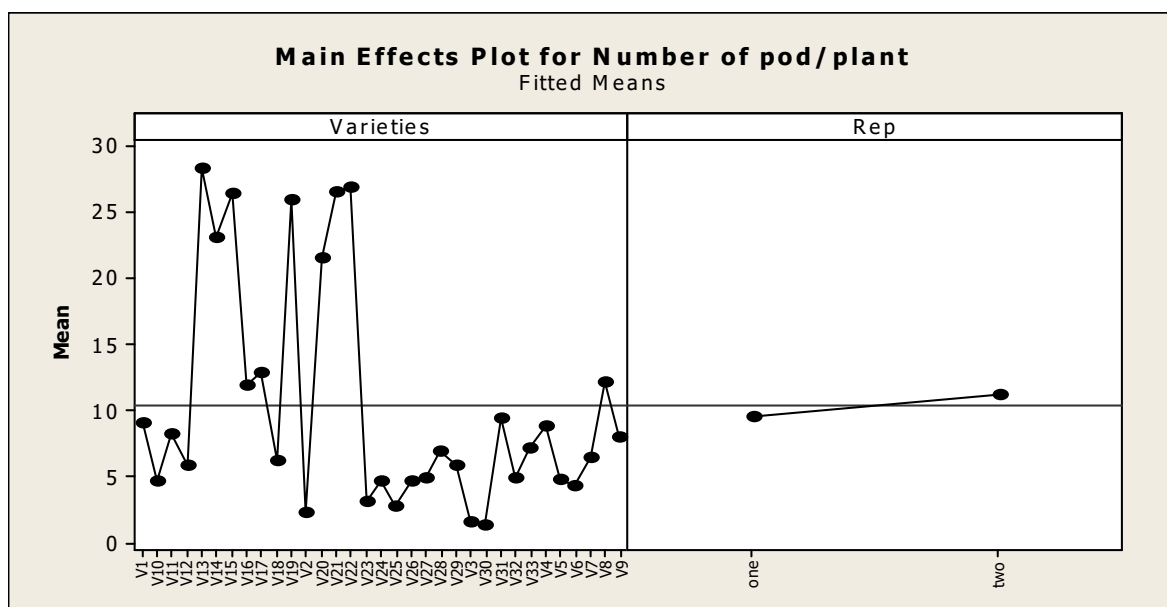


Figure 1. Main effects plot for pod per plant.

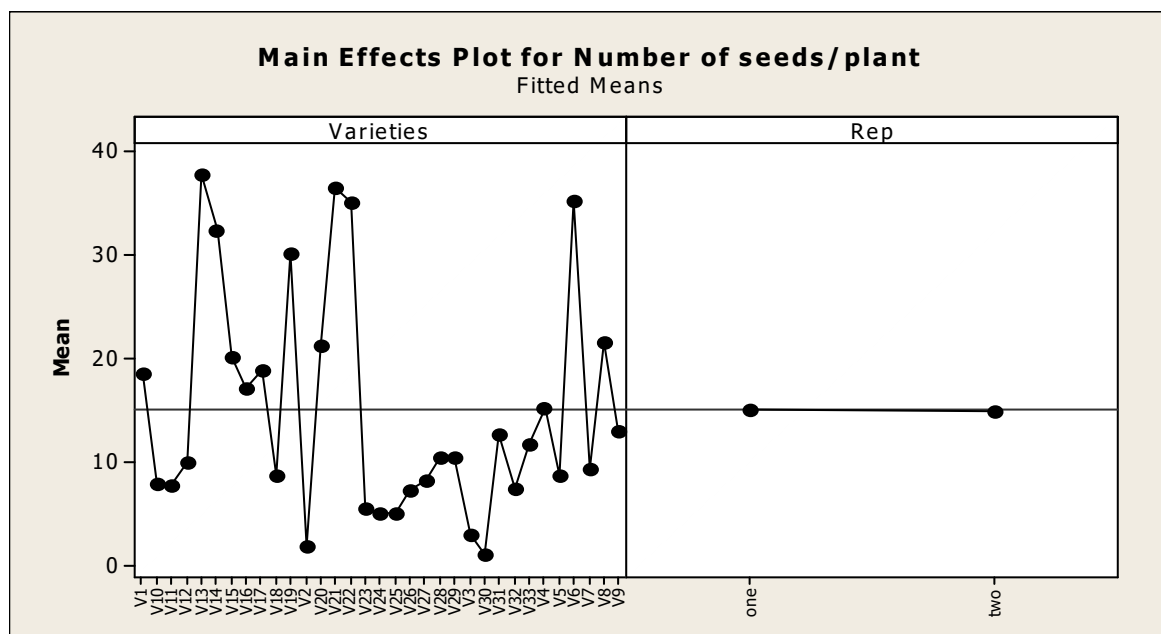


Figure 2. Main effects plot for seed per plant.

3.2 Plant survivorship and pathological indices

Eleven out of 33 accessions representing 33.3% had a survival rate of $\geq 70.0\%$ (Table 3). All 10 inbred lines were in this category except inbred line–

6 which performed below this threshold. The best accession in survival rate was inbred line–5 with the value of 93.3%. Inbred line–3 and 8 recorded 86.7% each. Among the landraces, AMU and BOD had survival rates $\geq 70.0\%$ while ADA had the

lowest score (23.3%). Variations in plant survivorship and pathological indices are shown in **Figures 3 and 4**, respectively. Analysis of pathological parameters showed those accessions that scored $\geq 15.0\%$ in disease incidences. At day 30, DSV disease was not pronounced since all accessions had incidences below the 15% threshold. About 20 out of 30 plant accessions (66.7%) showed no symptoms of DSV@30 while 13 (39.45%) showed symptoms. The frequency of DSV symptomatic plants declined at day 60 since only 5 (15.2%) accessions showed the symptoms of DSV@30. A total of 22 accessions (66.7%) showed no symptoms

of ELS@30 while 11 (33.3%) showed symptoms. Early leaf spot (ELS) was $\geq 15.0\%$ in inbred line-10 and KWK accessions where incidences were 31.3% and 30.9%, respectively. Late leaf spot (LLS@60) recorded high incidences in about 10 accessions, the highest being 52.1% in Agric-Duglu followed by Nada accessions. In all, 18 (54.5%) showed symptoms of LLS@60. Five accessions (15.5%) had pathological indices of zero indicating no traces of any disease of any type and they included one landrace (Agric-Dazhogwa) and four inbred lines-1, 2, 3, and 7. Pathological indices (Pi) were ≥ 5 in 11 (33.3%) accessions.

