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Genotype × Environment Interaction for Resistance to Early Leaf Spot of Groundnut Mini Core Collections in the Savannas of Nigeria

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ABSTRACT

Background: The genetic yield potential of groundnut (*Arachis hypogaea* L.) has been continuously challenged by several diseases including early leaf spot (ELS).

Methods: In the current study, we evaluated groundnut mini core collections under artificial and natural disease epiphytotics in six environments to identify stable elite sources for ELS resistance and pod yield. Mixed model analysis was done to adequately capture the variance component as a result of genotype (G), environment (E) and G × E interaction (GEI).

Result: Highly significant (p <0.001) effects for G and GEI on ELS and pod weight were observed. The parametric and non-parametric stability models ranked the genotype differently for their stability to ELS. The GGE biplot identified ICG 1519 as a stable genotype for the ELS resistance. For pod weight, ICG 8896 and ICG 7897 were consistently stable from all the stability models including the GGE biplot. ICG 9449 and ICG 4540 were identified as stable genotypes for both ELS and pod weight. These elite sources of ELS resistance identified in the current study will be useful in the development and deployment of groundnut varieties with resistance to ELS and high pod yielding potentials.

Key words: ELS, GEI, Groundnut, Pod weight, Resistance, Stability.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food legume grown worldwide and is considered to be a rich source of protein for both humans and livestock. Groundnut is a part of the mainstay to the livelihood of millions of small-holder farmers residing in semi-arid tropics (SAT) regions of the world. The largest producers of groundnut are China and India, followed by Nigeria. In Africa, Nigeria is the largest producer of groundnut (2.89 mt, 20.18%) followed by Sudan (2.88 mt, 20.16%) and the United Republic of Tanzania (0.94 mt, 6.57%) (FAOSTAT, 2020).

The genetic yield potential of groundnut cultivars has been continuously challenged by several diseases including early leaf spot (ELS) caused by *Cercospora arachidicola* (Subrahmanyam *et al*., 1980). ELS disease causes yield loss of up to 70%, resulting in a loss of approximately $600 million (Shaibu *et al*., 2020). While insecticides and fungicides have been used as part of integrated pest management approaches, breeding disease-resistant cultivars with high yield and good agronomic performance are the most economical and sustainable solution (Guo *et al*., 2013). In Nigeria, low productivity in groundnut has been attributed mainly to its exposure to a range of biotic stresses (Motagi, 2015). Thus, identification of nutrition-rich peanut cultivars possessing genetic resilience against ELS with enhanced pod yield is required to maintain sustained support to livelihood for millions of poor.

In multi-environment trials aimed at identifying superior genotypes for recommending to groundnut producers, stress factors such as diseases frequently induce genotype × environment interaction (GEI) that reduces the efficiency with which truly high yielding genotypes are selected (Padi, 2008). High and stable yields are therefore important to both groundnut producers and breeders although high genetic yield potential is frequently associated with decreased yield stability (Chaudhari *et al*., 2019; Shaibu *et al*., 2020). Reliance on low yielding varieties that are associated with higher yield stability, however, eliminates farmers' chances of exploiting the yield and economic potential of the crop.

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It should be noted that the genetics of a particular trait may vary with variation in plant material and the environment in which the materials are evaluated. Therefore, it is important to understand the genetics and stability of ELS resistance using the available breeding materials before starting a breeding program on ELS. The current study was conducted with a view of identifying groundnut germplasms that are stable for resistance to ELS disease.

MATERIALS AND METHODS

The research was carried out at Bayero University, Kano (BUK, Latitude 11°58′ N and Longitude 8°25′ E) in 2016, 2017 and 2019; and Institute for Agricultural Research, Samaru Zaria (SMR, Latitude 11°11′ N and Longitude 7°38′ E) in 2019. In 2019, one field in each location was artificially inoculated with ELS to increase the inoculum load of the disease. The laboratory culture spores of ELS were sprayed on groundnut germplasm grown in the field 30 days after sowing (DAS). The weather information recorded for the different environments is presented in Fig 1.

