

PLOS ONE

Exploring genotype by environment interaction on cassava yield and yield related traits using classical statistical methods

--Manuscript Draft--

Manuscript Number:	PONE-D-21-40030
Article Type:	Research Article
Full Title:	Exploring genotype by environment interaction on cassava yield and yield related traits using classical statistical methods
Short Title:	Accounting for genotype by environment interaction in cassava improvement
Corresponding Author:	Jean-Luc Jannink USDA-ARS / Cornell University Ithaca, UNITED STATES
Keywords:	genotype by environment interaction (GEI); target population of environment (TPE); linear-bilinear models; genotype sensitivity; yield; heritability; best linear unbiased predictor (BLUP)
Abstract:	Variety advancement decisions for root quality and yield-related traits in cassava are complex due to the variable genotype patterns by environment interactions (GEI). Therefore, studies focused on the dissection of the existing patterns of GEI using linear-bilinear models such as Finlay-Wilkinson (FW), additive main effect and multiplicative interaction (AMMI), and genotype and genotype by environment interaction (GGE) models are critical in defining the target population of environments (TPEs) for future testing, selection, and advancement. This study assessed thirty-six elite cassava clones in nine locations over three cropping seasons in the cassava breeding program of IITA based in Nigeria with a view of quantifying the GEI effects for root quality and yield-related traits. Genetic correlations coefficients and heritability estimates among environments depicted mostly intermediate to high values indicating high correlations with the major TPE. There was a differential clonal rankings among the environments indicating existence of GEI as also revealed by likelihood ratio test (LRT) which further confirmed statistical model with heterogeneity of error variances across the environments fit better. For all fitted models, we found the main effects of environment, genotype and their interaction to be significant for all observed traits except for dry matter content whose GEI sensitivities was marginally significant as reported in FW. We identified TMS14F1297P0019 and TMEB419 as two topmost stable genotypes with sensitivities value of 0.63 and 0.66 respectively using FW model. However, GGE and The AMMI stability value in conjunction with genotype selection index revealed that IITA-TMS-IBA000070 and TMS14F1036P0007 are the top-ranking genotypes combining both stability and yield performance measures. AMMI-2 model clustered the testing environments into 6 mega-environment based on winning genotypes for fresh root yield. Alternatively, we identified 3 clusters of testing environments based on genotypic blups derived from random GEI component.
Order of Authors:	Moshood Bakare Siraj Kayondo Cynthia Aghogho Marnin Wolfe Elizabeth Parkes Peter Kulakow Chiedozie Egesi Ismail Rabbi Jean-Luc Jannink
Additional Information:	
Question	Response

Financial Disclosure

Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the [submission guidelines](#) for detailed requirements. View published research articles from [PLOS ONE](#) for specific examples.

This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate.

Unfunded studies

Enter: *The author(s) received no specific funding for this work.*

Funded studies

Enter a statement with the following details:

- Initials of the authors who received each award
- Grant numbers awarded to each author
- The full name of each funder
- URL of each funder website
- Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript?
- **NO** - Include this sentence at the end of your statement: *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*
- **YES** - Specify the role(s) played.

* typeset

Competing Interests

Use the instructions below to enter a competing interest statement for this submission. On behalf of all authors, disclose any [competing interests](#) that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests.

This statement is **required** for submission

This research was generously funded by the Bill & Melinda Gates Foundation (<https://www.gatesfoundation.org>) and the United Kingdom's Foreign, Commonwealth & Development Office (FCDO) (<https://www.gov.uk/government/organisations/foreign-commonwealth-development-office>) through the "Next Generation Cassava Breeding project" (<https://www.nextgencassava.org>) (Grant INV-007637) managed by Cornell University.

Copyright: ©2021 Bakare et al. This is an open access research article distributed and licensed under the Creative Commons Attribution License (CC-BY), that permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

The funders gave support in the implementation of this study and encourage in publishing this work.

The authors have declared that no competing interests exist..

and **will appear in the published article** if the submission is accepted. Please make sure it is accurate and that any funding sources listed in your Funding Information later in the submission form are also declared in your Financial Disclosure statement.

View published research articles from [PLOS ONE](#) for specific examples.

NO authors have competing interests

Enter: *The authors have declared that no competing interests exist.*

Authors with competing interests

Enter competing interest details beginning with this statement:

I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]

* typeset

Ethics Statement

Enter an ethics statement for this submission. This statement is required if the study involved:

- Human participants
- Human specimens or tissue
- Vertebrate animals or cephalopods
- Vertebrate embryos or tissues
- Field research

Write "N/A" if the submission does not require an ethics statement.

General guidance is provided below. Consult the [submission guidelines](#) for detailed instructions. **Make sure that all information entered here is included in the Methods section of the manuscript.**

N/A

Format for specific study types

Human Subject Research (involving human participants and/or tissue)

- Give the name of the institutional review board or ethics committee that approved the study
- Include the approval number and/or a statement indicating approval of this research
- Indicate the form of consent obtained (written/oral) or the reason that consent was not obtained (e.g. the data were analyzed anonymously)

Animal Research (involving vertebrate animals, embryos or tissues)

- Provide the name of the Institutional Animal Care and Use Committee (IACUC) or other relevant ethics board that reviewed the study protocol, and indicate whether they approved this research or granted a formal waiver of ethical approval
- Include an approval number if one was obtained
- If the study involved *non-human primates*, add *additional details* about animal welfare and steps taken to ameliorate suffering
- If anesthesia, euthanasia, or any kind of animal sacrifice is part of the study, include briefly which substances and/or methods were applied

Field Research

Include the following details if this study involves the collection of plant, animal, or other materials from a natural setting:

- Field permit number
- Name of the institution or relevant body that granted permission

Data Availability

Authors are required to make all data underlying the findings described fully available, without restriction, and from the time of publication. PLOS allows rare exceptions to address legal and ethical concerns. See the [PLOS Data Policy](#) and [FAQ](#) for detailed information.

Yes - all data are fully available without restriction

A Data Availability Statement describing where the data can be found is required at submission. Your answers to this question constitute the Data Availability Statement and **will be published in the article**, if accepted.

Important: Stating 'data available on request from the author' is not sufficient. If your data are only available upon request, select 'No' for the first question and explain your exceptional situation in the text box.

Do the authors confirm that all data underlying the findings described in their manuscript are fully available without restriction?

Describe where the data may be found in full sentences. If you are copying our sample text, replace any instances of XXX with the appropriate details.

- If the data are **held or will be held in a public repository**, include URLs, accession numbers or DOIs. If this information will only be available after acceptance, indicate this by ticking the box below. For example: *All XXX files are available from the XXX database (accession number(s) XXX, XXX).*
- If the data are all contained **within the manuscript and/or Supporting Information files**, enter the following: *All relevant data are within the manuscript and its Supporting Information files.*
- If neither of these applies but you are able to provide **details of access elsewhere**, with or without limitations, please do so. For example:

Data cannot be shared publicly because of [XXX]. Data are available from the XXX Institutional Data Access / Ethics Committee (contact via XXX) for researchers who meet the criteria for access to confidential data.

The data underlying the results presented in the study are available from (include the name of the third party

All data files and analysis scripts are available online on github url address https://github.com/mab658/classical_analysis_GxE

and contact information or URL).

- This text is appropriate if the data are owned by a third party and authors do not have permission to share the data.

* typeset

Additional data availability information:

Exploring genotype by environment interaction on cassava yield and yield related traits using classical statistical methods

Moshood A. Bakare^{1*}, Siraj Ismail Kayondo², Cynthia I. Aghogho^{2,3}, Marnin D. Wolfe¹, Elizabeth Y. Parkes², Peter Kulakow², Chiedozie Egesi^{1,2,4}, Ismail Yusuf Rabbi², and Jean-Luc Jannink^{1*}

¹ Plant Breeding and Genetics Section, School of Integrative Plant Science, College of Agriculture and Life Sciences, Cornell University, 14853, Ithaca, NY USA.

² International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, Ibadan, Nigeria.

³ West Africa Centre for Crop Improvement, University of Ghana, PMB LG 30, Legon, Ghana

⁴ National Root Crops Research Institute Umudike, Umuahia, Nigeria

*Corresponding authors


E-mail: jeanluc.work@gmail.com (JJ)

Abstract

Variety advancement decisions for root quality and yield-related traits in cassava are complex due to the variable genotype patterns by environment interactions (GEI). Therefore, studies focused on the dissection of the existing patterns of GEI using linear-bilinear models such as Finlay-Wilkinson (FW), additive main effect and multiplicative interaction (AMMI), and genotype and genotype by environment interaction (GGE) models are critical in defining the target population of environments (TPEs) for future testing, selection, and advancement. This study assessed thirty-six elite cassava clones in nine locations over three cropping seasons in the cassava breeding program of IITA based in Nigeria with a view of quantifying the GEI effects for root quality and yield-related traits. Genetic correlations coefficients and heritability estimates among environments depicted mostly intermediate to high values indicating high correlations with the major TPE. There was a differential clonal rankings among the environments indicating existence of GEI as also revealed by likelihood ratio test (LRT) which further confirmed statistical model with heterogeneity of error variances across the environments fit better. For all fitted models, we found the main effects of environment, genotype and their interaction to be significant for all observed traits except for dry matter content whose GEI sensitivities was marginally significant as reported in FW. We identified TMS14F1297P0019 and TMEB419 as two topmost stable genotypes with sensitivities value of 0.63 and 0.66 respectively using FW model. However, GGE and The AMMI stability value in conjunction with genotype selection index revealed that IITA-

36 TMS-IBA000070 and TMS14F1036P0007 are the top-ranking genotypes combining both stability
37 and yield performance measures. AMMI-2 model clustered the testing environments into 6 mega-
38 environment based on winning genotypes for fresh root yield. Alternatively, we identified 3
39 clusters of testing environments based on genotypic blups derived from random GEI component.

