Faculty of Natural and Applied Sciences Journal of Applied Biological Sciences
Print ISSN: 3027-0685, e-ISSN: 3043-6494
www.fnasjournals.com
Volume 2; Issue 3; May 2025, Page No. 139-149.
DOI: https://doi.org/10.63561/jabs.v2i3.943

Genotypic Variation and Resistance to Early Leaf Spot in Groundnut: Insights from Inoculated and Non-Inoculated Field Screening with Multivariate Analysis

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Abstract
Early leaf spot (ELS), caused by *Cercospora arachidicola*, is a highly damaging foliar disease that significantly restricts groundnut (*Arachis hypogaea* L.) production in West Africa, often causing yield losses of over 50% due to severe leaf defoliation and decreased pod and fodder output. Developing host plant resistance provides a sustainable and affordable approach to managing ELS, particularly benefiting smallholder farmers. This study aimed to assess the genetic variation in resistance to ELS among 183 groundnut minicore genotypes under both inoculated and non-inoculated field conditions during the 2019 rainy season at two known disease hotspots of Teaching and Research farm of Bayero University Kano and Samaru, ABU Zaria. The experiment was laid out in a 14 × 13 alpha lattice design with two replications. Data were collected on agronomic and disease-related traits including days to 50% flowering, pod weight, seed weight, kernel yield, fodder weight, disease incidence, disease scores at 65 and 90 days after sowing (DAS), disease severity, shelling percentage, harvest index, and number of hills at harvest. The data were analyzed using ANOVA, Principal Component Analysis (PCA), and cluster analysis. Highly significant genetic differences ($p \leq 0.05$) were observed among the genotypes for both disease resistance and yield traits under both conditions, with no significant genotype × environment interaction. Genotypes such as SAMNUT 22, ICG 12991, ICG 3240, and ICG 4540 exhibited consistently low ELS incidence and high yield performance, identifying them as promising candidates for breeding programs. In contrast, SAMNUT 26, ICGV-IS 07213, and SAMNUT 24 showed high susceptibility to ELS. PCA revealed that the first two principal components accounted for over 75% of total variation, with pod and seed weights negatively associated with disease scores at 90 DAS. Cluster analysis grouped genotypes into four distinct clusters that did not align with eco-geographical origin, suggesting that genetic diversity is not strictly geography-dependent. These findings underscore the presence of exploitable genetic variability and the feasibility of incorporating ELS resistance into groundnut improvement pipelines. The identified resistant genotypes offer valuable genetic resources for developing high-yielding, disease-resistant groundnut cultivars suitable for West African production systems.

Keywords: Groundnut, Genetic Variability, Early Leaf Spot, Multivariate Analysis, Disease Incidence

Introduction

Groundnut (*Arachis hypogaea* L., 2n = 4x = 40) is an important oilseed and food crop primarily grown in tropical and semi-arid tropical areas across the globe. Being a self-pollinating annual legume, it carries significant nutritional and economic value. The cultivated groundnut is tetraploid (Janila et al., 2013), and is cultivated across more than 100 nations on approximately 32.72 million hectares (FAOSTAT, 2023). In Africa, groundnut production expanded significantly between 1990 and 2018 due to increased output in West African nations including Nigeria, Senegal, Ghana, Burkina Faso, and Mali. Nigeria, now the third-largest producer globally, contributed about 25% of Africa's groundnut production in 2014 (FAOSTAT, 2017).

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Groundnut seeds contain 48-55% oil and 26-28% protein and are rich in dietary fiber, vitamins, and minerals (Gonçalves *et al.,* 2023). The haulms and groundnut cake also serve as valuable animal feed. Additionally, groundnut enriches soil fertility through atmospheric nitrogen fixation. Despite its importance, groundnut productivity remains low in Africa, typically under 1 ton/ha compared to the global average of 2 tons/ha. A key challenge is foliar diseases, particularly early leaf spot (ELS) caused by *Cercospora arachidicola*, which is prevalent in West African semi-arid savannas (Kankam *et al.*, 2022). ELS can cause up to 70% yield loss, with global economic impacts estimated in the hundreds of millions of dollars annually (Kankam *et al.*, 2022). The disease leads to severe defoliation, reduced photosynthesis, and ultimately reduced pod development and grain yield. Traditional management approaches rely on fungicide application. However, these increase production costs and pose environmental risks, making them unaffordable for many smallholder farmers (Kumar *et al.*, 2021). Consequently, host plant resistance offers a more sustainable and cost-effective alternative. Breeding groundnut genotypes with genetic resistance to ELS is therefore a priority for improving yields in endemic regions. This study aimed to evaluate the resistance of various groundnut lines to early leaf spot under both inoculated and natural field conditions, with the goal of identifying promising resistant genotypes for agronomic and breeding programs.