One hundred and eighty-two groundnut mini core accessions including five check varieties were evaluated using 14 × 13 randomized incomplete block design with two replications. The description of the mini core collection along with the checks has been published previously (Shaibu *et al*., 2020). Single row plots measuring 4 m in length with inter- and intra-row spacing of 0.75 and 0.1 m, respectively were used. A basal dose of Nitrogen, Phosphorous and Potassium was applied to all the plots @ 20:40:40 kg ha^{-1}, of N:P_2O_5:K_2O at planting. Hand weeding was done using hoes at 3rd, 8th and 12th weeks after sowing (WAS) to prevent weed infestation and competition between plants and weeds.

The genotypes were categorized using a disease severity scale of 0-9 (Subrahmanyam *et al*., 1995) into resistant (≤ 3), moderately resistant (4-5), susceptible (6-7) and highly susceptible (>7) as described by Sudini *et al*. (2015).

Data analysis

To adequately capture the effect of genotype (G), environment (E) and GEI, a mixed model analysis was done using PROC MIXED in SAS (SAS Institute, 2015). The genotypes that had ≤ 3 disease severity scores for ELS in each location were selected to check their stability for their reaction against ELS and pod weight across the environments. The stability analysis was done using all the integrated stability methods in STABILITYSOFT online program (Pour Aboughadareh *et al*., 2019).

Also, GGE biplot analysis was done using "R" packages to visualize the GEI based on the following model:

$$Y_{ij} - \beta_j = b_j\alpha_i + \lambda_1\eta_{j1} + \Sigma_{ij}$$

Where,

Y_{ij} is the average yield of genotype i in environment j, β_j is the average yield of all genotypes in environment j, α_i is the main effect of genotype i, b_j is the regression coefficient of the environment-centered yields (*i.e*., Y_{ij}-β_j) within environment j on the genotype main effects (α_i). $\lambda_1 \Sigma_{i1}\eta_{j1}$ is the first principal component (PC) from singular value decomposition (SVD) of the residual. The values of λ_1, Σ_{j1} and η_{j1} are simultaneously obtained by subjecting the environment-centered yield (*i.e*. Y_{ij}-β_j) to SVD.

RESULTS AND DISCUSSION

Phenotypic variation for ELS and pod weight

The variance component analysis revealed highly significant (p <0.0001) genotype and GEI effects for ELS and pod weight (kg ha^{-1}) indicating that the genetic components and the GEI are more important in the reaction of groundnut genotypes against ELS and pod weight (Table 1). High variability in groundnut against ELS reaction has been previously reported (Shaibu *et al*., 2020; Zanjare *et al*., 2020). The significant G and GEI effects for resistance to ELS suggested the possibility of identifying resistant genotypes adapted specifically to a target environment and the need to deploy specifically adapted varieties in the future for more effective genetic control of ELS. The percent contribution of genotype to the total variability for ELS was 4.07% while the environment and GEI accounted for 55.84 and 5.11%, respectively. For pod weight, the genotype contributed 6.43% of the total variability while, the environment and the GEI contributed 5.95 and 18.20%, respectively.

The phenotypic and genotypic coefficient of variation (PCV and GCV) were moderate in each environment and the genetic advance as percent of the mean (GAM) ranged from 6.97% (BUK 2017) to 23.85% (BUK 2016) (Table 2). Similar PCV, GCV and GAM for late leaf spot (LLS) resistance in groundnut have been previously reported

Table 1: Variance component analysis for ELS and pod weight across environments.