40 **Introduction**

41 Cassava (*Manihot esculenta* Crant ) one of the most important food crops worldwide,
42 particularly in sub-Saharan Africa [1,2]. It is known to be a significant source of carbohydrates in
43 the diet of millions of people in developing countries. It is cultivated under diverse edaphic and
44 climatic conditions throughout the world [3] due to its efficient carbohydrate production [4] among
45 staple root crops. Cassava is a food security crop grown predominantly by smallholders for
46 subsistence due to its adaptability to survive in drought-prone areas under marginal conditions
47 where other crops may not thrive [1,5]. In comparison to other crops, Sayre et al. [5] reported that
48 cassava is mostly grown under marginal conditions, making it produce more energy per unit area
49 with limited human input than other crops. Cassava is getting much attention because of its
50 mechanisms to cope with diverse environmental conditions [6]. Cassava shows a strong genotype
51 by environment interaction (GEI) effects [7], which makes selection for superior genotypes a
52 difficult task for cassava breeders. Therefore, selection for a superior genotype requires the cassava
53 breeding program to take into consideration the GEI effect. Detailed evaluation of the magnitude
54 and significance of GEI is of utmost importance to ensure greater precision in the release of high
55 yielding and stable genotypes [7].

56 Crop phenotypes are well known to be influenced by environmental conditions [8]. This
57 can result in differential genotypic responses across the testing environments resulting in GEI
58 variability. The phenotypic panel for evaluating GEI is often called a multi-environment trial
59 (MET). In METs where genetic lines are often evaluated over many years and locations within a
60 target population of environments (TPE), there is usually an important cross-over interaction
61 (COI), and a GEI term needs to be explored to study the non-additivity of effects.

62 Among several statistical models devised for exploring the empirical genotypic mean
63 response across environments and for studying and interpreting GEI in agricultural field trials are:

64 Linear models, bilinear models, and linear-bilinear models [9]. Typical examples of fixed-effect
65 linear-bilinear models such as the Sites Regression (SREG) [10] and the Additive Main Effect and
66 Multiplicative Interaction (AMMI) models [11,12] are used for investigating patterns of genotypic
67 response across environments. In these models, biplots can be used to visualize the patterns of
68 genotypic responses and environments [13,14] that allow the breeders to identify high or low
69 performing genotype(s) with broad or specific adaptation for a given trait of interest. A form of
70 the fixed-effect linear model called a factorial regression (FR) model, and a form of the bilinear
71 model, called partial least squares (PLS) regression, allow integrating external environmental and
72 genotypic covariates into the model and can be used to identify weather conditions causing GEI
73 or the genomic segments (e.g., molecular markers) influencing GEI [9].

74 AMMI is one of the commonly used fixed-effect linear-bilinear models that models the
75 complex structure of GEI. It is a hybrid statistical model combining analysis of variance (ANOVA)
76 to model main effects of genotype and environment and principal component analysis (PCA) to
77 decompose complex GEI structure into Interactive Principal Component Axes (IPCAs) through
78 singular value decomposition. In this model, the percentage of GEI variation explained by IPCAs
79 decreases, with the first IPCA accounting for the highest percentage of GEI variation. The AMMI
80 biplot of first IPCA scores against the mean of genotypic performance visualizes both genotypes
81 and environments through which genotypes with broad or specific adaptation can be identified.
82 Genotypes with IPCA score in the vicinity of zero are considered to be stable across environments.
83 However, genotypes with scores that deviate from zero for a given IPCA are unstable relative to
84 the determinants of that IPCA but may exhibit specific adaptation if they are identified as close to
85 a particular environment in the AMMI biplot. AMMI is often preferable to a linear regression
86 approach in the sense of being parsimonious as it requires fewer degrees of freedom to explain

87 GEI. The AMMI model can be further used to delineate the testing environments with the best
88 genotypes into mega environment using principal component axes scores and AMMI stability
89 values (ASV) [15]. The AMMI stability value (ASV) for a genotype is defined to be the distance
90 from the coordinate point for that genotype to the origin in a two-dimensional space of the first
91 two Interactive principal component analysis scores (IPCA1 and IPCA2, [16]. Because IPCA
92 scores account for different amounts of variation in the GEI sum of squares a weighted value must
93 be assigned in the assessment of stability using the AMMI model. Genotypic stability alone does
94 not provide a sufficient yardstick for selection as stable genotypes might not necessarily give the
95 highest yield performance. Mahmodi et al. [17] and Tumuhimbise et al. [2] used a genotype
96 selection index (GSI) which is sum of genotypic yield rank across environments and ASV rank to
97 identify high yielding and stable genotypes. This index implicitly values yield and stability equally.
98 A low GSI value signifies a desirable genotype with high average yield performance and high
99 stability [17].





100 The Site Regression (SREG) model, also called Genotype Main Effect plus Genotype-
101 Environment Interaction (GGE), is a modification of AMMI model where the bilinear term
102 combines the genotype main effect (G) and the GEI effect in a multiplicative term. It allows
103 breeders to explore total genetic rather than exclusively GEI variation. GGE allows the finding of
104 GEI in terms of crossover resulting from changes in genotypic ranking across the environments
105 [18]. Unlike AMMI biplots that approximate only GEI, the genotypic scores in a GGE model
106 describe the G and GEI jointly to approximate overall performance of (G + GE) interaction.

107 This study's principal objectives were: (i) To identify stable and high-yielding cassava
108 clones adapted to broad and/or specific environments; (ii) To determine the relative importance of
109 sources of variation influencing key agronomic traits; and (iii) To identify mega environments for

110 Nigerian cassava; iv) To provide a clear road map for other researchers pursuing these types of
111 objective.

112 **Materials and methods**

113 **Clonal material and experimental field design**

114 Thirty-six (36) advanced cassava clonal lines were evaluated, corresponding to 31
115 experimental lines and five standard checks (Table 1) as in the uniform yield trial (UYT), an
116 advanced stage of the II  breeding program. These clones were evaluated across 20 trials grown
117 in nine (9) locations across different agro-ecological zones in Nigeria from 2017 to 2020 (Table
118 2). The geographical coordinate system describing the location of each field trial in space is given
119 in terms of latitude and longitude. Weather data were collected across the testing environments
120 from an online database, www.awhere.com. Mean temperature over crop growth cycle across the
121 environments ranged from 26.8  for Zaria20 to 28.9  for Mokwa20. Meanwhile, the total
122 precipitation varied between 944.2 for Kano19 and 3208.4 for Onne19. Average relative humidity
123 varied across Each  trial was established as a Randomized Complete Block Design (RCBD) in three
124 replicates. The experimental plot consisted of 6 rows of length 5.6 m with an inter-row spacing of
125 1 m and intra-row spacing of 0.8 m. The locations used varied from one cropping season to another
126 as did the number of trials, resulting in an unbalanced data structure.

127 **Table 1.** Thirty-six cassava clonal lines evaluated across 20 environments in Nigeria

Clone	Pedigree
IITA-TMS-IBA000070	
IITA-TMS-IBA30572	58308 X BRANCA DE SANTA CATARINA
IITA-TMS-IBA980581	
IITA-TMS-IBA982101	IITA-TMS-IBA951181