Table 3. Survivorship, pathological indices, and overall yield

Accessions	Survival rate (S)	DSV30	ELS30	DSV60	LLS60	Pathological index (Pi)	Yield (kg/ha)
CBD	56.7	3	11	4	0	4.5	169.1 ± 29.6
AMU	73.3	0	0	3	26.5*	7.4	34.42 ± 2.92
AAG	43.3	1.5	0	1.5	12.5	3.9	35.2 ± 15.7
AAL	46.7	0	0	3	0	0.8	80.3 ± 11.0
CBL	32	0	0	1.5	10	2.9	48.75 ± 2.08
AKA	40	3	12.8	4	7.2	6.8	41.1 ± 10.8
NAD-1	50	1.5	5	4	34*	11.1	32.3 ± 5.2
CB-1	36.7	0	0	5	20*	6.3	108.4 ± 10.8
AKO	36.7	0	0	1.5	16.7*	4.6	48.5 ± 10.8
NAD-IS	53.3	0	0	4	21.6*	6.4	60.4 ± 10.1
KPG	60	3	7.9	3	0	3.5	76.8 ± 15.7
ADA	23.3	0	0	0	0	0	33.3 ± 10.7
IL-1	70	0	0	0	0	0	466.4 ± 17.8
IL-2	76.7	0	0	0	0	0	481.0 ± 126
IL-3	86.7	0	0	0	0	0	469.4 ± 19.1
IL-4	80	0	0	1.5	0	0.4	144.3 ± 16.6
IL-5	93.3	0	0	1.5	0	0.4	297 ± 22.5
IL-6	46.7	0	0	1.5	0	0.4	52.5 ± 6.7
IL-7	76.7	0	0	0	0	0	377.0 ± 27.0
IL-8	86.7	0	0	1.5	0	0.4	428.4 ± 26.1
IL-9	70	0	0	1.5	0	0.4	404.0 ± 17.0
IL-10	83.3	3.5	31.3*	1.5	0	9.1	516.8 ± 46.9
BOD	76.7	1.5	3.1	1.5	3.4	2.4	57.5 ± 6.5
AGG	43.3	1.5	12.5	4	12.5	7.6	34.4 ± 6.58
KWK	50	3.5	30.9*	5	20*	14.9	22.2 ± 2.3
AGO	56.7	0	0	1.5	11.1	3.2	51.9 ± 11.9
AKN	56.7	1.5	3.4	4	24.3*	8.3	54.8 ± 3.9
ADE	43.3	1.5	3.9	4	11.1	5.1	78.5 ± 15.7
NAD-D	56.7	1.5	0	1.5	10	3.3	74.7 ± 11.2
AKD	26.7	0	0	6	0	1.5	4.0 ± 1.6
AGD	36.7	0	0	6	52.1*	14.5	103.6 ± 6.7
AGI	56.7	0	0	3	12.5	3.9	60.2 ± 5.3
BAZ	66.7	1.5	8.3	3	20*	8.2	73.9 ± 4.9

Note: incidences ≥ 15 ; Pi ≥ 5

Pearson's R (survivorship and yield) = 0.711, $R^2 = 50.6\%$

Pearson's R (pathology and yield) = -0.386, $R^2 = 14.9\%$

The regression equation is Yield (kg/ha) = -146 - 7.94 × Pi + 5.8 × S

$F = 17.7$, $P = 0.000$ ($P < 0.05$)

Key: IL= inbred line

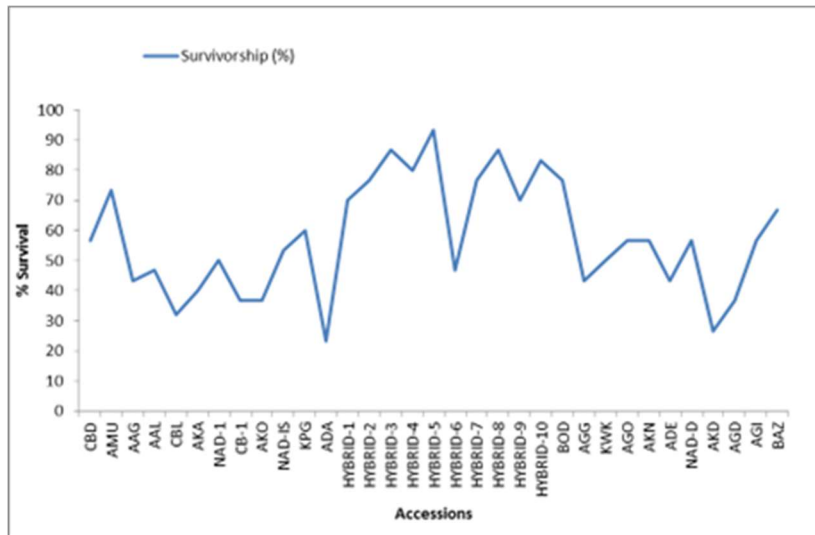


Figure 3. Plant survivorship plot.