Random effect	Early leaf spot			Pod weight (kg ha^{-1})		
	Variance component	Wald p-value	Per cent of total	Variance component	Wald p-value	Per cent of total
Replication	0.03	0.3534	0.68	-243.08	0.1106	0.00
Genotype (G)	0.16	<.0001	4.07	32483.51	0.0001	6.43
Environment (E)	2.13	0.1148	55.84	30069.35	0.1332	5.95
G × E	0.20	<.0001	5.11	91941.30	<.0001	18.20
Residual	1.31		34.30	350682.76		69.42
Total	3.82		100.00	505176.93		100.00

Table 2: Descriptive statistics of ELS for the individual environment.

Environment	Mean	Min	Max	CV (%)	Genotypic CV (%)	Phenotypic CV (%)	GAM (%)
BUK_16	2.77	1	5	30.83	18.87	30.75	23.85
BUK_17	3.60	2	6	22.50	8.73	22.54	6.97
BUK_19I	4.66	1	8	38.09	14.70	35.41	12.57
BUK_19U	6.19	2	8	20.99	8.92	20.68	7.93
SMR_19I	6.01	1	9	23.71	13.56	23.68	16.00
SMR_19U	6.27	1	8	21.91	9.42	22.01	8.30

I = inoculated; U = uninoculated; CV = coefficient of variation; GAM = genetic advance as percent of mean.

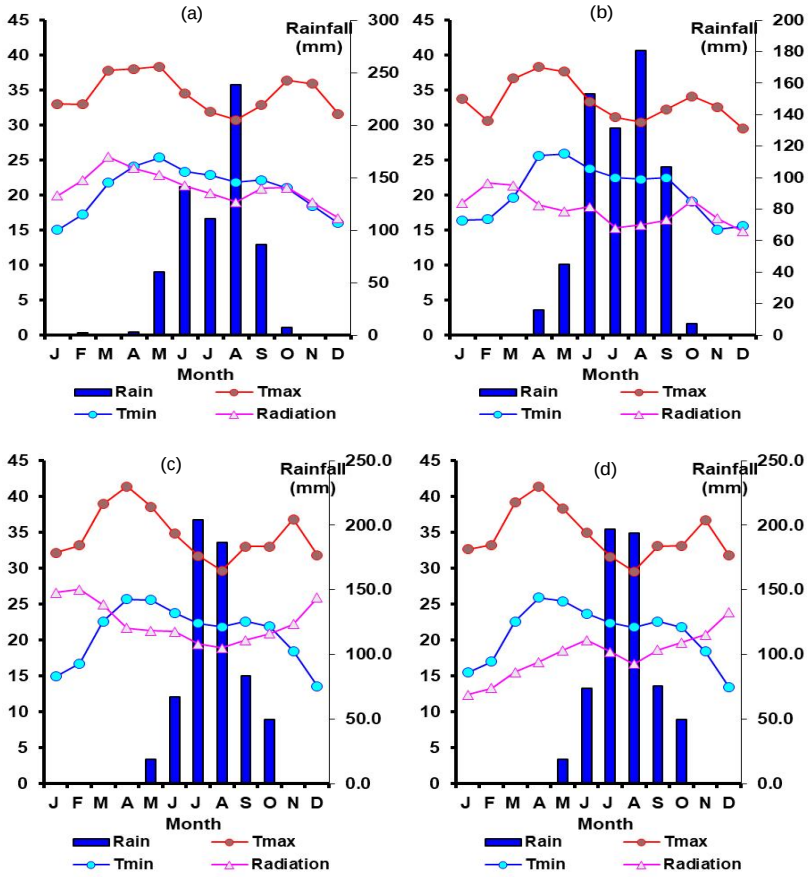

Fig 1: Weather information of the experimental locations. (a) BUK 2016 (b) BUK 2017 (c) BUK 2019 and (d) Samaru 2019. The alphabets J to D represent January to December, respectively.

(John *et al*., 2006; Vishnuvardhan *et al*., 2013; Chaudhari *et al*., 2019).