Materials and Methods

Experimental Sites

Field trials were conducted during the 2019 rainy season at two locations known for high early leaf spot (ELS) pressure: Bayero University Kano Research Farm, Kano and Institute for Agricultural Research farm Samaru, Zaria. The experimental sites are located in the Sudan and northern Guinea savanna ecology of Nigeria and are recognized hotspots for ELS epidemics due to their conducive environmental conditions.

Plant Materials

A total of 183 groundnut (*Arachis hypogaea* L.) minicore accessions were used in the study. These genotypes were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kano station.

Experimental Design and Treatments

The trial was conducted using a 14 × 13 alpha lattice design with two replications at each location. The study was carried out under two treatment conditions: Inoculated plots (artificially infested with ELS pathogen) and non-inoculated plots (natural infection conditions). Each plot consisted of a single row, 4 meters in length, with 0.75 m spacing between rows and 0.30 m between plants. Two seeds were sown per hill.

Artificial Inoculation

To ensure uniform ELS pressure in the inoculated plots, artificial inoculation was performed at 30 DAS (days after sowing) by spraying a spore suspension of *Cercospora arachidicola* using a hand sprayer between 5–6 PM to favor spore viability and disease development.

Fertilizer and Crop Management

Fertilization followed ICRISAT's standard protocol: a combination of single superphosphate (SSP) at 200 kg/ha and NPK (15:15:15) at 100 kg/ha, applied at a 2:1 ratio by side drilling 2–3 weeks after sowing (WAS).
Weeding was done manually at the 3rd, 8th, and 12th WAS. Soil remolding was also carried out to facilitate peg penetration and proper pod development.

Data Collection

The following traits were recorded:

i. Phenological Traits: Days to 50% flowering
ii. Yield Components: Pod weight (kg/ha), seed weight (kg/ha), fodder weight, Shelling percentage, Harvest index.
iii. Disease Parameters: Disease incidence (%), Disease scores (65 and 90 DAS) and Disease severity (visual rating scale)

Disease scoring was conducted using a modified 9-point scale, where 1 indicated no symptoms and 9 indicated severe defoliation.

Statistical Analysis

Data from both sites were combined and analyzed using Analysis of Variance (ANOVA) via the General Linear Model (GLM) procedure in SAS software (version 9.4). Mean separation was conducted using the Least

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Significant Difference (LSD) test at a 5% significance level. To evaluate trait relationships and classify genotypes according to their performance, Principal Component Analysis (PCA) and cluster analysis were performed on centered trait means using Singular Value Decomposition (SVD).

Results

Table 4.: Mean square of groundnut genotypes for agronomic and disease traits for early leaf spot disease in groundnut under inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season

Source	DF	DS65	DS90	DI	DS	HI	SP%	PW	
Replication	1	0.011	0.005	239.9	55.36	0.004	142.2	284.4	
Rep (Block)	26	0.019	0.008	93.86	76.37	0.013	76.02*	3E+05	2I
Genotypes (G)	182	0.03**	0.027**	96.83	140.1**	0.013	54.61	4E+05*	2E-
Location (L)	1	2.6**	0.036**	29008**	418.2*	1.148**	451.1**	4E+06**	2E-
G x L	182	0.021	0.009	92.19	80.13	0.011	55.55	3E+05	2I
Error		0.021	0.008	102	79.01	0.011	50.67	3E+05	2I

*****, and ****** Significant at 0.05 and 0.01 probability levels, respectively.
Days to 50% flowering: DFF, Pod weight: PW, Seed weight: SW, Fodder weight: FW: Disease incidence: DI, Disease scoring: DS 65 and DS 90, Disease severity: DS, Shelling percentage: sp%, Harvest Index: HI

Table 2: Mean square of groundnut genotypes for agronomic and disease traits for early leaf spot disease in groundnut under non inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season