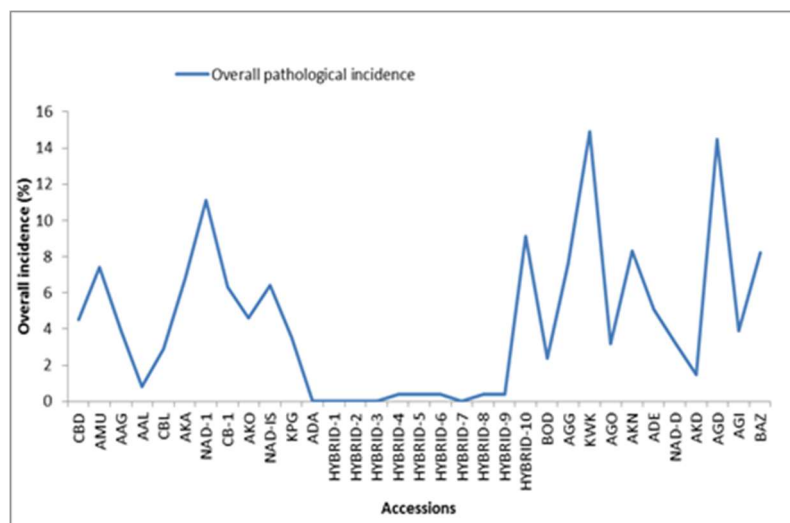


Figure 4. Plant pathological indices.

3.3 Plant yield and regression analysis

Overall yield (kg/ha) ranged from 4.0 ± 1.6 in Akwashiki-Doma to 516.8 ± 46.9 kg/ha in inbred line-10. Significant variation was observed in yield (kg/ha) among varieties ($F = 16.04$, $P < 0.05$) and between blocks ($F = 5.15$, $P < 0.05$). The top five high yielding accessions were inbred line-10 (517 kg/ha), inbred line-2 (481 kg/ha), inbred line-3 (469 kg/ha), inbred line-1 (466 kg/ha) and inbred line-8 (428 kg/ha). The main effect plot revealed eight accessions (24%) whose yields were above the 150 kg/ha benchmark (Figure 5). A strong positive correlation was established between yield (kg/ha) and plant survivorship (S) ($R = +0.711$) where the coefficient of determination R^2 was estimated as 50.6%. A weak negative relationship was established between yield and pathological indices

(P_i) ($R = -0.4$) where the coefficient of determination R^2 was estimated as 15%. The model for yield was fit and significant ($F = 17.7$, $P < 0.05$). The regression equation for yield is given as: Yield (kg/ha) = $-146 - 7.94 \times P_i + 5.88 \times S$. Based on the trend analysis plot (Figure 6) for yield, MAPE was 270.0 while MAD was 135.9. The linear trend model of yield is given as: Yield (kg/ha) = $149.3 + 0.165 \times t$. Result shows that plant susceptibility to diseases depends on the type of variety ($\chi^2_{(32)} = 127.67$, $P = 0.00$) as shown in Figure 7. The observed pathological indices were below the expected values in all Inbred lines except in inbred line-10. Landraces including Agric-Alwaza, Chikabuhu-Lafia, Kpoklo-Gude, Agric-Dazhogwa Bomboyi-Dugu, Akwashiki-Doma and Agric-Igbavo had minimal pathological indices below the expected values.

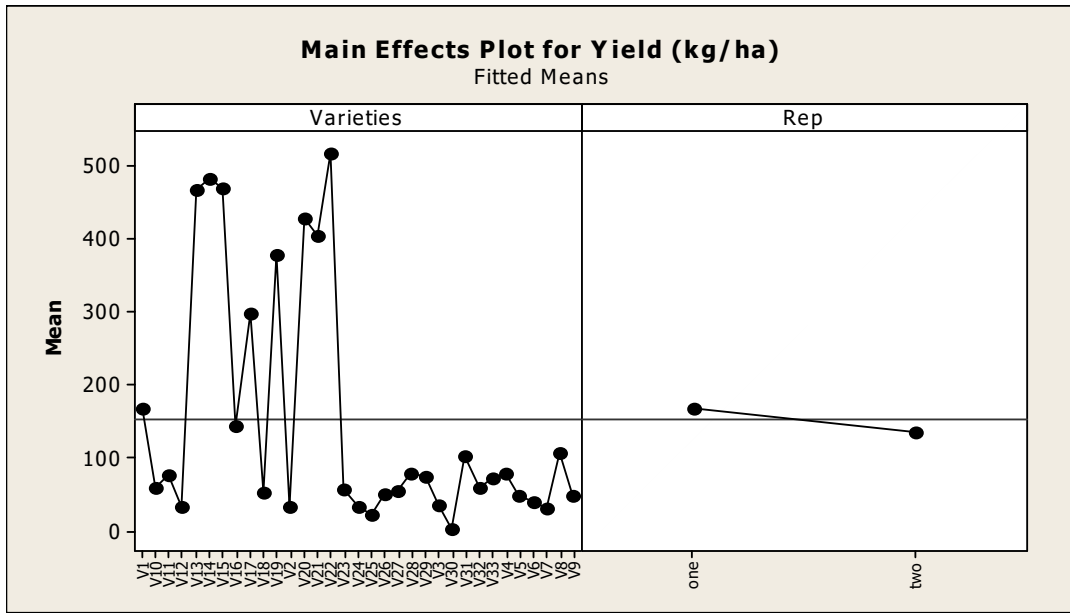


Figure 5. Main effects plot for yield.