Disease reactions of genotypes against ELS

The number of genotypes in each environment for resistant and moderately resistant categories was highly variable (Fig 2). This highlighted the important role of GEI and the polygenic nature of ELS. This result was similar to that of Chaudhari *et al*. (2019) who showed that the reaction of groundnut to LLS was highly variable in different environments owing to the complex nature of the disease resistance, which is governed by polygenes with additive gene effects. The number of genotypes in the resistant and moderately resistant categories respectively was 62 and 73 (BUK 2016), 50 and 71 (BUK 2017), 25 and 100 (BUK 2019I), 5 and 18 (BUK 2019U), 8 and 22 (SMR 2019I) and 8 and 12 (SMR 2019U). ICG 3240 and ICG 4540 had ELS score ≤ 3 in all the environments. One of the improved varieties used (Samnut 22) also had an ELS score of ≤ 3 in all environments except at BUK 2017 where it had a score of 4. The other improved varieties used had varied responses to ELS in different environments. In 2019, at both BUK and SMR, six genotypes including Samnut 22 had ELS score of ≤ 3.

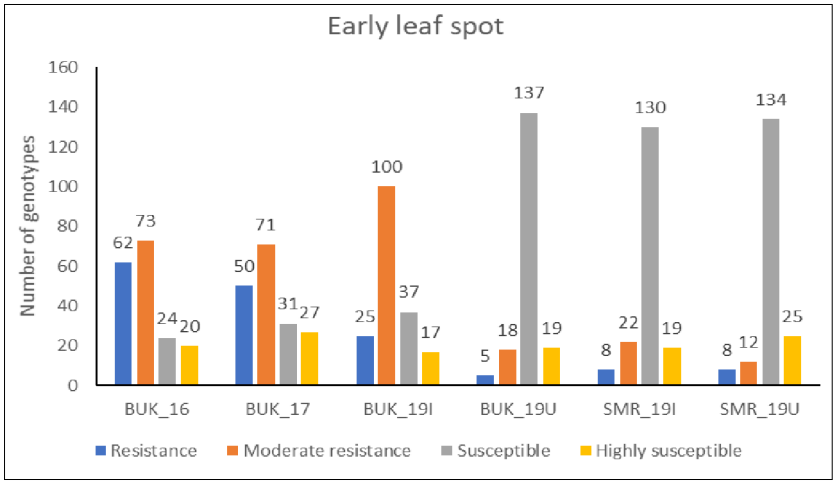

Fig 2: Categorization of genotypes based on reaction against ELS at 90 DAS in the individual environment.

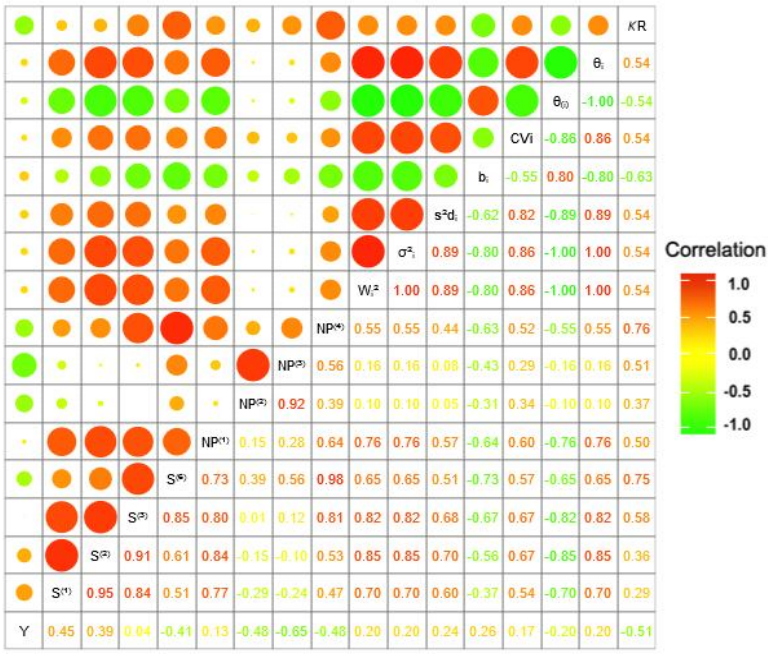

Fig 3: Correlation among parametric and non-parametric stability parameters for ELS pooled across environments.