Source	DF	DS65	DS90	DI	DS	HI	SP%	PW	
Replication	1	0.011	0.859**	1004**	3445**	0.002	2170**	13792	
Rep (Block)	26	0.009	0.011	104.4	46.37	0.007	54.97	3E+05	
Genotypes (G)	182	0.017**	0.033**	131.4	139.7**	0.009	58.45	4E+05	
Location (L)	1	0.031**	3.949**	5250**	20728**	2.291**	383**	1E+07**	7
G x L	182	0.011	0.019	134.6	90.94	0.008	63.11	3E+05	
Error		0.009	0.019	139.2	95.39	0.007	65.28	3E+05	

*****, and ****** Significant at 0.05 and 0.01 probability levels, respectively.
Days to 50% flowering: DFF, Pod weight: PW, Seed weight: SW, Fodder weight: FW: Disease incidence: DI, Disease scoring: DS65 and DS90, Disease severity: DS, Shelling percentage: sp%, Harvest Index: HI

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Table 3: Mean performance of groundnut genotypes for agronomic and disease traits for early leaf spot disease in groundnut under inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season (combined data)

Genotype	Days to 50% flowering	Disease scoring at 65 DAS	Disease scoring at 90 DAS	Fodder weight (kg/ha)	Seed weight (kg/ha)	Pod weight (kg/ha)	Shelling percentage	Harvest index	Disease severity at 90 (%)	Disease incidence at 90 (%)
SAMNUT 22	28.22	0.87	1.85	9962.01	868.33	1220.77	31.66	0.17	28.89	17.74
ICG 12991	29.40	1.12	2.16	13191.69	1088.29	1576.64	28.72	0.20	29.47	22.97
ICG 4763	26.82	1.81	2.43	9288.53	530.99	726.73	30.03	0.07	27.01	31.56
ICG 3240	28.79	1.41	2.50	9668.22	976.68	1348.17	26.44	0.13	27.00	40.04
ICG 4540	28.58	1.62	2.96	16681.79	1136.78	1563.59	29.78	0.14	32.83	30.16
ICG 6643	29.74	1.44	3.85	2897.21	221.20	298.25	27.14	0.10	42.70	39.05
ICGV-IS 15380	28.28	2.41	5.02	5858.94	301.83	429.36	28.43	0.07	55.32	36.33
ICG 8896	29.87	2.21	5.05	3598.36	231.01	323.13	29.68	0.07	56.17	36.32
ICG 1519	29.80	2.30	5.06	6350.93	437.23	624.87	29.82	0.11	56.22	46.93
ICG 9809	29.10	2.44	5.09	5501.95	370.90	527.74	26.07	0.08	56.62	34.72
ICG 5195	28.68	3.29	7.26	3752.12	100.02	161.45	35.62	0.05	80.63	24.87
ICG 3436	28.42	1.97	7.33	3934.47	577.31	788.13	29.71	0.21	82.18	35.37
ICG 334	26.84	2.08	7.36	5446.13	328.41	445.85	27.62	0.11	81.81	35.66
ICG 7463	29.22	2.88	7.38	5911.11	189.16	271.57	31.10	0.08	81.78	32.58
ICG6654	28.27	2.55	7.40	4219.71	476.82	712.83	28.91	0.20	82.32	15.69
ICGV IS 13840	29.50	2.18	7.46	3537.43	443.52	655.99	30.22	0.20	82.79	40.12
SAMNUT 24	29.81	2.87	7.50	5844.33	605.79	877.30	29.43	0.19	83.36	15.44
SAMNUT 26	29.90	2.78	7.63	3827.04	467.06	696.26	31.06	0.13	84.08	25.25
ICG 3140	29.08	2.80	7.71	3676.95	626.10	857.57	25.97	0.16	85.66	24.21
ICGV-IS 07213	29.77	2.31	8.01	4433.45	256.00	410.13	32.25	0.09	89.10	31.27

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Table 4: Mean performance of groundnut genotypes for agronomic and disease traits for early leaf spot disease in groundnut under non inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season (combined data)