TMEB419	
TMS13F1021P0008	IITA-TMS-IBA010903 X IITA-TMS-IBA030075
TMS13F1114P0001	IITA-TMS-IBA070126 X IITA-TMS-IBA000355
TMS13F1182P0002	IITA-TMS-IBA011412 X TMEB419
TMS13F1461P0002	IITA-TMS-MM990268 X IITA-TMS-IBA000355
TMS13F2061P0005	(IITA-TMS-IBA070004 X IITA-TMS-IBA070520 X SM3361-30)-11
TMS13F2207P0001	IITA-TMS-KAN930061 X IITA-TMS-IBA960249
TMS14F1001P0004	TMS13F1303P0001 X TMS13F1020P0002
TMS14F1016P0006	TMS13F1307P0011 X TMS13F1108P0007
TMS14F1022P0006	TMS13F1307P0020 X TMS13F1106P0006
TMS14F1035P0004	TMS13F1095P0009 X TMS13F1307P0008
TMS14F1035P0007	TMS13F1095P0009 X TMS13F1307P0008
TMS14F1036P0007	TMS13F1109P0009 X TMS13F1307P0020
TMS14F1049P0001	TMS13F1391P0039 X TMS13F1306P0003
TMS14F1120P0003	TMS13F1309P0001 X TMS13F1333P0003
TMS14F1131P0001	TMS13F1087P0002 X TMS13F1176P0003
TMS14F1194P0002	TMS13F1101P0007 X TMS13F1307P0020
TMS14F1195P0005	TMS13F1106P0006 X TMS13F1307P0020
TMS14F1208P0007	TMS13F1106P0006 X TMS13F1020P0002
TMS14F1223P0007	TMS13F1106P0006 X TMS13F1108P0007
TMS14F1224P0004	TMS13F1106P0006 X TMS13F1212P0055
TMS14F1262P0002	TMS13F1063P0009 X TMS13F1307P0008
TMS14F1285P0017	IITA-TMS-IBA961632 X IITA-TMS-IBA000070
TMS14F1291P0011	IITA-TMS-IBA030055A X IITA-TMS-IBA961632
TMS14F1297P0019	IITA-TMS-IBA020431 X IITA-TMS-MM970806
TMS14F1300P0008	IITA-TMS-ZAR930151 X IITA-TMS-MM970043
TMS14F1303P0012	I IITA-TMS-ZAR930151 X IITA-TMS-IBA930134
TMS14F1306P0015	IITA-TMS-IBA030060 X IITA-TMS-MM970043
TMS14F1306P0020	IITA-TMS-IBA030060 X IITA-TMS-MM970043
TMS14F1310P0004	IITA-TMS-IBA030060 X IITA-TMS-IBA930265
TMS14F1311P0020.	IITA-TMS-IBA030060 X IITA-TMS-ZAR930151
TMS14F1312P0003	IITA-TMS-IBA930134 X IITA-TMS-ZAR930151

129 **Table 2.** Climatic characteristics of the experimental sites showing the geographical coordinates, range and mean of temperature,
 130 precipitation, and relative humidity.

Climatic factors	Abuja	Ago-owu	Ibadan	Ikenne	Kano	Mokwa	Onne	Otobi	Ubiaja	Umudike	Zaria
Latitude (°)	9.67 N	7.25 N	7.37 N	6.87 N	12.00 N	9.28 N	4.72 N	7.10 N	6.64 N	5.48 N	11.09 N
Longitude (°)	7.39 E	4.32 E	3.94 E	3.71 E	8.59 E	5.05 E	7.15 E	8.08 E	6.39 E	7.54 E	7.71 E
Min - Max (Mean) temperature (°C)											
2018	-	23.9 - 32.6 (28.3)	23.6 - 32.2 (27.9)	24.3 - 32.1 (28.2)	-	23.6 - 33.9 (28.8)	-	-	-	-	-
2019	-	23.9 - 32.1 (28.0)	23.7 - 32.2 (27.9)	24.4 - 32.2 (28.3)	20.9 - 34.1 (27.5)	23.7 - 33.9 (28.8)	21.8 - 29.5 (25.7)	23.1 - 32.2 (27.7)	.	23.1 - 30.9 (27.0)	-
2020	22.8 - 32.9 (27.8)	23.7 - 32.3 (28.0)	23.5 - 32.2 (27.8)	24.3 - 32.4 (28.3)	-	23.5 - 34.3 (28.9)	21.9 - 29.7 (25.8)	-	22.6 - 31.5 (27.0)	-	20.5 - 33.2 (26.8)
Total precipitation (mm)											
2018	-	1082.1	1175.9	1295.4	-	1215.7	-	-	-	-	-
2019	-	1647.4	1439.0	1653.2	944.2	1250.5	3208.4	1409.1	-	2795.6	-
2020	1920.0	1673.8	1479.1	1681.3	-	1068.5	3199.9	.	2050.0		1322.8
Relative humidity (%)											
2018	-	56.9 - 88.5 (72.7)	57.5 - 88.4 (72.9)	61.6 - 91.0 (76.3)	-	46.3 - 77.9 (62.1)	-	-	-	-	-
2019	-	59.4 - 90.9 (75.2)	58.2 - 90.1 (74.2)	62.3 - 92.5 (77.4)	31.2 - 68.3 (49.8)	45.4 - 78.9 (62.1)	62.8 - 95.0 (78.9)	53.8 - 86.8 (70.3)	-	62.1 - 93.5 (77.8)	-

2020	51.8 - 86.0 (68.9)	58.0 - 91.7 (74.9)	56.4 - 90.6 (73.5)	60.3 - 93.1 (76.7)	-	44.8 - 79.4 (62.1)	61.8 - 95.6 (78.7)	-	56.7 - 91.5 (74.1)	-	33.7 - 71.6 (52.6)
Agroecological Zone	Southern Guinea savanna	Derived savanna	Forest savanna transition	Humid forest	Northern Guinea savanna	Southern Guinea savanna	Humid forest	Southern Guinea savanna	Humid forest	Humid forest	Northern Guinea savanna

131

132 **Statistical analysis**

133 **Data quality control and single trial analysis**

134 Before formal genotype by environment analysis, the empirical distribution of the observed
135 agronomic traits was visualized by individual environment (i.e., location by year combination) in
136 boxplots using the ggplot2 package [19] in R [20]. A linear mixed model was fitted to the
137 individual trials to estimate clonal variance components and broad-sense heritability. The Proc
138 Mixed procedure of Statistical Analysis Software (SAS) software version 9.4 [21] was used to fit
139 the following model:

$$y = \mu + X_1 r + X_2 cov + Z_1 g + \epsilon \quad (1)$$

140 where y is the vector ($n \times 1$) of observed phenotypic values, in which n is the number of
141 observations; μ is the intercept (overall mean); r is the vector ($j \times 1$) of fixed effects of j replicates;
142 cov denotes the proportion of plant stands harvested, a covariate for all traits except dry matter
143 content; g is the vector ($i \times 1$) of random effects of i^{th} genotype with its associated design matrix
144 Z_i , and ϵ is a residual term which is assumed to follow Gaussian distribution.

145 Quality of each trial was assessed by calculating the coefficient of variation (CV), broad-
146 sense heritability (H^2), and experimental accuracy (Ac) proposed by [22] using the following

147 expressions: $CV\% = \frac{\hat{\sigma}_e}{\bar{y}} \times 100$, $H^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}$, and $Ac = \sqrt{1 - \frac{\overline{PEV}}{\hat{\sigma}_g^2}}$

148 in which $\hat{\sigma}_e$ is the estimated residual standard deviation, \bar{y} is the estimated overall mean for a
149 trait; $\hat{\sigma}_g^2$ is the estimated genetic variance $\hat{\sigma}_e^2$ is the estimated error variance, and \overline{PEV} is the
150 average of prediction error variance.

151 This analysis identified three trials with low heritability ($H^2 < 0.1$), low accuracy (Ac < 0.4) and
152 high CV (CV > 40.5). These trials were removed from further analysis (Figs 2 and 3).

153 **Joint G×E analysis of multiple trials**

154 Following single trial analysis to identify and eliminate poor quality trials, we carried out
155 a combined analysis of trials using a linear mixed model framework on data consisting of g
156 genotypes evaluated across e environment in r replicates within each environment. The model
157 fitted for each agronomic trait was:

$$y_{ijk} = \mu + g_i + e_j + b_{k(j)} + ge_{ij} + cov + \epsilon_{ijk} \quad (2)$$

158 where y_{ijk} is a phenotypic vector of the observed agronomic trait of i^{th} genotype in k^{th} replicate
159 within j^{th} environment; μ is a fixed intercept, g_i is the effect of i^{th} genotype considered to be
160 random, e_j is the random effect of j^{th} environment, $b_{k(j)}$ is the random block effect within j^{th}
161 environment, ge_{ij} is the random interaction effect of i^{th} genotype and j^{th} environment, cov denotes
162 the proportion of plant stands harvested as covariates; and ϵ_{ijk} is the vector of the random residual
163 term. The random effects in the model are postulated to follow a multivariate normal distribution
164 with means and variances defined as:

$$165 g \sim N(0, \sigma_g^2 I), e \sim N(0, \sigma_e^2 I), b \sim N(0, \sigma_b^2 I), ge \sim N(0, \sigma_{ge}^2 I), \text{ and } \epsilon \sim N(0, \sigma_\epsilon^2 I)$$

166 where 0 is the expected value (mean) of zero; σ_g^2 is the genetic variance; σ_e^2 is the
167 environmental variance; σ_b^2 is the block variance nested with j^{th} environment; σ_{ge}^2 is the variance
168 of genotype-by-environment interaction σ_ϵ^2 is the residual variance; and I is the identity matrix,
169 with order equal to the number of observations. We calculated the percentage of total phenotypic
170 variance explained by each random effect to determine how significant it was in influencing the
171 variability in each trait. Broad-sense heritability on plot mean basis across all environments was
172 derived from variance components estimate as

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_{ge}^2/e) + (\sigma_e^2/er)} \quad (3)$$

173 where e is the number of environment, r is the number of replicates of genotypes per environment,
 174 and other terms were described above. Out of 17 trials, 12 had replicates of 3 and others have 2
 175 replicates each. Therefore, the harmonic mean was calculated to be approximately 2.6 and it was
 176 used as a good representative of the number of replicates across all the trials while computing the
 177 heritability.