Stability of ELS reaction across the environments

An important objective of resistance breeding is to identify genotypes with durable resistance irrespective of the environment (Chaudhari *et al*., 2019). To identify consistent sources of ELS resistance, 48 genotypes that had ELS scores of ≤ 3 (except for the six checks) were subjected to stability analysis using different parametric and non-parametric statistics (Table S1). Wricke's ecovalence (Wi^2) had positive correlations with all other stability parameters except for the regression coefficient (bi) and the GE variance component (Fig 3). Shukla's stability variance (σ^2i) also had a similar trend with Wi^2. Based on Wi^2, σ^2i and GE variance component, ICG 14106 was the most stable variety for ELS resistance. Six of the stability parameters ranked ICG 14106 as the most stable genotype while four ranked it as the second stable genotype. ICG 4540 was identified as the most stable genotype based on deviation from regression (S^2di) and coefficient of variance (CVi) stability parameters. The few discrepancies observed in the ranking of the genotypes among the parametric and non-parametric statistics could have arisen due to the differences in the analytical methods (Pour Aboughadareh *et al*., 2019).

The first two PCs of the GGE biplot accounted for 58.27% of the total variation. This shows that most of the variabilities from the GEI were explained by the first two PCs. From the biplot of the relationship among the environments, BUK19I and SMR19I were related (Fig 4a). The similarity observed in the GGE biplot between BUK19I and SMR19I might be due to the creation of artificial

Table S1: Stability parameters for ELS across the four environments.

Code	Genotype	Wricke's ecovalence	Shukla's stability variance	Deviation from regression	Coefficient of variance	Kang's rank-sum	GE variance component
1	4540	9	9	1	1	32	9
2	3240	42	42	37	44	48	42
3	12991	30	30	15	8	40	30
4	SAMNUT22	32	32	9	3	41	32
5	9449	47	47	48	48	46	47
6	14985	39	39	36	39	35	39
7	11542	20	20	34	14	27	20
8	8494	38	38	41	41	42	38
9	4763	41	41	3	4	45	41
10	1668	12	12	23	23	27	12
11	4998	26	26	29	27	22	26
12	6263	36	36	39	34	34	36
13	15236	27	27	31	32	12	27
14	8896	23	23	32	36	32	23
15	14179	4	4	6	24	20	4
16	15380	10	10	20	21	22	10
17	1519	3	3	12	18	19	3
18	12509	17	17	30	7	16	17
19	442	8	8	17	5	11	8
20	13787	5	5	10	16	14	5
21	114	43	43	44	42	44	43
22	7897	28	28	27	40	35	28
23	131096	44	44	46	45	43	44
24	6402	31	31	25	38	25	31
25	6643	40	40	42	19	37	40
26	15415	45	45	47	43	39	45
27	5236	34	34	40	30	37	34
28	13943	46	46	43	46	46	46
29	1478	48	48	45	47	29	48
30	3584	16	16	7	37	22	16
31	15403	29	29	35	28	25	29
32	6813	22	22	22	22	14	22
33	10384	24	24	33	29	30	24
34	4684	2	2	5	17	1	2
35	5774	37	37	38	26	30	37
36	12682	18	18	4	31	13	18
37	4670	21	21	19	33	7	21
38	SAMNUT26	35	35	13	35	20	35
39	14106	1	1	2	12	10	1
40	7463	25	25	21	25	17	25
41	9315	13	13	16	11	8	13
42	3436	33	33	28	20	18	33
43	Exdakarred	6	6	18	2	3	6
44	J11	11	11	14	13	4	11
45	SAMNUT23	7	7	11	9	2	7
46	SAMNUT24	19	19	24	15	9	19
47	SAMNUT25	14	14	8	10	6	14
48	TAG24	15	15	26	6	5	15