Genotype	Days to 50% flowering	Disease scoring at 65 DAS	Disease scoring at 90 DAS	Fodder weight (kg/ha)	Seed weight (kg/ha)	Pod weight (kg/ha)	Shelling percentage	Harvest index	Disease severity at 90 (%)	Disease incidence at 90 (%)
SAMNUT 22	30.10	0.96	2.05	6269.62	723.81	1290.70	40.02	0.17	28.31	42.21
ICG 12991	28.62	1.11	2.13	3826.72	619.45	876.02	28.76	0.18	29.35	60.30
ICG 4540	27.15	1.99	2.29	4101.20	850.93	1144.18	27.61	0.34	25.24	37.10
ICG 3240	28.25	1.98	2.34	5369.00	582.20	894.17	34.10	0.14	26.25	32.40
ICG 4763	26.12	1.79	2.55	8313.66	1451.64	1990.90	25.27	0.25	28.35	25.98
ICG 9449	28.30	1.75	3.61	5737.40	471.90	680.25	33.82	0.11	40.29	35.97
ICGV 11542	30.86	2.02	3.77	8807.85	438.87	616.09	30.10	0.07	41.89	32.45
ICGV-IS 14985	27.69	1.95	3.77	6532.58	119.37	203.55	39.67	0.03	41.83	37.80
ICG 5236	31.11	2.34	4.11	5238.50	281.04	409.08	32.86	0.08	45.65	32.25
ICGV-IS 15380	27.68	1.80	4.14	6154.99	700.74	967.00	28.59	0.14	46.05	36.48
ICGV-IS 09992	29.44	2.73	6.95	5613.68	382.81	511.68	40.68	0.13	77.23	52.59
ICG 3673	28.11	2.36	6.96	5740.67	499.16	657.39	25.47	0.19	77.34	43.11
ICGV-IS 15415	29.78	2.36	7.06	6121.61	571.08	769.39	27.58	0.14	78.64	47.57
ICG 6344	27.70	2.06	7.07	4516.34	277.22	463.74	37.98	0.11	78.54	34.69
ICG 3436	28.33	2.34	7.07	4544.42	322.52	468.89	35.60	0.08	78.35	32.36
ICG 513	30.66	2.04	7.12	4911.50	208.34	117.08	36.68	0.04	78.83	57.64
SAMNUT 24	28.83	2.23	7.12	6616.37	644.21	833.15	26.72	0.14	79.23	41.92
ICG 9666	28.08	2.21	7.28	3349.08	477.45	600.45	31.63	0.16	81.11	23.19
ICGV-IS 07213	28.81	2.08	7.34	9395.75	420.66	587.17	27.21	0.08	81.30	43.37
SAMNUT 25	27.73	2.22	7.46	3079.35	501.82	647.01	27.02	0.20	82.83	64.91

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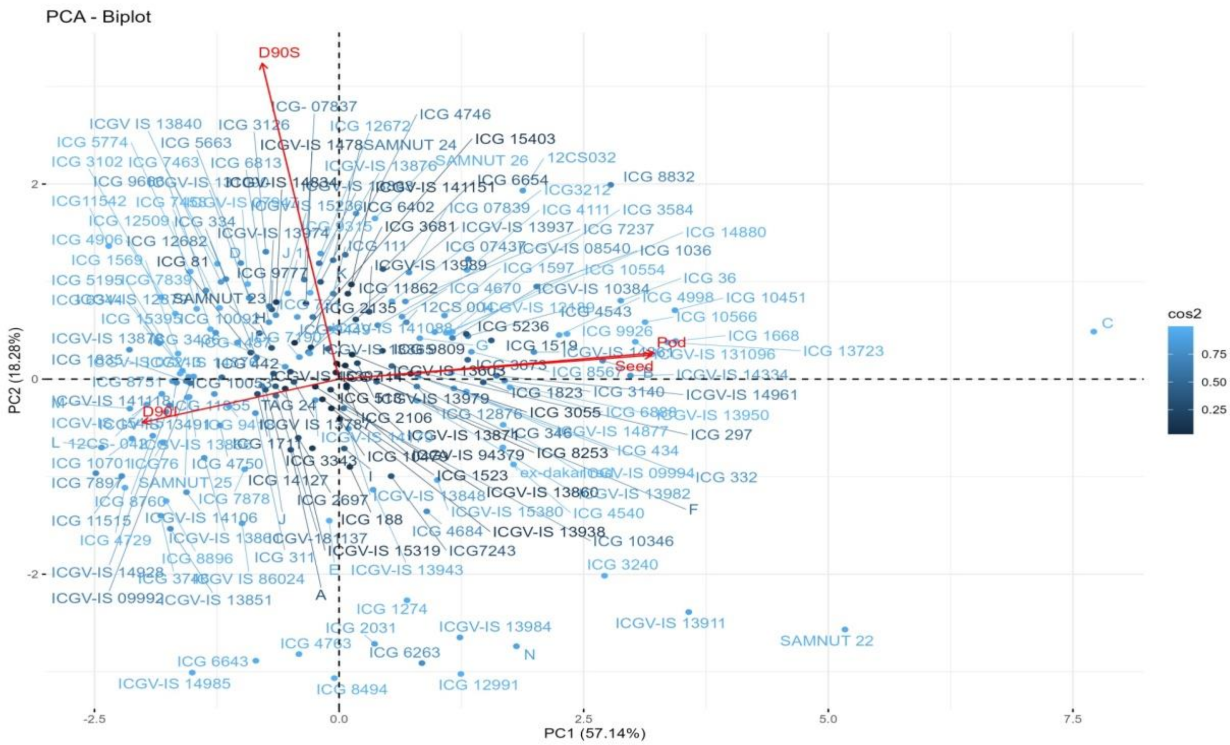