178 We further ascertained the presence or absence of GEI by fitting both the reduced model
 179 without the GEI term and a full model that included the GEI term. The likelihood ratio test (LRT)
 180 was carried out on each of the agronomic traits to determine if there was a significant improvement
 181 in fitting a full model. In the same manner, we tested for the homogeneity versus heterogeneity of
 182 error variance across trials. Finally, we further partitioned the GEI variance into a repeatable
 183 component as genotype by location (GL), and non-repeatable components as genotype by year
 184 (GY) and genotype by location by year (GLY). In the presence of significant GEI, we assessed its
 185 pattern by fitting Finlay Wilkinson (FW), Additive main effect and multiplicative interaction
 186 model (AMMI), and genotype and genotype by environment (GGE) models on the two-way
 187 genotype environment adjusted means using the statgenGxE package [23] in R [20], as described
 188 below.

189 **Finlay-Wilkinson regression**

190 The Finlay-Wilkinson regression approach [24] was used to model GEI by regressing mean
 191 phenotypic performance of individual genotypes on an environmental index and determine the
 192 heterogeneity of associated slopes. The environmental index was the mean of all clones for a trait
 193 in an environment. Without explicit environmental information, an environment can be

194 characterized by the mean performance of all genotypes in that environment [25]. This method
 195 requires two steps: (i) Compute the environmental mean, and (ii) Estimate intercept and slope for
 196 each genotype by regressing genotypic performance on the environmental index. Prior to fitting
 197 the Finlay Wilkinson model, trait values were scaled to mean of zero and standard deviation of
 198 one following the equation below to allow comparison of means square error (MSE) values across
 199 traits, which are measured in different scale of units as:

$$y_{ij_{standardized}} = [y_{ij} - mean(Y)]/sd(Y) \quad (4)$$

200 where y_{ij} is the adjusted phenotypic mean value of i^{th} genotype in j^{th} environment and Y is the
 201 overall mean of adjusted phenotypic response of all clones in all environments. This
 202 standardization of each trait necessitated that the MSE values reflected variability and not the
 203 absolute scale of a given unit [26]. Then for each trait, we fitted the Finlay-Wilkinson model as

$$y_{ij} = \mu + g_i + \beta_i e_j + \epsilon_{ij} \quad (5)$$

204 in which y_{ij} is as described above but scaled, μ is overall mean, g_i is the genotypic intercept, β_i is
 205 a slope representing the sensitivity of i^{th} genotype. The average value of β_i is 1; $\beta_i > 1$ indicates
 206 genotypes with a higher than average sensitivity, and $\beta_i < 1$ indicates genotypes that are less
 207 sensitive than average, e_j is the environment sample mean, and ϵ_{ij} is a random error term associated
 208 with i^{th} genotype evaluated in j^{th} environment.

209 The three key parameters arising from this approach are: the genotypic intercept, which
 210 expresses the general performance of a genotype, the slope which measures the sensitivity of a
 211 genotype, and the residual variance which is a deviation from the regression line denoting the
 212 stability. The residual is genotypic-specific. Therefore, some lines have high residual, while others
 213 are low. To quantify and compare trait sensitivity to GEI, the variance of slope and that of MSE
 214 resulting from Finlay-Wilkinson model were used [26]

215 **AMMI analysis**

216 The observed traits' G×E interaction was analyzed using the Additive Main effect and
217 Multiplicative interaction (AMMI) model. AMMI is a fixed effect linear-bilinear model which
218 analyses the main effect of genotype and environment using ANOVA and the multiplicative effect
219 using principal component analysis (PCA) in a single model [27]. Each of the agronomic traits
220 was subjected to AMMI analysis by fitting the model

$$y_{ij} = \mu + g_i + e_j + \sum_{k=1}^K (\lambda_k \alpha_{ik} \gamma_{jk}) + \epsilon_{ij} \quad (6)$$

221 where y_{ij} is the mean performance of i^{th} genotype in j^{th} environment; μ is the intercept; g_i is fixed
222 effect of i^{th} genotype; e_j is the fixed effect of j^{th} environment. The GEI component is decomposed
223 into K multiplicative terms ($k = 1, 2, \dots, K$), each multiplicative term is a product of k^{th} eigenvalue
224 (λ_k); genotypic score (α_{ik}); and environmental scores (γ_{jk}); and ϵ_{ij} is the residual GEI not captured
225 by the model and some error deviation.

226 We computed AMMI Stability Value (ASV) for each genotype relative to the influence of
227 IPCA1 and IPCA2 scores based on their interaction sum of squares according to Purchase [16]
228 using the formula:

$$ASV = \sqrt{\left[\left(\frac{SS_{IPCA1}}{SS_{IPCA2}} \right) \times IPCA1 \right]^2 + IPCA2^2} \quad (7)$$

229 where (SS_{IPCA1}/SS_{IPCA2}) was the weight assigned to the *IPCA1* value by dividing the *IPCA1* SS by
230 the *IPCA2* SS; and the *IPCA1* and *IPCA2* scores were the genotypic score derived from the AMMI
231 model. A large positive ASV value indicates a genotype that is more adapted to particular
232 environments. A small (close to zero) ASV value indicates a stable genotype across environments
233 [16].

234 We also calculated genotype selection index (GSI) for each genotype as the sum of genotypic rank
235 based on mean yield across environments (RY) and rank of AMMI stability value (RASV):

$$GSI_i = RASV_i + RY_i \quad (8)$$

236 The genotype with the lowest GSI value is considered the most valuable [28].

237 **GGE analysis**

238 Genotype main effect and Genotype by Environment (GGE) analysis is a modification of
239 AMMI analysis. Unlike AMMI, only the environment is fitted as a main effect in the GGE model.
240 This brings about fitting principal component analysis jointly on genotype main effect and
241 genotype by environment interaction as a sum of multiplicative terms. The GGE analysis does the
242 job of fitting the principal component model with two components to the two-way genotype by
243 environment table of mean centered per environment with genotypes as object and environments
244 as variable [18]. Like AMMI, the principal component scores can be exploited in constructing
245 biplots. The GGE model or AMMI analysis could be used at differentiating mega-environment
246 [29] even though this is debated in the literature in terms of preference. The observed traits were
247 subjected to GGE analysis by fitting GGE model as

$$y_{ij} = \mu + e_j + \sum_{k=1}^2 (\lambda_k \alpha_{ik} \gamma_{jk}) + \epsilon_{ij} \quad (9)$$

248 where each term is similar to the AMMI model.

249 **Mega-environment delineation**

250 In the context of GEI, a mega-environment is defined to be a group of environments sharing
251 a common best performing genotype. In principle, it also follows that different genotypes are
252 adapted to different mega-environments and GEI variation between the mega-environments is

253 higher than variation within the mega-environments [30]. We determined mega-environments
254 based on the AMMI-2 model of order 2. The environments were clustered using the *gxeMegaEnv()*
255 function of the *statgenGxE* package [23] based on the fitted values from AMMI-2 model.
256 Environments that share the common best genotype belong to the same mega-environment.

257 The version of GGE biplot graphic called “which won where” plot is also a tool for the
258 delineation of a mega-environment. In the case of delineating mega-environment through GGE
259 biplot analysis, the resulting mean value in the graphics is related to mega-environment mean and
260 not grand mean, and it supports in identifying genotypes with broad or narrow adaptation to some
261 environments or groups of environments [31]. The “which won where” biplot includes an irregular
262 polygon whose vertices mark the genotypes that are furthest from the origin in all directions such
263 that the polygon encompasses all genotypes in the biplot. Lines are also drawn originating from
264 the biplot’s origin and intercepting the polygon’s sides perpendicularly [32]. The lines originating
265 from the origin split the biplot into sections and the genotype at the vertex of every section had the
266 optimal yield performance in environments contained in that section. Each section in effect defines
267 a mega-environment.

268 **Cultivar superiority index**

269 Further assessment of stability of each clone was determined after testing the significance
270 of GEI. We quantified yield stability across the testing environments using a univariate stability
271 estimate called cultivar-superiority measure [33]. It is a measure of stability by superiority index
272 and it is defined as a function of the sum of the squared differences between a cultivar’s mean
273 performance and the best cultivar’s mean, where the sum is across trials. Lin and Binns [33]
274 proposed the calculation of superiority index using expression:

$$P_i = \sum_{j=1}^n (X_{ij} - M_j)^2 / 2n \quad (10)$$

275 where P_i is the superiority index of i^{th} cultivar; X_{ij} is the yield of i^{th} cultivar in the j^{th} environment;
 276 M_j is the highest yield response got among the cultivars in the j^{th} environment; and n is the number
 277 of environments. This expression was further decomposed as

$$P_i = \left[n(\bar{X}_{i.} - \bar{M})^2 + \sum_{j=1}^n (X_{ij} - M_j + \bar{M})^2 \right] / 2n \quad (11)$$

278 where $\bar{X}_{i.} = \sum_{j=1}^n X_{ij}/n$, and $\bar{M} = \sum_{j=1}^n M_j/n$, $\bar{X}_{i.}$ = mean yield of i^{th} cultivar in n environments
 279 and \bar{M} = mean of maximum response in the n environments. According to Lin and Binns [33],
 280 the first section of P_i expression quantifies genetic deviation and the second section signifies
 281 GEI. Cultivars with the lowest values of the index P_i incline to be more stable, and in proximity
 282 to the best cultivar in each environment.