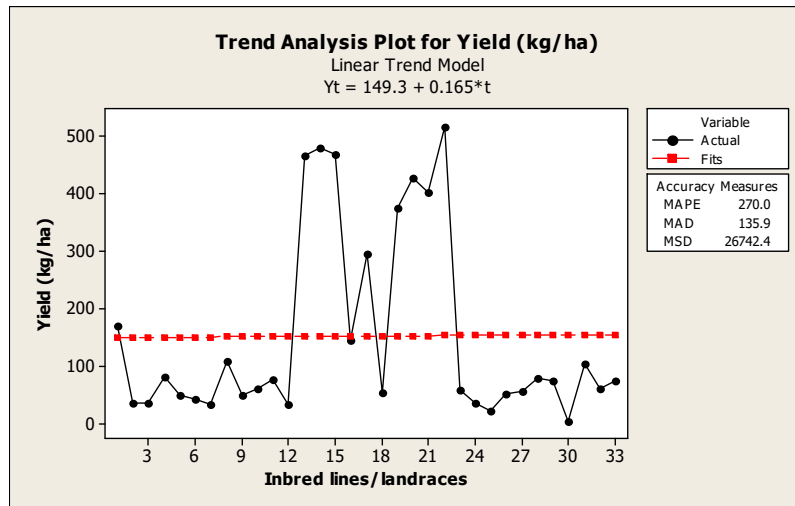


Figure 6. Trend analysis for groundnut yield.

Note: F (Variety) = 16.04, $P = 0.00$ ($P < 0.050$); F (Block) = 5.15, $P = 0.030$

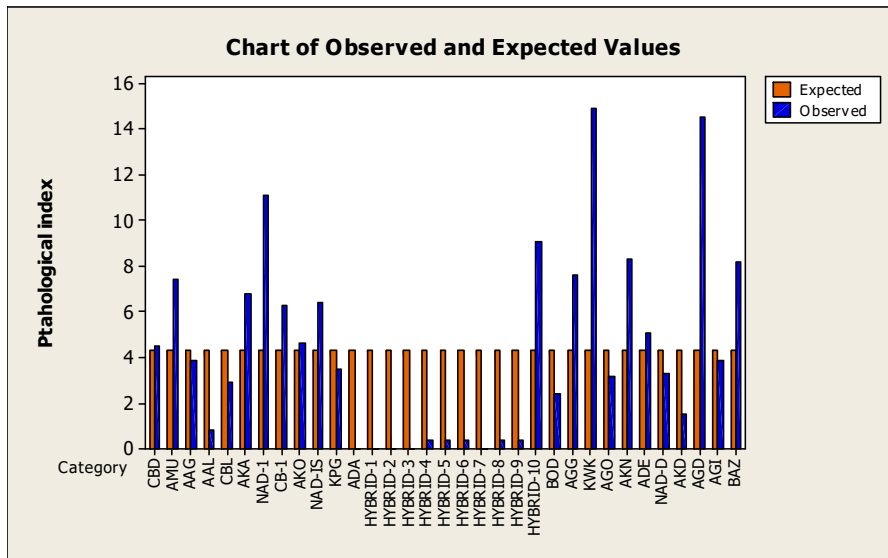


Figure 7. Test of dependency (variety and pathology).

Note: $\chi^2_{(32)} = 127.67$, $P = 0.00$

3.4 Cluster analysis

The 33 accessions were clustered on the basis of plant survivorship, pathological parameters and overall yield (Figure 8). Dendrogram gave two clusters. The first clusters comprised 8 accessions all of which were inbred line where inbred lines–5, 10 and 8 were distinct accessions. Inbred line–5 had the highest survival rate (93.3%). Inbred line–10 was the best yielding accession (517 kg/ha) while inbred line–8 also possessed high survivorship and high yield with minimal traces of diseases. Similarity coefficient ranged from 28.57 to 100. The genomic DNA extracted from groundnut seedlings, the product of the optimization stage and amplified products of the primers are shown in Plates

1–2. The binary plot of multiplex RAPD primers (Figure 9) revealed amplified bands in 29 varieties and while 4 varieties had no bands including Nada-Isgugu, inbred lines–4, 9 and 10. Amplified bands were present only in four landraces accessions including Agric-Musha, Chika buhu-Lafia, Chika buhu and Bomboyi-Dugu. The dendrogram generated by molecular data (Figure 10) gave three groups among the accessions whose genetic similarity ranged from 41.4 to 100.0. The first group comprised four genetically distinct landraces: Agric-Musha, Chika buhu-Lafia, Chika buhu and Bomboyi-Dugu. In the second group, 3 out of the 4 clustered members were inbred lines–4, 9 and 10. The third group comprised 25 accessions, a mix of inbred lines and landraces.

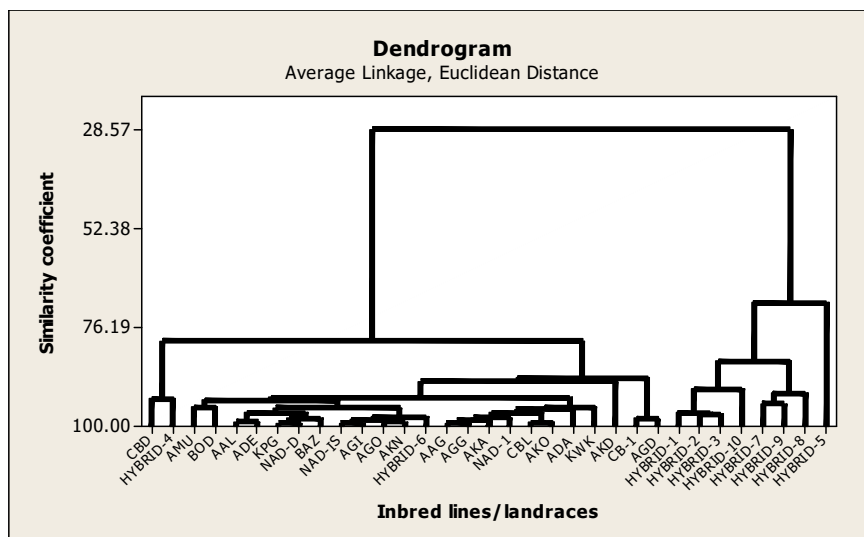


Figure 8. Dendrogram showing the clustering pattern among accessions.