Figure 1: Principal component analysis showing the diversity among groundnut genotypes based on yield and disease traits under inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season.

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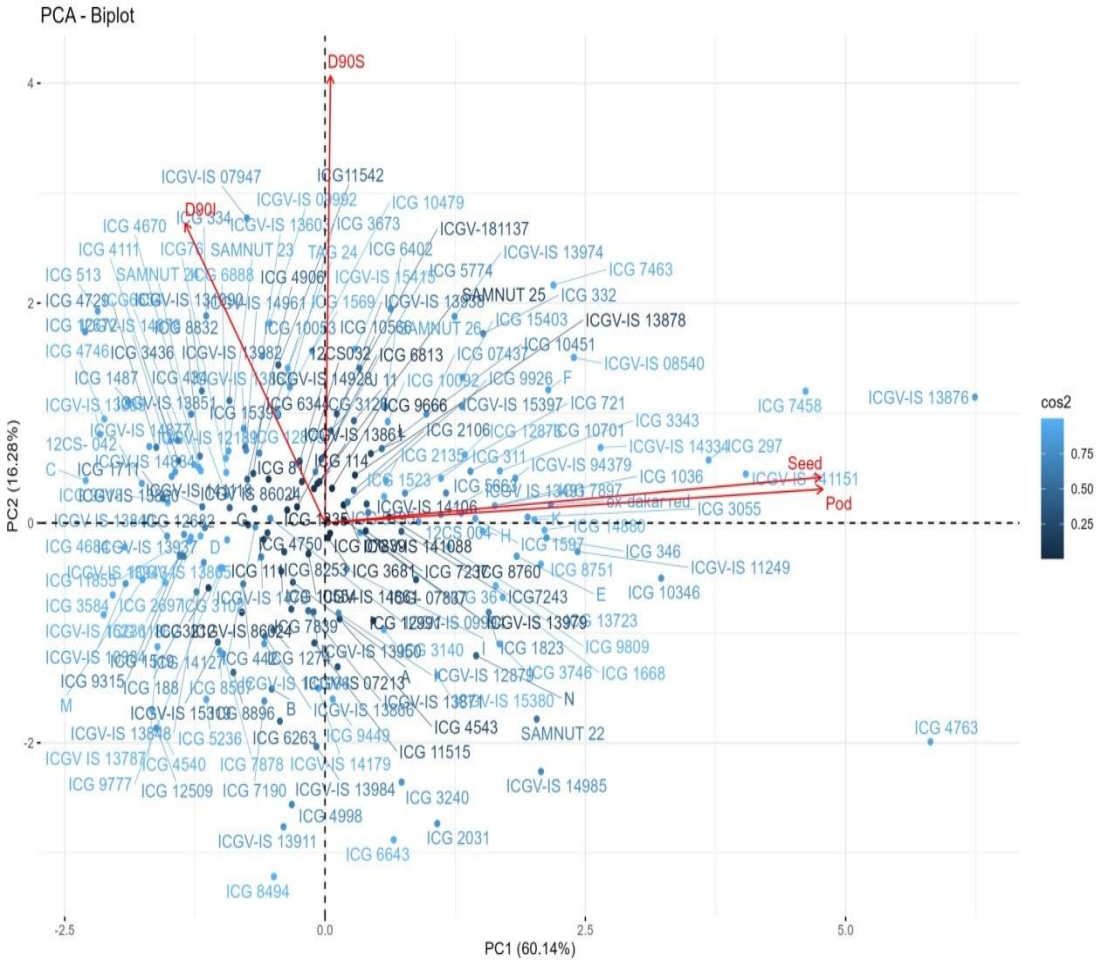

Figure 2: Principal component analysis showing the diversity among groundnut genotypes based on yield and disease traits under non inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season.