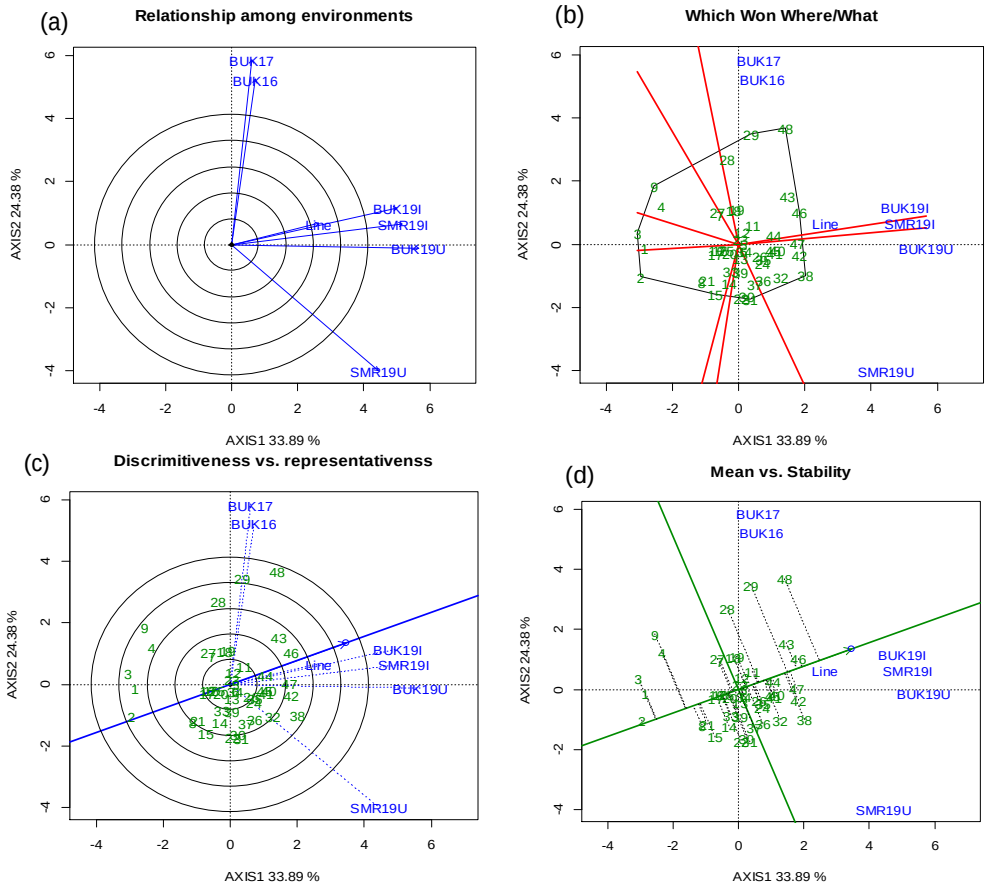

Fig 4: GGE biplot analysis for disease severity scores to ELS at 90 DAS. (a) relationship among environments (b) polygon view of biplot showing ranking of genotypes based on which won where (c) discrimitiveness vs representativeness of environments for an ideal test environment for disease severity of ELS and (d) GGE biplot showing ranking of genotypes for mean and stability of disease severity scores of ELS.