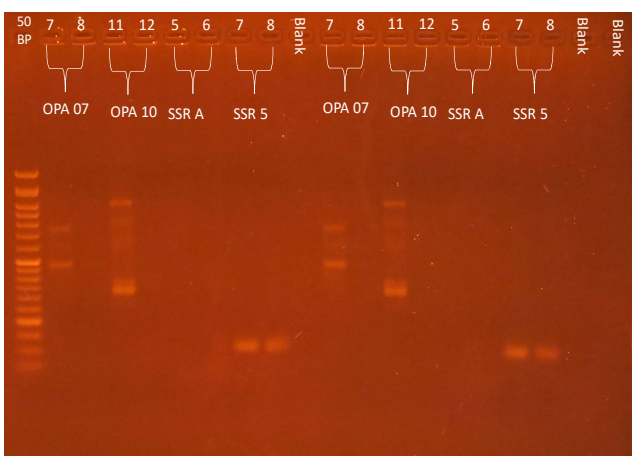


Plate 1. Optimization of primers and protocols.

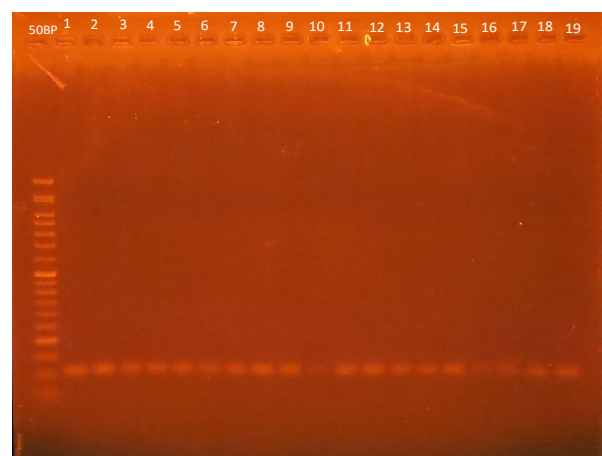


Plate 2. Amplification by SSR, multiplex primer (V1-19).

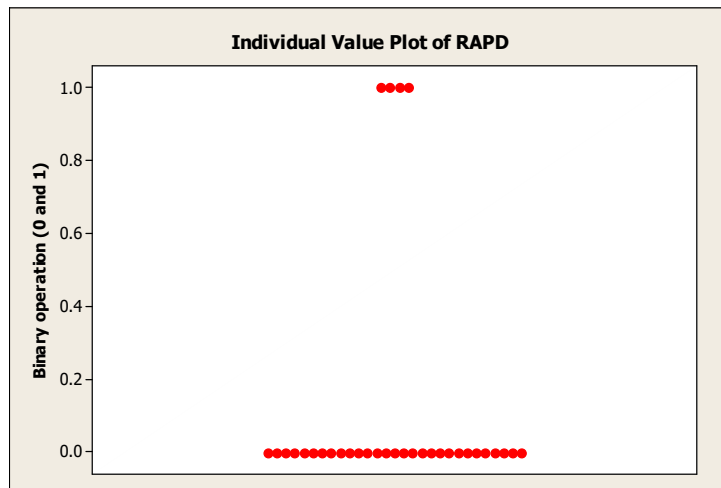


Figure 9. Binary plot of RAPD amplification.

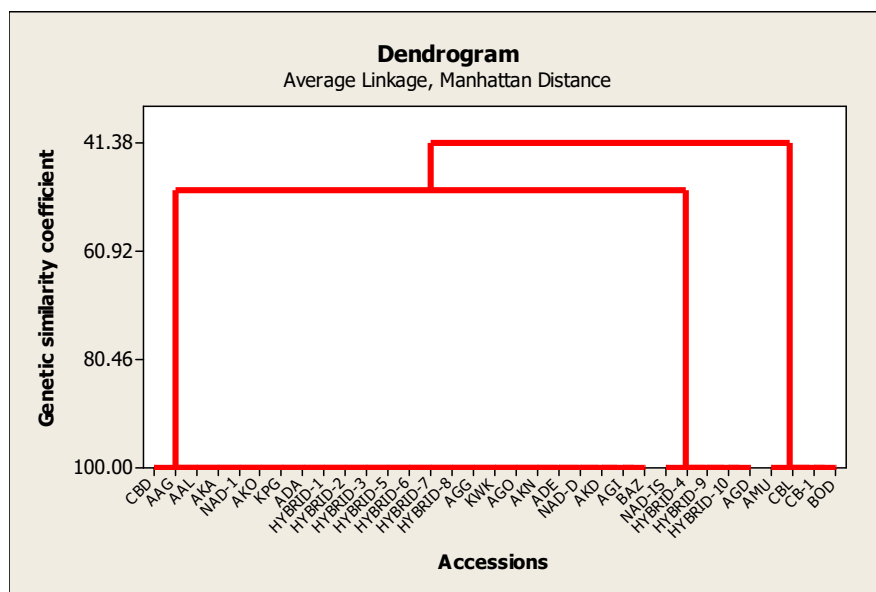


Figure 10. Dendrogram of molecular data.

4. Discussion

Characters displayed huge variability among the collections, especially the pod sizes and the overall grain yield. This finding is in tandem with previous reports on groundnut that pod and seed yield accounted for the highest diversity that formed the basis of the selection of superior genotypes and subsequent improvement of weaker types^[16–18]. The established variability among the collections is supported by the low coefficient of similarity ranging from 29 to 100 and 41 to 100 in the phylogenetic analyses of morphological and molecular data, respectively. The present investigation supports previous studies^[19,20] on the existence of divergent morphological and genetic characters in *Arachis hypogea*. Unlike the low variability

reported in some works^[21,22], this outcome agrees with other studies reporting high variability in groundnut^[23,24].