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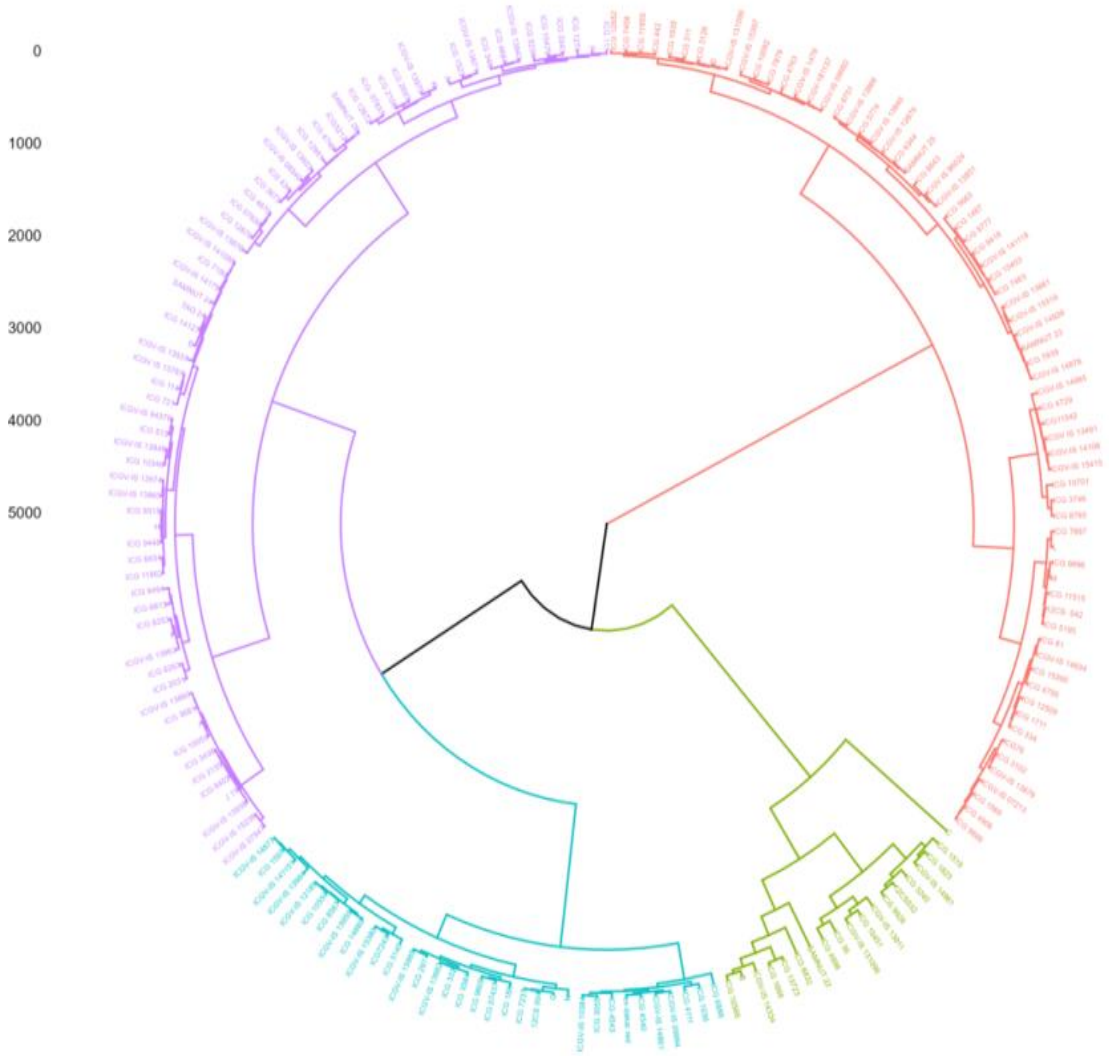

Figure 3: Dendogram showing grouping of groundnut genotypes for yield and disease traits under inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season.