283 **Representative of target population of environments**

284 We considered all environments in the study to be the target population of environments
 285 (TPE). We identified testing environments that best represented the TPE by following these steps:
 286 i) calculate environment-specific genotypic BLUPs by fitting genotype effect as random, ii)
 287 calculate genotypic BLUPs across all environments which represent a TPE, iii) calculate the
 288 Pearson correlation between environment-specific genotypic BLUPs and genotypic BLUPs across
 289 all environments as a measure of breeding value accuracy, and iv) estimate environment-specific
 290 heritability based on the Cullis approach which involves the variance of a difference between
 291 genotypes. Cullis et al. [34] proposed to compute heritability as

$$H_{Cullis}^2 = 1 - \frac{\bar{V}_{\Delta..}^{BLUP}}{2\sigma_g^2} \quad (12)$$

292 where $\bar{V}_{\Delta..}^{BLUP}$ is the mean variance of a difference of two genotypic BLUPs and σ_g^2 is the genetic
 293 variance, and v) rank heritability and Pearson correlation value and take the sum of their rank. We
 294 use both high genetic correlation and heritability estimate as indicators for identifying a good
 295 representative for the TPE.

296 To determine the number of testing environments representing the entire TPE, we
 297 randomly sampled subsets of 1 to 16 environments from the phenotypic data repeatedly for 50
 298 times. For each sampling, a model was fitted to obtain genotypic best linear unbiased prediction
 299 (BLUP). Then, for each sampling environment, Pearson correlation was obtained between the
 300 BLUP and the BLUP derived from all the environments. We further calculated the average
 301 correlation coefficient as a breeding value accuracy relative to overall environments. The point at
 302 which the line plot showing the trends of breeding values accuracy relative to all TPE and sampled
 303 environments reaches a plateau is used to determine optimal number of environments to represent
 304 TPE.

305 To provide further insights into the relatedness or grouping of the current testing environments
 306 based on key traits, we extracted environment-specific genotypic BLUPs from the random G×E
 307 effect component of the joint analysis. Then, we further carried out a Pearson correlation analysis
 308 among the environments. Thereafter, the clustering of the environments was carried out based on
 309 a distance matrix derived from correlation matrix using ward.D2 linkage method [35]. Intuitively,
 310 we examined the resulting dendrogram from the clustering to identify environments that joined
 311 together with the smallest distance as a cluster group

312 **Results**

313 **Phenotypic data description and single trial analysis**

314 The distribution of the phenotypic values of observed traits of the 36 genotypes revealed
315 that all observed traits approximated a normal distribution across the testing environments
316 satisfying the assumption of normality in classical statistical methods (Fig 1). We observed a range
317 in variation of fresh root yield (t/ha) from low performance environments (Onne19, Onne20,
318 Ubiaja20), to high performance environments (Ibadan19, Otobi19, Ago-owu20, Ikenne20). The
319 boxplots further revealed the heterogeneity of variability for the observed traits across the
320 environments indicating the presence of GEI.

321 **Fig 1. Box plot displaying the distribution of dry matter content (dm %), dry yield (dyld**
322 **t/ha), fresh root yield (fyld t/ha), harvest index (hi), and top yield (tyld t/ha) of 36 genotypes**
323 **evaluated.**

324 The plots resulting from data quality control of single-trial analysis (S1 and S2 Figs.) showed that
325 the three trials: 18UYT36setAKN, 19UYT36setAZA, and 19UYT36setAMK should be removed
326 based on thresholds set for CV, H^2 , and Accr. The mean fresh root yield across the remaining 17
327 trials ranged from 6.52 t/ha (19UYT36setAON) in Onne20 to 47.49 t/ha (18UYT36setAOT) in
328 Otobi19 with an overall mean of 27.46 t/ha (S1 Table). The summary statistics for other traits like
329 dry matter content, dry yield, top yield, and harvest index from each testing environment are also
330 reported (S1 Table). In addition, visualization of the distribution of derived parameters such as
331 broad-sense heritability, coefficient of variation, experimental accuracy, and residual variance for
332 all traits across all trials were showed (S3 Fig). We observed smallest variability in dry matter
333 content and then harvest index across all trials relative to other traits.

334 **Combined analysis of multiple trials**

335 The likelihood ratio test (LRT) statistics identified presence of significant GEI and error
336 variance heterogeneity across testing environments for all observed traits (S2 and S3 Tables). The
337 percentage of phenotypic variance attributed to each model term for each trait was reported (Fig 2
338 and S4 Table). The environment had a significant effect on all traits ($P < 0.01$) and captured largest
339 percentage of total phenotypic variance ranging from 48.6% in harvest index to 63.9% in top
340 yield. The genotypic effect was highly significant ($P < 0.001$) for each trait and explained
341 percentage of phenotypic variance between 2.6% (harvest index) and 12.6% (dry matter content).
342 The GEI term was also highly significant ($P < 0.001$) and accounted for 5.3% (top yield) to 12.5%
343 (harvest index) of phenotypic variance. We observed relatively high GEI variance compared to
344 genetic variance for fresh root yield. In contrast, genetic variance for top yield was higher than
345 GEI variance indicating environmental conditions played a lesser role in influencing top yield (Fig
346 2 and S4 Table). The replication nested within environment captured between 2.3-5.6% of the
347 phenotypic variance, which was the smallest relative to other source of variation. It was highly
348 significant ($P < 0.01$) for all traits. Residual term was the second largest source of variation after
349 the environment effect. It accounted between 18.8-30.8% of the phenotypic variance (Fig 2 and
350 S4 Table). The broad-sense heritability estimates varied from 0.64 for harvest index to 0.92 for
351 dry matter content (S4 Table).

352 **Fig 2 Barchart plot displaying the percentage of total phenotypic variance attributed to each**
353 **effect for each trait across 17 trials.**

354 Further decomposition of GEI term into repeatable component (GL) and non-repeatable
355 component (GY and GLY) revealed that GY component was not significant for all traits except
356 dry matter content ($P < 0.001$). It accounted for the smallest portion of phenotypic variance ranging
357 from approximately 0.2 to 5.6% (S5 Table). Repeatable GL component explained between 3.7-

358 48.6% percentage of phenotypic variance. In comparison to GL and GY components, the GLY
359 term was highly significant ($P < 0.001$) and accounted for largest portion of phenotypic variance
360 for all traits except for harvest index where GL explained largest portion of phenotypic variance.
361 The significance of GLY is an indication that for all traits, genotypic response to conditions
362 particular to a specific location depends on year of evaluation and vice versa..

363 **Finlay-Wilkinson regression**

364 The genotypic and environmental main effects of the Finlay-Wilkinson (FW) model were
365 highly significant ($P \leq 0.001$) for all observed traits (S6 Table). Significant differences in
366 regression slope (sensitivity) among genotypes on the environmental mean was found for all traits
367 except dry matter content (S6 Table). In other words, there was variation in genotypic response
368 for all traits but not dry matter with respect to changes in environment mean.

369 The genotypic sensitivity values were ranked from the most stable (low sensitivity value) to the
370 least stable (high sensitivity value) for each trait (S7 Table). The FW model identified
371 TMS14F1297P0019, TMEB419, TMS14F1120P0003, TMS13F1461P0002, and
372 TMS14F1312P0003 as the top 5 most stable genotypes for fresh root yield with sensitivities values
373 of 0.638, 0.663, 0.721, 0.786, and 0.813 respectively.

374 Trait sensitivity to GEI was quantified by the variance of the slopes and the variance of
375 MSEs. The top yield had the lowest median MSE of all traits (median = 0.146) and the variance
376 of MSE (variance=0.007) (Fig 3 and S8 Table). Meanwhile, the slope variance varied from 0.023
377 (top yield) to 0.058 (harvest index) with the corresponding slope median values of 1.001 and 1.010
378 respectively, (Fig 3 and S9 Table).

379 **Fig 3. Boxplots showing distribution of MSE and slope resulting from Finlay Wilkinson**
380 **model for the evaluation of 36 elites cassava clones across 17 environments for 5 traits.**

381 **AMMI analysis**

382 The AMMI analysis revealed significant variation in the main effects of genotype (G),
383 environment (E) and their interactions (GEI) ($P < 0.001$) for all observed traits (S10 Table). The
384 partition of total sum of squares (TSS) showed that the environment main effect accounted for
385 highest amount of variation varying from 48.2% (harvest index) to 76.1% (top yield).

386 The decomposition of variation in GEI for fresh root yield showed that the first and the second
387 interactive principal components (IPCs) captured 21.6% and 15.7% and accounted for 4.5% and
388 3.3% of the TSS. For dry matter content, the first two IPCs accounted for 21.0% and 15.9% of
389 GEI SS and 4.3% and 3.3% of TSS. Finally, the partition of variation in GEI for top yield revealed
390 the first and second principal components explained 26.5% and 17.6% and accounted for 4.0%
391 and 2.7% of TSS respectively (S10 Table).