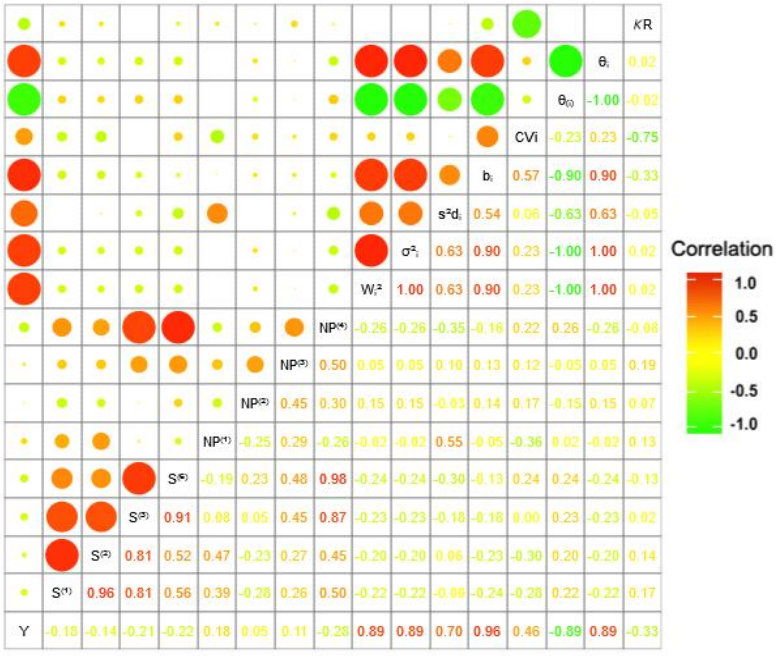

Fig 5: Correlation among parametric and non-parametric stability parameters for pod weight pooled across environments.