Seed infestation due to diseases was high (18%). This could be interpreted as the presence of 18 contaminated seeds from every 100 seeds harvested. There is every possibility that other seeds might be contaminated with time during storage, if not controlled, because the seeds are packaged together. Although the sources of the diseases were not investigated in this work, may be due to pathogens of viral or bacterial or fungal origin. The economic losses and detrimental health hazards associated with the consumption of contaminated seeds are well documented globally^[13,17,25,26]. For example, aflatoxin is a common carcinogenic toxin

produced by a fungus (*Aspergillus flavus*) that causes aspergilliosis when ingested in contaminated seeds^[13,17]. Therefore, the collections should be investigated further to determine the real causes of seed infestation and identify sources that are susceptible and resistant to diseases of health concern. Also, yield losses can be prevented by cultivating highly resistant seeds that are resistant to specific diseases.

The Inbred lines are well adapted to the field environmental conditions as revealed in their high survivorship where 9 out of the 11 accessions selected for high adaptability were all products of groundnut breeding. The two landraces selected for their high rate of survival are Agric-Musha and Bomboyi-Dugu. The relationship between plant adaptability as physiological attributes and genetic factors is well established in literature^[2]. It can be inferred that the selected genotypes are vigorous, highly tolerant to stresses and well suitable for cultivation. They could serve as a template for improving groundnut accessions for tolerance to environmental conditions, although some of them are products of ongoing breeding work to achieve certain specific objectives. Apart from high adaptability, the inbred lines were noted for disease resistance with few exceptions while most of the landraces were susceptible to diseases of groundnut as evident in the high incidence of LLS (late leaf spot) in some landraces. Previous reports have shown that groundnut disease causes losses of up to 100% pod yield if infection occurs before flowering^[8,27–29]. The most outstanding landraces in terms of disease tolerance was the Agric-Dazhogwa but it should be subjected to further trials in a different environment before a conclusion can be drawn. However, this landrace is likely to possess the genes for disease resistance and, therefore, a potential candidate for a resistance breeding programme.

This study has identified genotypes that possess high yield. Top on the list was the INBRED LINE-10 made from a cross between Samnut 24 and ICGV-91328 yielding 517 kg/ha. Four other high yielding selections were inbred lines. This shows that the collections used in this work are bred for tolerance, resistance and yield qualities as

shown from the overall performances. They may complement the resilient landraces identified in this work including Agric-Musha, Bomboyi-Dugu and Agric-Dazhogwa by initiating a good breeding programme to achieve high yielding and resilient landraces. Apart from Agric-Dazhogwa, notable landraces that demonstrated some elements of tolerance to diseases include: Agric-Alwaza, Chika buhu-Lafia, Kpoklo-Gude, Bomboyi-Dugu, Akwashiki-Doma and Agric-Igbavo. The present study is in agreement with well-established reports^[8,29,30] that disease susceptibility depends on the type of plant variety. Hence, the use of host resistant varieties is the most effective, economical and sustainable way to control groundnut disease^[7].

The present outcome agrees with other authors who described the groundnut plant as a crop with a moderate level of character association in quantitative traits including yield^[24,31,32]. This work revealed the complexity of yield factor and it has further confirmed earlier reports on the complex character association of yield and other agronomic traits^[24]. This complexity in yield determination may be due to the polygenic nature of the inheritance of yield traits since the genes that control genes and disease resistance in many crops are polygenic, hence they are studied through QTL (quantitative trait loci) analysis. Quantitative traits are controlled by interactions and additive effects of many genes^[24,33]. In this study, plant survivorship determined yield by 51% while disease infestation affected yield by 15% only, most significantly the BNR@30 and DSV@60*RUST@60 interaction. The implication is that the remaining 34% in yield variability is attributed to other known and unknown factors. It further shows that the use of highly vigorous, resilient and disease resistant varieties is not a complete guarantee to achieve high yield in groundnut. The interpretation of this model is corroborated by the fact that the best genotype in yield component (Samnut 24 and ICGV-91328) coded as INBRED LINE-10 was not resistant to diseases as shown in the high incidence of 31% in early leaf spot disease, thus suggesting the need for resistance breeding on this variety. Khedikar^[8] reported that breeding for disease resistance was linked to undesirable traits like low

pod yield and small seed size. This assertion partly explains why inbred line–10 possessed a high yield but low resistance.

Generally, the outcome of this work is not in tandem with the report of Khedikar^[8] since most of the high yielding Inbred lines are also disease resistant. Molecular markers applied on different groundnut breeds are channeled towards breeding for yield or resistance/tolerance to some biotic and abiotic challenges^[1,5,34,35]. This present study has established a model where groundnut yield and its trend could be predicted using a simple regression analysis that involves survivorship and pathological indices. This type of model aligns with previous models in soybeans^[36]. More complex multi-factorial models may help provide useful information to unravel the complex nature of factors affecting the overall survival and yield of the groundnut crop^[13]. The outcome of molecular marker studies achieved through a multiplex of SSR primers linked to genes for resistance to a particular disease has identified 4 accessions that lacked the genes of interest. The inbred line–10 pointed out through morphological data as a susceptible cultivar is among the four accessions without the genes. The two varieties reported as resilient landraces (Agric-Musha, Bomboyi-Dugu) in the morphological data have been revealed as distinct accessions using RAPD analysis. Therefore, morphological and molecular data are complimentary. However, the actual level of similarity among the accessions was 41% as revealed by molecular data.