392 The AMMI-2 biplot revealed how the genotypes and environment are interrelated based
393 on fresh root yield (Fig 4a). The genotypes close to each other in this biplot have a tendency to
394 have a relatively similar yield in the tested environments. Meanwhile, genotypes far apart within
395 the plot tend to differ in yield or have a unique pattern of yield response across the environments.
396 Genotypes in the vicinity of the origin are not sensitive to environmental interaction and those
397 distant from the origin are sensitive and have large interaction.

398 **Fig 4. Polygon view of (a) AMMI2 model and (b) GGE2 model for fresh root yield (t/ha).**

399 The mean fresh root yield (t/ha) value of cassava genotypes averaged over testing
400 environments indicated that genotype IITA-TMS-IBA000070 had the highest fresh root yield
401 (37.9 t/ha) and genotype TMS14F1120P0003 had the lowest yield (22.5 t/ha, S11 Table). The
402 IPC1 and IPC2 scores signify the adaptability of a genotype over environments and the relationship
403 between genotype and environment (S11 Table). Genotypes with large scores in absolute value

404 (e.g., TMS14F1297P0019 and TMEB419) have high interactions and are unstable, whereas
405 genotypes with scores close to zero (IITA-TMS-IBA000070, TMS14F1036P0007) have low
406 interactions and are stable.

407 The AMMI stability value (ASV) ranged from 2.60 to 40.34, averaging 15.39 across the
408 36 cassava genotypes. The genotypes IITA-TMS-IBA000070 (2.60), TMS14F1306P0020 (3.28),
409 TMS14F1223P0007 (3.88), and TMS14F1306P0015 (5.68) had the lowest ASV values, while
410 TMEB419 (40.34), TMS14F1297P0019 (39.21), and TMS14F1300P0008 (30.41) had the highest
411 values (S11 Table). Stability is not the only yardstick for selection, as the most stable genotype
412 would not necessarily give the best yield performance. Therefore, the genotype selection index
413 showed that IITA-TMS-IBA000070 and TMS14F1036P0007 were the two best when combining
414 both stability and yield performance measures (S11 Table).

415 **Identifying mega environments**

416 The fitted fresh root yield values from the AMMI2 model were used to cluster the
417 seventeen testing environments into six mega environments, one for each of the winning genotypes
418 “IITA-TMS-IBA000070”, “IITA-TMS-IBA980581”, “TMS14F1016P0006”,
419 “TMS14F1036P0007”, “TMS14F1285P0017”, and “TMS14F1300P0008” (S12 Table). The
420 genotypes IITA-TMS-IBA000070 and TMS14F1016P0006 had broad adaptation to eight and four
421 environments, respectively. However, genotypes IITA-TMS-IBA980581, TMS14F1285P0017,
422 and TMS14F1300P0008 had specific adaptation to environments Abuja20, Mokwa18, and
423 Ibadan18, respectively. TMS14F1036P0007 was the best genotype in environments Ago-Owu19
424 and Ibadan20.

425 **GGE analysis**

426 The GGE model showed a significant main effect of environment and combined genotype
427 and genotype by environment interaction effect ($P \leq 0.001$) for the observed traits (S13 Table).
428 The partition of TSS which includes sum of squares (SS) of environment and genotype and
429 genotype by environment interaction indicated that environment explained a larger percentage of
430 variation for all observed traits relative to GGE component except for harvest index. The variation
431 explained by the GGE component ranged from 23.9% (top yield) to 51.8% (harvest index). For
432 fresh root yield, the first and second IPCs accounted for 9.6% and 4.4% of TSS and explained
433 33.3% and 15.3% of GGE variation, respectively with a cumulative total of 48.6%. For dry matter
434 content, the first two IPCs captured 17.4% and 4.3% of TSS and explained 47.6% and 11.8% of
435 GGE variation, with a cumulative total of 59.4%. For the top yield, the first two IPCs explained
436 11.3% and 3.1% of TSS and captured 47.4% and 12.8% of GGE variation resulting to cumulative
437 total of 60.2%.

438 GGE biplots based on symmetric scaling of genotype and environment were used to
439 estimate the pattern of environments in relation to genotypes (Fig 4b). The first principal
440 component of environment had both negative and positive scores indicating a difference in yield
441 performance across environments resulting in cross-over GEI.

442 **Fig 4. Polygon view of (a) AMMI2 model and (b) GGE2 model for fresh root yield (t/ha).**

443 The three models revealed that environment effect accounted for almost the same percentage of
444 total phenotypic variation for the observed traits (Fig 5). Likewise, the genotypic effect of FW
445 and AMMI models explained nearly the same percentage of total phenotypic variation for each
446 measurable trait. The interaction factor of GGE model includes main effect of genotype and
447 genotype by environment interact resulting in a larger percentage of total phenotypic variation in

448 comparison to other models. The GEI component of AMMI captured larger percentage of variation
449 than FW, which explained relatively low variation for all the traits. For all the observed traits, the
450 residual term of the AMMI model was the lowest while for FW it was the highest (Fig 5).

451 **Fig 5. Percentage of total variation captured by each factor from fitting additive main effect**
452 **and multiplicative interaction (AMMI), Finlay Wilkinson (FW), and genotype and genotype**
453 **by environment (GGE) models to yield related traits on 36 elite cassava genotypes evaluated**
454 **in 17 environments. Note that the variation attributed to genotype x environment factor for**
455 **GGE model includes that of genotype.**

456 **Cultivar superiority index**

457 Mean performance and index values of cultivar-superiority stability estimates were
458 presented for fresh root yield, dry matter content, and dry yield to assess genotypes' stability
459 across the testing environments (S14 Table). Among the 36 cassava genotypes, 19 had mean fresh
460 root yield above grand mean of 29.5 t/ha. The remaining genotypes had average fresh root yield
461 below grand mean. Dyke et al. [36] pointed out that a stable genotype tends to sustain a constant
462 yield performance across testing environments. Consequently, genotypes with above mean
463 performance and are stable by outcome of this stability measures are desirable.

464 Superiority index value P_i is defined as the deviation of the i^{th} genotype relative to the genotype
465 with maximum performance in each environment. The top ranked 5 stable genotypes for fresh root
466 yield that tends to be closer to the best genotype in each environment were identified with lowest
467 P_i value include IITA-TMS-IBA000070, TMS14F1036P0007, TMS14F1016P0006,
468 TMS14F1262P0002, and TMS14F1035P0004, most of which would be attributed to genetic
469 deviation [33]. These genotypes also have relatively high fresh root yield above grand average
470 yield of 29.5 t/ha and their corresponding dry matter ranged from 31.3% for TMS14F1016P0006
471 to 37.4% for TMS14F1035P0004 (S4 Fig and S14 Table). Top ranked 5 genotype showing

472 consistent performance for dry matter content include TMS14F1035P0004, TMS14F1306P0015,
 473 TMS14F1291P0011, TMS14F1195P0005, and TMS14F1049P0001 (S5 Fig and S14 Table).
 474 However, it is superiority index associated to dry yield that showed genotypes with consistent
 475 performance for both fresh root yield and dry matter content and top ranked 5 genotypes were
 476 TMS14F1036P0007, IITA-TMS-IBA000070, TMS14F1035P0004, TMS14F1262P0002, and
 477 TMS13F2207P0001 (S6 Fig and S14 Table).

478 **Representative of target population of environments**

479 The correlation coefficient of each environment's BLUPs with genotypic BLUPs of all
 480 environments in the TPE for fresh root yield ranged from 0.33 (Ibadan18) to 0.73 (Ago-Owu19)
 481 with corresponding heritability estimates of 0.71 and 0.80, respectively (Table 3). The top ranked
 482 5 environments showing high correlation with TPE and high heritability estimate include Ago-
 483 Owu19 (0.73, 0.80), Ikenne18 (0.69, 0.82), Ibadan19 (0.68, 0.73), Ago-Owu18 (0.56, 0.74), and
 484 Ikenne20 (0.67,0.68) (Table 3).

485 **Table 3.** Correlation coefficient (r) of environment_specific BLUPs with all target population of
 486 environment (TPE) and environment-specific heritability (H²) based on the Cullis method [34] for
 487 fresh root yield (t/ha).