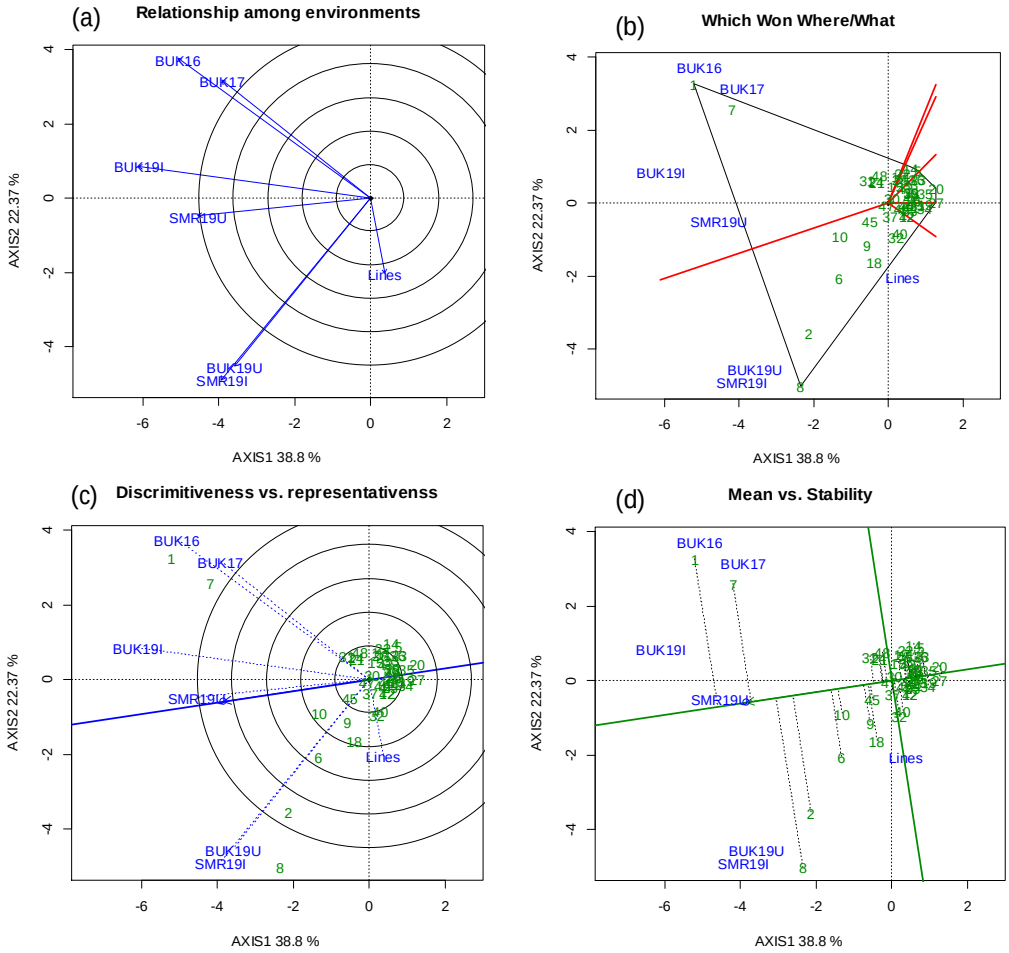

Fig 6: GGE biplot analysis for pod weight. (a) relationship among environments (b) polygon view of biplot showing ranking of genotypes based on which won where (c) discrimitiveness vs representativeness of environments for an ideal test environment for pod weight and (d) GGE biplot showing ranking of genotypes for mean and stability of pod weight.

epiphytotics by ELS spore inoculation in these two environments. The which won where/what biplot of the data showed that line 48 (TAG24) was the most resistant genotype among the selected genotypes at BUK in 2016 and 2017 (Fig 4b). Lines 1 (ICG 4540) and 3 (ICG 12991) had superior performances (resistant) in all the environments. The discriminativeness and repres entativeness of BUK19I and SMR19I (Fig 4c) were largely due to the inoculation with ELS carried out in these environments. Therefore, for cultivar evaluation against ELS resistance, the test environments should contain the right inoculum. Line 2 (ICG 3240) was the most resistant and stable genotype (Fig 4d) and other genotypes such as J11 and Samnut 24 were also stable but had ELS scores >5 across the environments. Samnut 22 (line 4) which showed resistance to ELS was, however, not stable. All the stability model used consistently identified ICG 1519 as a stable genotype. Therefore, ICG1519 can be used as an elite source of resistance against ELS in groundnut breeding programs in Nigeria.

Stability of pod weight across the environments

The correlations between the parametric and non-parametric statistics were majorly negative (Fig 5). Wricke's ecovalence had positive correlations with regression coefficient (bi), deviation from regression (s^2di) and Shukla stability variance (σ^2i). Based on Wricke's ecovalence, ICG 7897 was the most stable genotype followed by ICG 8494, ICG 8896, ICG 7463 and ICG 9449. Kang's rank-sum statistic ranked ICG 9449 and ICG 8494 as the most stable genotypes followed by ICG 8896, ICG 12991 and ICG 7897.

The first two PCs of the GGE biplot explained 61.17% of the observed variations. The relationship among environments biplot (Fig 6a) showed that BUK16 and BUK17 are highly related. From the which won where/what biplot, lines 1 (ICG 4540) and 7 (ICG 11542) were ideal for BUK16 and BUK17 environments (Fig 6b). Lines 8 (ICG 8494) and 2 (ICG 3240) were ideal for BUK19U and SMR19I environments. SMR19U was the most discriminative and representative environment for the evaluation of the genotypes for pod weight (Fig 6c). The stability of the

genotypes was determined by their projections onto the average-tester coordinate y-axis single-arrow line. The greater the absolute length of the projection of a genotype, the less stable it is. Lines 4 (Samnut 22), 30 (ICG 3584) and 44 (J11) were stable (Fig 6d). ICG 9449 and ICG 4540 were identified to be stable for both ELS and pod weight.

CONCLUSION

The incidence of ELS in the savannas of Nigeria is an ongoing challenge that necessitated the efforts to identify stable ELS resistant groundnut genotypes. The genetic composition and GEI played important roles in the reaction of the genotype against ELS and expression of pod weight. Here, we identified elite stable sources against ELS resistance under natural and artificial ELS epiphytotic environments. These identified sources of resistance can be used in the development and deployment of ELS resistant groundnut varieties that are high yielding. Furthermore, the presence of high genetic variability in the mini core also makes them important materials for genome-wide association studies for the identification of markers and genomic regions/genes that are linked to ELS resistance and pod weight of groundnut.

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