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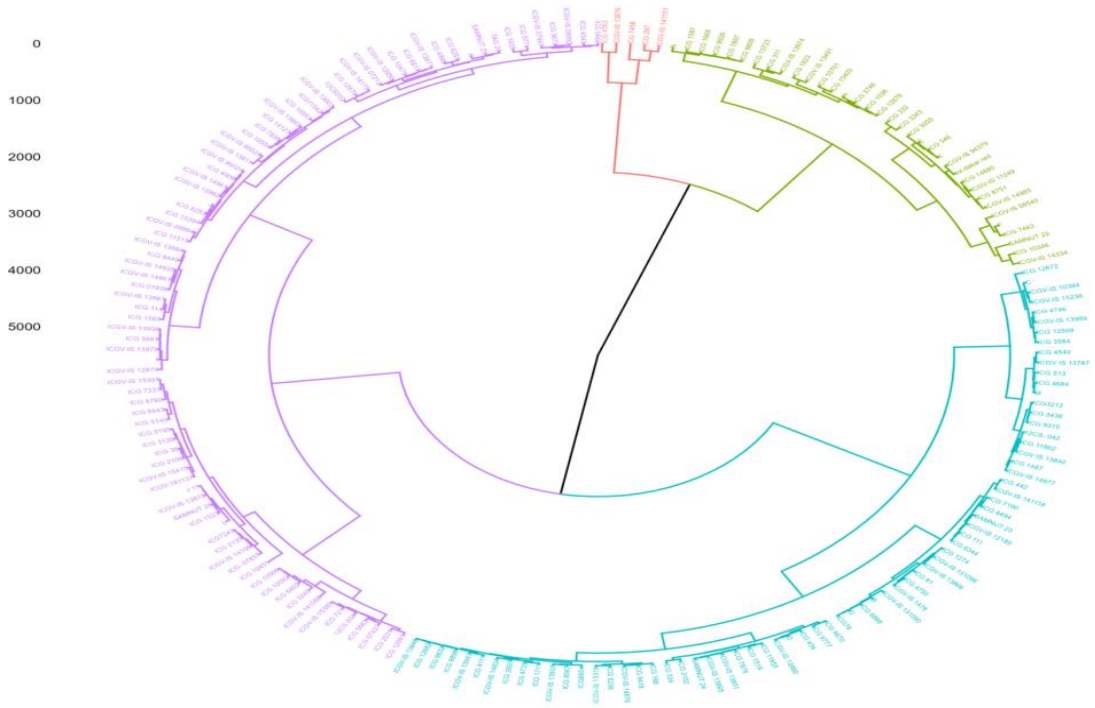

Figure 4: Dendogram showing grouping of groundnut genotypes for yield and disease traits under non inoculated condition evaluated at BUK and Samaru during 2019/2020 raining season.

Discussion

Disease Response Under Inoculated Conditions

The combined analysis of variance (ANOVA) from BUK and Samaru under inoculated conditions revealed significant variation among genotypes for most disease and yield-related traits (Table 1). Specifically, highly significant differences ($p \leq 0.01$) were observed among genotypes for disease scores at 65 and 90 DAS, disease severity at 90 DAS, and seed weight. Significant differences ($p \leq 0.05$) were recorded for pod weight, while there was no significant genotype × environment (G×E) interactions observed for any traits, indicating stable performance across locations. These results confirm the presence of genetic variability for disease resistance and yield potential, consistent with earlier findings that genetic diversity facilitates breeding gains in groundnut (Falconer & Mackay, 1996).

Disease Response Under Non-Inoculated Conditions

Under natural (non-inoculated) conditions, ANOVA showed a highly significant differences ($p \leq 0.01$) among genotypes for disease scores and severity at both 65 and 90 DAS (Table 2), while there was no significant G×E interactions, reaffirming genotype stability across environments. This suggests that even under natural pressure, genotypes expressed distinguishable levels of resistance or susceptibility to ELS. This result is supported by findings of Shaibu et al. (2021), who evaluated groundnut minicore collections under both natural and artificial disease pressure in multiple environments, revealing significant genotypic differences and stability of ELS resistance.

Mean Performance of Genotypes

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Under inoculated conditions, disease score at 65 DAS ranged from 0.87 to 3.29, indicating resistance at early stages (Table 3). At 90 DAS, genotypes such as SAMNUT 22 exhibited strong resistance (score: 1.85; severity: 27.00%), while ICGV-IS 07213 was most susceptible (score: 8.01; severity: 89.10%). For yield, ICG 4540 had the highest seed weight (1136.78 kg/ha), and ICG 12991 had the highest pod weight (1576.64 kg/ha).

Under non-inoculated conditions (Table 4), all the genotypes under investigation showed initial resistance at 65 DAS (scores 0.96–2.73). however, at 90 DAS, SAMNUT 26 was most susceptible (score: 7.46; severity: 82.83%), while ICG 4540, ICG 9666, and SAMNUT 22 displayed the strongest resistance. These findings align with previous studies that document wide genetic variability for foliar disease resistance and yield in groundnut (Zongo *et al.*, 2019).