Environment	r	(H ²)	rank(r)	rank(H ²)	Sum ranks
Ago-Owu19	0.73	0.80	1	2	3
Ikenne18	0.69	0.82	2	1	3
Ibadan19	0.68	0.73	4	5	9
Ago-Owu18	0.56	0.74	9	4	13
Ikenne20	0.67	0.68	5	8	13
Onne19	0.67	0.69	6	7	13
Ago-Owu20	0.68	0.61	3	11	14
Mokwa18	0.47	0.74	12	3	15

Ibadan20	0.61	0.51	7	15	22
Umudike19	0.61	0.56	8	14	22
Ibadan18	0.33	0.71	17	6	23
Ikenne19	0.54	0.57	10	13	23
Mokwa19	0.46	0.61	13	10	23
Ubiaja20	0.42	0.62	15	9	24
Otobi19	0.44	0.58	14	12	26
Abuja20	0.49	0.27	11	16	27
Onne20	0.40	0.26	16	17	33

489

490 As for dry matter content, the environments revealed a higher range of correlation coefficient with
491 TPE relative to fresh root yield varying from Onne20 (0.48) to Ikenne20 (0.85) with corresponding
492 heritability estimate of 0.57 and 0.88, respectively (S15 Table). The top ranked 5 environments to
493 represent the TPE for showing high correlation and high heritability included Ikenne20 (0.85,
494 0.88), Ikenne18 (0.79, 0.78), Onne19 (0.72, 0.87), Ubiaja20 (0.73, 0.82), and Umudike (0.78,
495 0.77). For top yield there was higher variability in the correlation coefficient with TPE ranging
496 from 0.14 (Mokwa19) to 0.83 (Ubiaja20) with heritability estimates of 0.32 and 0.71, respectively
497 (S16 Table). The top ranked 5 environments to represent the TPE were Ikenne19 (0.82, 0.81),
498 Otobi19 (0.83, 0.78), Ikenne18 (0.79, 0.82), Ago-Owu20 (0.79, 0.76), and Ubiaja20 (0.83, 0.71).
499 A line graph provides further insights into the number of environment(s) that is likely to be
500 sampled to represent TPE and their corresponding breeding value accuracy compared to all the
501 environment for fresh root yield, dry matter content, and top yield (Fig 6). Regardless of the
502 number of environments sampled, the breeding value accuracy of fresh root yield is lower
503 compared to dry matter content and top yield. As revealed in Fig 6, sampling of five (5)
504 environments is likely to represent TPE where fresh root yield has an approximate breeding value


505 accuracy of 0.84 lower than dry matter content and top yield with breeding value accuracy of 0.92
506 and 0.91 respectively.

507 **Fig 6. A line graph showing the estimated breeding value accuracy against the number of**
508 **sampling environments for dry matter content (dmc), fresh root yield (fyld), and top yield**
509 **(tyld).**

510 The relatedness among the testing TPE for fresh root yield revealed the grouping of the testing
511 TPE into three cluster groups such that environments within a cluster are more similar and
512 dissimilar from environments in other cluster (S7 Fig). As for dry matter content, the TPEs were
513 grouped into 4 clusters (S8 Fig). However, 6 cluster groups of TPE were identified for top yield
514 out of which 3 clusters have one environment each (S9 Fig).

515 **Discussion**

516 This study demonstrated the application of classical ANOVA in a linear mixed model
517 framework and linear-bilinear models such as Finlay-Wilkinson, additive main effect and
518 multiplicative interaction model, and genotype plus genotype environment models towards
519 identifying stable genotypes, mega-environments and environments representative of the TPE.

520 The large sum of squares and significant effect of environment on the observed agronomic
521 traits as shown by FW, AMMI, and GGE models demonstrated that the field trials were conducted
522 under diverse environmental conditions causing variation in cassava genotypes yield and other
523 yield-related traits. The significant variation of GEI effect found for the observed agronomic traits
524 indicated that neither genotype nor environment effect can independently capture all the variation
525 observed. This resulted in diverse performance of the genotypes in the testing environments. This
526 variation is useful when proposing to examine GEI, as well as to assess the stability of genotypes.
527 We found that the AMMI model attributed largest percentage of treatment sum of squares to
528 environment (71.1%) for fresh root yield. This finding was contrary to Tumuhimbise et al. [2] who
529 reported that genotype accounted for largest percentage of treatment sum of squares (48.5%). The
530 disparity in the result may be due to the fact that our study evaluated 36 genotypes in 17
531 environments compared to 12 genotypes in 3 environments in  Tumuhimbise et al. [2]. Also
532 contrary to our findings, Jiwuba et al. [37] reported that GEI accounted for largest percentage of
533 treatment sum of squares (43.80%) in their study where 60 genotypes were evaluated over 6
534 environments.

535 As for top yield, the environment captured largest percentage of total variability (76.1%)
536 from the AMMI model. This was contrary to findings from Jiwuba et al. [37] who reported that
537 environment accounted for the lowest percentage of total variation (11.9%) for the biomass. The

538 disparity in the result may be because they evaluated fewer environments (6) in their study
539 compared to 17 environments in our study. Unlike fresh root yield and top yield, the percentage
540 of total sum of squares attributed to G (16.1%) was relatively close to GEI (20.6%) for dry matter
541 content, indicating that there was similarity in dry matter response of some of the genotypes across
542 environments. However, all the linear bilinear models explored in this study revealed that the
543 environment accounted for much greater variation on DMC compared to genotypic effect. This
544 may be due to the fact this is a UYT study, so that genotypes have already been strongly selected,
545 so that genetic variability is reduced. In contrast, Benesi et al. [38] reported that genotypic
546 influence on dry matter content is much higher than for the environment.

547 AMMI, like the FW model, revealed a significant genotypic effect for the observed
548 agronomic traits, signifying the presence of genetic variation in IITA cassava germplasm. This is
549 similar to what Nduwumuremyi et al. [39] reported about the existence of significant genetic
550 variation in Rwandan germplasm.

551 The limitation of classical ANOVA is that it does not provide insight into the complex
552 pattern of GEI, which necessitates further use of linear bilinear models. The strength of AMMI
553 and GGE models is that they concurrently visualize genotypes and environments using biplots that
554 expedite the interpretation of GEI. On biplots, a genotype in the vicinity of an environment with a
555 large IPC score is expected to display a higher performance in that environment in comparison to
556 its mean performance, and conversely for genotypes located far from that environment on the
557 biplot.

558 **Conclusion**

559 The classical statistical methods used in this study found highly significant genotype x
560 environment interaction, a major challenge confronting cassava breeders in the course of breeding
561 for high yielding and stable varieties. We also observed highly diverse environments, with
562 environment effects accounting for a large percentage of observed variation in the agronomic traits
563 as these traits are polygenic in nature. There were 6 mega-environments identified from 17 testing
564 environments as a function of winning genotypes.

565 The Finlay-Wilkinson, AMMI, and GGE are fixed effect models and they may not be an
566 appropriate approach to use when estimating quantitative genetic parameters in the presence of
567 unbalanced data and/or when jointly analyzing heterogeneous trial designs. Such circumstances
568 require a mixed model approach where different variance covariance structures can be explored.
569 In addition, these models assumed homogeneity of error variances across the testing environments
570 which may be misleading as error variances were heterogeneous as revealed through likelihood
571 ratio tests. None of these linear bilinear models can account for relatedness among the genotypes,
572 e.g., using relatedness matrices from pedigree and/or molecular data.

573 Though the same genotypes were evaluated across the testing environments (trials or
574 location by year combination), there were locations (Abuja, Otobi, Umudike, and Ubiaja) where
575 this study was carried out in just one out of three cropping seasons. This caused unbalancedness
576 in the data structure. Therefore, the outcome of delineating the testing environments into mega-
577 environments may be misleading. In future studies, it may be advisable to have more than one
578 cropping season of data from such locations to ensure having well-defined mega environments.
579 To get a clearer picture of locations that are representative of the TPE, future studies may require

580 many years of historical data. To better understand the factors influencing the GEI, it is advisable
581 to explicitly exploit soil and weather data.

582 **Acknowledgements**

583 The authors thank the UK's Foreign, Commonwealth & Development Office (FCDO) and
584 the Bill & Melinda Gates Foundation (Grant INV-007637, <http://www.gatesfoundation.org>) for
585 their financial support. We also extend our appreciation to the dedicated staff of the International
586 Institute of Tropical Agriculture (IITA) of the cassava breeding program that assisted with the
587 fieldwork, screen house evaluation and laboratory analyses.