Results are consistent with the findings of Wang *et al.*^[24] who stated that molecular markers provide the genetic fingerprint that reveals true genetic convergence and divergence among varieties of a species since it is not influenced by environmental factors unlike in morphological studies. This is because they represent landmarks on DNA that are linked to various genes controlling. Moreover, SSR (Simple Sequence Repeats) markers are highly distinguishing microsatellites while both RAPD and SSR markers are highly polymorphic^[13,24]. It was observed that morphological expression could be an interplay or interaction of environment and genetic constitution in line with the

genotype \times environment effect called $g \times e$ effect postulated by geneticists^[22,31,32,37]. From the foregoing, all landraces that possess quality agronomic characteristics are well noted. This outcome is in agreement with other reports in that landraces are a valuable source of genetic diversity and possess useful traits for breeding^[18,37–39]. As such, they can be introduced into groundnut breeding programmes to incorporate unique genes such as resistance to biotic and abiotic stresses; and quality attributes.

5. Conclusion

In this study, yield related characters displayed considerable variability among the collections, especially the pod sizes and the overall grain yield. The established variability among the collections is supported by the low coefficient of similarity ranging from 29 to 100 in the analysis of morphological data. Seed infestation due to diseases was high (18%). The Inbred lines were noted for their high survivorship, good yield, and disease resistance. The line Samnut 24 \times ICGV–91328 had the highest yield (517 kg/ha) but was susceptible to diseases. Among the landraces, Agric-Musha, Bomboyi-Dugu and Agric-Dazhogwa were selected for high survivorship and disease resistance. This study has established a model where groundnuts yield and its trend could be predicted using a simple regression analysis that involves survivorship and pathological indices. Morphological and molecular data are complementary. The actual level of similarity among the accessions was 41% as revealed by molecular data. The selected inbred lines and landraces are valuable genetic resources that may harbor beneficial traits for breeding. Those accessions that possess quality agronomic traits should be presented to the growers.

Author contributions

Conceptualization, LOO and CUA; software, OJO and SST; validation, LOO; formal analysis, OJO and DP; investigation, IJ and OJO; resources, LOO and DP; data curation, OJO; writing—original draft preparation, IJ and CUA; writing—review and editing, IJ, CUA, OJO and LOO; visualization,

IJ; supervision, CUA, OJO and LOO; project administration, LOO; funding acquisition, LOO. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors wish to acknowledge the ICRISAT for their genetic resources. The management of the Molecular Biology Laboratory, Federal University of Agriculture Makurdi Nigeria is duly acknowledged.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Upadhyaya HD, Reddy LJ, Gowda CLL, Singh S. Identification of diverse groundnut germplasm. Sources of early maturity in a core collection. *Field Crop Research* 2006; 97(3): 261–271. doi: 10.1016/j.fcr.2005.10.010.
2. Gulluoglu L, Basal H, Onat B, *et al.* The effect of harvesting on some agronomic and quality characteristics of peanut grown in the Mediterranean region of Turkey. *Turkish Journal of Field Crops* 2016; 21: 224–232. doi: 10.17557/tjfc.20186.
3. Pandey MK, Monyo E, Akins PO, *et al.* Advances in *Arachis* genomics for peanut improvement. *Biotechnology Advances* 2012; 30(3): 639–651. doi: 10.1016/j.biotechadv.2011.11.001.
4. FAO. FAO statistical database [Internet]. Rome: Food and Agriculture Organization of the United Nations; 2017 [accessed 2023 Aug 31]. Available from: <http://www.fao.org/faostat>.
5. ICRISAT. Annual report 2015—Building climate smart farming communities [Internet]. Telangana: International Crops Research Institute for the Semi-Arid Tropics; 2015 [accessed 2023 Aug 31]. Available from: <http://www.icrisat.org>.
6. Frimpong A, Nyarko G, Bayor H, Apeliga JA. Effects of different seed treatment methods on the seedling vigor and biomass production of groundnuts in Ghana. *Pakistan Journal of Biological Sciences* 2004; 7(6): 1024–1028. doi: 10.3923/pjbs.2004.1024.1028.
7. Subrahmanyam P, Naidu RA, Reddy LJ, *et al.* Resistance to groundnut rosette disease in wild *Arachis species*. *Annals of Applied Biology* 2001; 139: 45–50. doi: 10.1111/j.1744-7348.2001.tb00129.x.
8. Khedikar PK. Molecular tagging and mapping of resistance to late leaf spot and rust in groundnut (*Arachis hypogaea* L.) [PhD thesis]. Dharwad: University of Agricultural; 2008. p. 154.
9. Abah RC. An application of geographic information system in mapping flood risk zones in a north central city in Nigeria. *African Journal of Environmental Science and Technology* 2013; 7(6): 365–371. doi: 10.5897/AJEST12.182.
10. Nigerian Population Commission (NPC)—Information on Nigerian population [Internet]. Abuja: NPC; 2016. Available from: <https://nationalpopulation.gov.ng/>.
11. Jambunathan R. Groundnut quality characteristics. In: Hall SD, Sudhir P, Rajan V, *et al.* (editors). *Uses of tropical grain legumes*. Proceedings of a Consultants' Meeting; 1989 Mar 27–30; Patancheru, India. Patancheru: ICRISAT Center; 1991. p. 267–275.
12. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). ICRISAT annual report 2015: Building climate-smart farming communities [Report]. Patancheru: ICRISAT; 2016.
13. Olasan JO, Aguoru CU, Omoigui LO, *et al.* Molecular genetic studies and QTL mapping for aflatoxin resistance in selected groundnut (*Arachis hypogaea* L.) breeding lines. *Agricultural and Biological Sciences Journal* 2020; 6(4): 202–210.
14. IBPGR/ICRISAT. Descriptors for groundnut (Revised). Rome: IBPGR Secretariat; 2021. p. 7–15.
15. Omoigui LO, Ishyaku MF, Gwoda BS, *et al.* Suitability and use of two molecular markers to track race-specific resistance *Striga gesnerioides* in Cowpea (*Vigna unguiculata* (L.) Walp.). *Africa Journal of Biotechnology* 2015; 14(27): 2179–2190. doi: 10.5897/AJB2015.14627.
16. Husain F, Mallikarjuna N. Genetic diversity in Bolivian landrace lines of groundnut (*Arachis hypogaea* L.). *Indian Journal of Genetics and Plant Breeding* 2012; 72: 384–389.
17. Kanyika BTN, Lungu D, Mweetwa AM, *et al.* Identification of groundnut (*Arachis hypogaea* L.) SSR markers suitable for multiple resistance traits for QTL mapping in African germplasm. *Electronic Journal of Biotechnology* 2015; 18: 61–67. doi: 10.1016/j.ejbt.2014.10.004.
18. Corrado G, Rao R. Towards the genomic basis of local adaptation in landraces. *Diversity* 2017; 9(4): 51. doi: 10.3390/d9040051.
19. Chandra K, Pandya SM. Morphological characterization of *Arachis* species section *Arachis*. *Plant Genetic Resources Newsletter* 2000; 121: 38–41.
20. Christenhusz MJM, Byng JW. The number of known plants species in the world and its annual increase. *Phytotaxa* 2016; 261(3): 201–217. doi: 10.11646/PHYTOTAXA.261.3.1.
21. Malti RK, Wesche-Ebeling P. The peanut (*Arachis hypogaea*) crop. Enfield: Science Publishers Inc.; 2002.
22. Janila P, Nigam SN, Manish KP, *et al.* Groundnut improvement: Use of genetic and genomic tools. *Frontiers in Plant Science* 2013; 4(23): 1–33. doi: 10.3389/fpls.2013.00023.
23. Balota MT, Isleib G, Tallury S. Variability for drought related traits of Virginia-type peanut cultivars and advanced breeding lines. *Crop Science*