Principal Component Analysis (PCA) and Trait Associations

Principal Component Analysis (PCA) was conducted on the mean values of the entire training population, producing a reduced-dimension model that highlights the observed differences among the genotypes within the population. The biplot was constructed by joining the vertices of the genotypes that were furthest from the biplot origin. PCA reduced the dataset into key components that explained over 75% of total phenotypic variation under both treatment conditions (Fig 1 and Fig 2). The PC1 was the most influential, driven primarily by seed weight and pod weight. Disease score at 90 DAS was negatively associated with yield traits, indicating that resistance is positively correlated with higher productivity. Genotypes such as ICGV-IS 141151, ICGV-IS 13876, and ICG 7458 clustered as high-yielding and disease-resistant, while SAMNUT 26 and ICGV-IS 07213 were grouped among the susceptible lines. This is consistent with reports that yield and disease resistance are often negatively correlated, requiring careful selection in breeding programs (Shaibu *et al*., 2020).

Cluster Analysis

Four distinct clusters were observed under both inoculated and non-inoculated conditions (Fig. 3 and Fig. 4). Under inoculated conditions, Clusters I, II, III, and IV consisted of 56.5%, 24.2%, 18.8%, and 0.31% of the genotypes, respectively. Similarly, under non-inoculated conditions, the clusters contained 50.57%, 25.87%, 23.1%, and 0.43% of the genotypes. Genotypes did not form distinct clusters based on specific traits; rather, they were widely distributed across all clusters.

Moreover, clustering did not align with eco-geographical origin, supporting the conclusion that genetic diversity is not solely determined by geographic location consistent with recent findings on the weak association between geography and genetic variation in groundnut (Banla *et al*., 2020). This underscores the need to prioritize molecular characterization over geographic assumptions in germplasm utilization.

Principal Component Analysis (PCA) showed that the first two principal components accounted for over 75% of the total genetic variation under both inoculated and non-inoculated conditions, with PC1 contributing the most. Similar results were reported by Shaibu *et al.* (2020), where major components explained most of the trait variation in groundnut diversity panels. Seed and pod weights were highly positively correlated, while disease scoring at 90 DAS and disease incidence showed moderate correlation but remained distinct traits. PCA revealed a strong negative relationship between yield traits and disease severity, indicating that increased resistance enhances yield potential. Daudi *et al*. (2021) reported similar findings. These results highlight the value of multivariate analyses in identifying promising genotypes for breeding programs. Such approaches are supported by recent studies emphasizing the integration of phenotypic and genotypic data for selecting groundnut lines with both resistance and high yield potential (Shaibu *et al.*, 2021).

Conclusion

This study revealed significant genetic variability among 183 groundnut genotypes for early leaf spot (ELS) resistance and agronomic traits under both inoculated and non-inoculated field conditions. The presence of highly significant differences in disease scores, severity, and yield-related traits confirms the existence of exploitable genetic diversity crucial for breeding programs. Genotypes such as SAMNUT 22, ICG 12991, and ICG 3240 consistently exhibited low ELS incidence and severity, identifying them as promising candidates for resistance

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breeding. Conversely, SAMNUT 26, ICGV-IS 07213, and SAMNUT 24 were highly susceptible and are less suitable for deployment in ELS-prone environments. Principal Component Analysis (PCA) revealed that the first two components accounted for over 75% of the total variation, with disease traits showing a strong negative association with yield traits, indicating that resistance is linked to better productivity. Cluster analysis grouped genotypes independently of eco-geographical origin, emphasizing that genetic diversity is not solely determined by geography and reinforcing the need for molecular selection approaches.

Based on these findings, we recommend the use of SAMNUT 22, ICG 12991, and ICG 3240 as parent lines in breeding programs targeting ELS resistance and yield improvement. Their stability across both inoculated and non-inoculated conditions supports their utility in diverse environments. To accelerate the breeding process, molecular characterization and marker-assisted selection should be employed to validate resistance traits. Further multi-location and multi-season trials are also advised to confirm the consistency of resistance and agronomic performance across varying conditions.

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Cite this article as:
Lawan, Z. M., Usman, A., Mohammed, M. S., Umar, M. L., Ajeigbe, H. A., & Shittu, E. A. **(**2025**)**. Genotypic variation and resistance to early leaf spot in groundnut: insights from inoculated and non-inoculated field screening with multivariate analysis**.** *FNAS Journal of Applied Biological Sciences, 2*(3), 139-149