- 2012; 52(6): 2702–2713. doi: 10.2135/crop-sci2012.03.0207.
24. Wang H, Lei Y, Yan L, *et al.* Development and validation of simple sequence repeat markers from *Arachis hypogaea* transcript sequences. *The Crop Journal* 2018; 6: 172–180. doi: 10.1016/j.cj.2017.09.007.
 25. Afolabi CG, Ezekiel CN, Kehinde IA, *et al.* Contamination of groundnut in Southwest Nigeria by aflatoxin in relation to processing. *Journal of Phytopathology* 2014; 163(4): 279–286. doi: 10.1111/jph.12317.
 26. Ahmed O, Olayinka BU, Garuba T, *et al.* Germination of several groundnut cultivars in relation to incidence of fungi. *Science World Journal* 2017; 12(1): 38–41.
 27. Monfort WS, Culbreath AK, Stevenson KL, *et al.* Effects of reduced tillage, resistant cultivars, and reduced fungicide inputs on progress of early leaf spot of peanut (*Arachis hypogaea*). *Plant Disease* 2004; 88: 858–864. doi: 10.1094/PDIS.2004.88.8.858.
 28. Waliyar F, Kumar PL, Ntare BR, *et al.* A century of research on groundnut rosette disease and its management. Information bulletin no. 75 [Report]. Telangana: International Crops Research Institute for the Semi-Arid Tropics; 2007.
 29. Okello DK, Akello LB, Tukamuhabwa P, *et al.* Groundnut rosette disease symptoms types distribution and management of the disease in Uganda. *Africa Journal of Plant Science* 2014; 8(3): 153–163.
 30. Kayondo SI, Rubaihayo PR, Ntare BR, *et al.* Genetics of resistance to groundnut rosette virus disease. *African Crop Science Journal* 2014; 22(1): 21–29.
 31. Teklu DH, Kebede SA, Gebremichael DE. Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in *Sesame* genotypes. *Asian Journal of Agricultural Research* 2014; 8(4): 181–194.
 32. Bayat M, Amirnia R, Rahimi M. Phenotypic and genotypic relationships between traits in saffron (*Crocus sativus* L.) as revealed by path analysis. *Journal of Applied Research on Medicinal and Aromatic Plants* 2017; 5: 33–40. doi: 10.1016/j.jar-map.2016.10.001.
 33. Andargie M, Pasquet RS, Muluvi GM, *et al.* Quantitative trait loci of flowering time related traits identified in recombinant inbred lines of cowpea (*Vigna unguiculata*). *Genome* 2013; 56(5): 289–294. doi: 10.1139/gen-2013-0028.
 34. Huang L, He H, Chen W, *et al.* Quantitative trait locus analysis of agronomic and quality-related traits in cultivated peanut (*Arachis hypogaea* L.). *Theoretical and Applied Genetics* 2015; 128: 1103–1115. doi: 10.1007/s00122-015-2493-1.
 35. Zhang X, Zhang J, He X, *et al.* Genome-wide association study of major agronomic traits related to domestication in peanut. *Frontiers in Plant Science* 2017; 8: 1611. doi: 10.3389/fpls.2017.01611.
 36. Hymowitz T, Newell CA. Taxonomy of the genus *Glycine*, domestication and uses of soybeans. *Economic Botany* 1981; 35(3): 272–288. doi: 10.1007/BF02859119.
 37. Singh S, Prakash A, Chakraborty NR, *et al.* Genetic variability, character association and divergence studies in *Jatropha curcas* for improvement in oil yield. *Trees* 2016; 30(4): 1163–1180. doi: 10.1007/s00468-016-1354-0.
 38. Varshney RK, Kudupa H, Roorkiwal M. *et al.* Advances in genomics and molecular breeding of three legume crops of semi-arid tropics using next-generation sequencing and high-throughput genotyping technologies. *Journal of Biosciences* 2013; 37(5): 811–820. doi: 10.1007/s12038-012-9228-0.
 39. Lopes MS, El-Basyoni I, Baenziger PS. *et al.* Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *Journal of Experimental Botany* 2015; 66: 3477–3486. doi: 10.1093/jxb/erv122.