588

589 **References**

- 590 1. Egesi CN, Ilona P, Ogbe FO, Akoroda M, Dixon A. Genetic variation and genotype X
591 environment interaction for yield and other agronomic traits in cassava in Nigeria. *Agron J.*
592 2007;99(4):1137–42.
- 593 2. Tumuhimbise R, Melis R, Shanahan P, Kawuki R. Genotype × environment interaction
594 effects on early fresh storage root yield and related traits in cassava. *Crop J.* 2014;2(5):329–
595 37.
- 596 3. Kvitschal MV, Filho PSV, Scapim CA, Gonçalves-Vidigal MC, Sagrilo E, Pequeno MG,
597 Rimoldi F. Comparison of methods for phenotypic stability analysis of cassava (*Manihot*
598 *esculenta crantz*) genotypes for yield and storage root dry matter content. *Braz Arch Biol*
599 *Technol.* 2009;52(1):163–75.
- 600 4. Kawano K. Thirty years of cassava breeding for productivity - Biological and social factors
601 for success. *Crop Sci.* 2003;43(4):1325–35.
- 602 5. Sayre R, Beeching JR, Cahoon EB, Egesi C, Fauquet C, Fellman J, Fregene M, Gruissem
603 W, Mallova S, Manary M, Maziya-Dixon B, Mbanaso A, Schachtman DP, Sirtunga D,
604 Taylor N, Vanderschuren H, Zhang P. The BioCassava Plus Program: Biofortification of
605 Cassava for Sub-Saharan Africa. *Annu Rev Plant Biol.* 2011;62(1):251–72.
- 606 6. Ceballos H, Kulakow P, Hershey C. Cassava Breeding: Current Status, Bottlenecks and the
607 Potential of Biotechnology Tools. *Trop Plant Biol.* 2012;5(1):73–87.
- 608 7. Kvitschal MV, Vidigal Filho PS, Scapim CA, Gonçalves-Vidigal MC, Pequeno MG,
609 Sagrilo E, Rimoldi F. Evaluation of phenotypic stability of cassava clones by AMMI
610 analysis in northwestern Paraná state. *Crop Breed Appl Biotechnol.* 2006;6(3):236–41.
- 611 8. Gregorius HR, Namkoong G. Joint analysis of genotypic and environmental effects. *Theor*
612 *Appl Genet.* 1986;72(3):413–22.
- 613 9. Crossa J. From Genotype × Environment Interaction to Gene × Environment Interaction.
614 *Curr Genomics.* 2012;13(3):225–44.
- 615 10. Crossa J, Cornelius PL. Sites regression and shifted multiplicative model clustering of
616 cultivar trial sites under heterogeneity of error variances. *Crop Sci.* 1997;37(2):406–15.
- 617 11. Gauch HG, Zobel RW. Identifying mega-environments and targeting genotypes. *Crop Sci.*
618 1997;37(2):311–26.
- 619 12. Gauch HGJr. Model Selection and Validation for Yield Trials with Interaction. Published
620 by : International Biometric Society Stable URL : <http://www.jstor.org/stable/2531585>.
621 2016;44(3):705–15.
- 622 13. Gabriel. The biplot graphic display of matrices with application to principal component
623 analysis. *Biometrika.* 1971;58:453–67.

- 624 14. Kempton RA. The use of biplots in interpreting variety by environment interactions. *J Agric*
625 *Sci.* 1984;103(1):123–35.
- 626 15. Hagos HG, Abay F. AMMI and GGE biplot analysis of bread wheat genotypes in the
627 northern part of Ethiopia. *J Plant Breed Genet.* 2013;1(1):12–8.
- 628 16. Purchase J. Parametric analysis to describe genotype by environment interaction and yield
629 stability in winter wheat. PhD Thesis. 1997;83–83.
- 630 17. Mahmodi N, Yaghotipoor A, Farshadfar E. AMMI stability value and simultaneous
631 estimation of yield and yield stability in bread wheat ('Triticum aestivum' L.). *Aust J Crop*
632 *Sci.* 2011;5(13):1837–44.
- 633 18. Yan W, Hunt LA, Sheng Q, Szlavnic Z. Cultivar evaluation and mega-environment
634 investigation based on the GGE biplot. *Crop Sci.* 2000;40(3):597–605.
- 635 19. Wickham H. *ggplot2: Elegant Graphics for Data Analysis.* [Internet]. New York: Springer-
636 Verlag; 2016. Available from: <https://ggplot2.tidyverse.org>
- 637 20. R Core Team. *R: A language and environment for statistical computing.* R Foundation for
638 Statistical Computing, Vienna, Austria. 2018;
- 639 21. SAS Institute Inc. *SAS/STAT(®) 13.3 User's Guide.* Cary, NC: SAS Institute Inc.; 2013.
- 640 22. Mrode RA. *Linear Models for the prediction of animal breeding values.,* Third ed. CABI,
641 Oxfordshire, UK.; 2014.
- 642 23. Bart-Jan van R. *statgenGxE: Genotype by Environment (GxE) Analysis.* R package version
643 1.0.4. 2021; Available from: <https://cran.r-project.org/package=statgenGxE>
- 644 24. Finlay KW Wilkinson, GN. THE ANALYSIS OF ADAPTATION IN A PLANT-
645 BREEDING PROGRAMME and G. N. WILKINSON? By K. W. FINLAY*. *Aust J Agric*
646 *Res.* 1963;14(1958):742–54.
- 647 25. Malosetti M, Ribaut JM, van Eeuwijk FA. The statistical analysis of multi-environment
648 data: Modeling genotype-by-environment interaction and its genetic basis. *Front Physiol.*
649 2013;4 MAR(March):1–17.
- 650 26. Falcon CM, Kaeppler SM, Spalding EP, Miller ND, Haase N, AlKhalifah N, Bohn M,
651 Buckler ES, Campbell DA, Ciampitti I. Relative utility of agronomic, phenological, and
652 morphological traits for assessing genotype- by- environment interaction in maize inbreds.
653 *Crop Sci.* 2020;60(1):62–81.
- 654 27. Gauch HGJr. Model Selection and Validation for Yield Trials with Interaction. *Biometrics.*
655 1988;44(3):705–15.
- 656 28. Farshadfar E, Sutka J. Biplot analysis of genotype-environment interaction in durum wheat
657 using the ammi model. *Acta Agron Hung.* 2006;54(4):459–67.

- 658 29. Yan W, Kang MS, Ma B, Woods S, Cornelius PL. GGE biplot vs. AMMI analysis of
659 genotype-by-environment data. *Crop Sci.* 2007;47(2):643–55.
- 660 30. Hongyu K, Silva F, Oliveira A, Sarti DA, Araújo L, Dias C. Comparação entre os modelos
661 AMMI e GGE-Biplot para os dados de ensaios multi-ambientais. *Rev Bras Biom.*
662 2015;33(2):139–55.
- 663 31. Yan W, Kang MS. GGE biplot analysis: A graphical tool for breeders, geneticists, and
664 agronomists. CRC Press, Boca Raton, FL.; 2003.
- 665 32. Yan W. GGE biplot vs. AMMI graphs for genotype-by-environment data analysis. *J Indian*
666 *Soc Agric Stat.* 2011;65(2):181–93.
- 667 33. Lin C-S, Binns MR. A superiority measure of cultivar performance for cultivar× location
668 data. *Can J Plant Sci.* 1988;68(1):193–8.
- 669 34. Cullis BR, Smith AB, Coombes NE. On the design of early generation variety trials with
670 correlated data. *J Agric Biol Environ Stat.* 2006;11(4):381–93.
- 671 35. Ward JH. Hierarchical Grouping to Optimize an Objective Function. *J Am Stat Assoc.*
672 1963;58(301):236–44.
- 673 36. Dyke G, Lane P, Jenkyn J. Sensitivity (stability) analysis of multiple variety trials, with
674 special reference to data expressed as proportions or percentages. *Exp Agric.*
675 1995;31(1):75–87.
- 676 37. Jiwuba L, Danquah A, Asante I, Blay E, Onyeka J, Danquah E, Egesi C. Genotype by
677 Environment Interaction on Resistance to Cassava Green Mite Associated Traits and
678 Effects on Yield Performance of Cassava Genotypes in Nigeria. *Front Plant Sci.*
679 2020;11:572200.
- 680 38. Benesi I, Labuschagne M, Dixon A, Mahungu N. Genotype x environment interaction effects
681 on native cassava starch quality and potential for starch use in the commercial sector. *Afr*
682 *Crop Sci J.* 2004;12(3):205–16.
- 683 39. Nduwumuremyi A, Melis R, Shanahan P, Theodore A. Interaction of genotype and
684 environment effects on important traits of cassava (*Manihot esculenta* Crantz). *Crop J.*
685 2017;5(5):373–86.

686

687 **Supporting information**

688 **S1 Fig.** This is the scatter plot of coefficient of variation and heritability.

689 **S2 Fig.** This is the scatter plot of experimental accuracy and heritability.

690 **S3 Fig.** This is the boxplot showing distribution of observed traits for some parameter estimates.

691 **S4 Fig.** This is the scatter plot of cultivar superiority index and mean fresh root yield.

692 **S5 Fig.** This is the scatter plot of cultivar superiority index and mean dry matter content.

693 **S6 Fig.** This is the scatter plot of cultivar superiority index and mean dry yield.

694 **S7 Fig.** This is clustering of environments based on genotypic blups of fresh root yield.

695 **S8 Fig.** This is clustering of environments based on genotypic blups of dry matter content.

696 **S9 Fig.** This is clustering of environments based on genotypic blups of top yield.

697 **S1 Table.** This is the summary statistics of individual trials

698 **S2 Table.** This is the likelihood ratio test of absence versus presence of GEI

699 **S3 Table.** This is the likelihood ratio test of homogeneity versus heterogeneity of error
700 variances.

701 **S4 Table.** This is the ANOVA table showing the variance component estimates.

702 **S5 Table.** This is ANOVA table showing the partition of GEI variance component.

703 **S6 Table.** This is the ANOVA table resulting from Finlay-Wilkinson (FW) model.

704 **S7 Table.** This is the genotypes ranking based on sensitivities values from FW model.

705 **S8 Table.** This is minimum, median, maximum, and variance of MSE from FW model.

706 **S9 Table.** This is minimum, median, maximum, and variance of slopes from FW model.

707 **S10 Table.** This is the combined ANOVA table resulting from AMMI model.

708 **S11 Table.** This is the ranking of genotypes based on AMMI stability value and genotype
709 selection index.

710 **S12 Table.** This is the table showing mega-environment based on AMMI2 model.

711 **S13Table.** This is the combined ANOVA table resulting from GGE model.

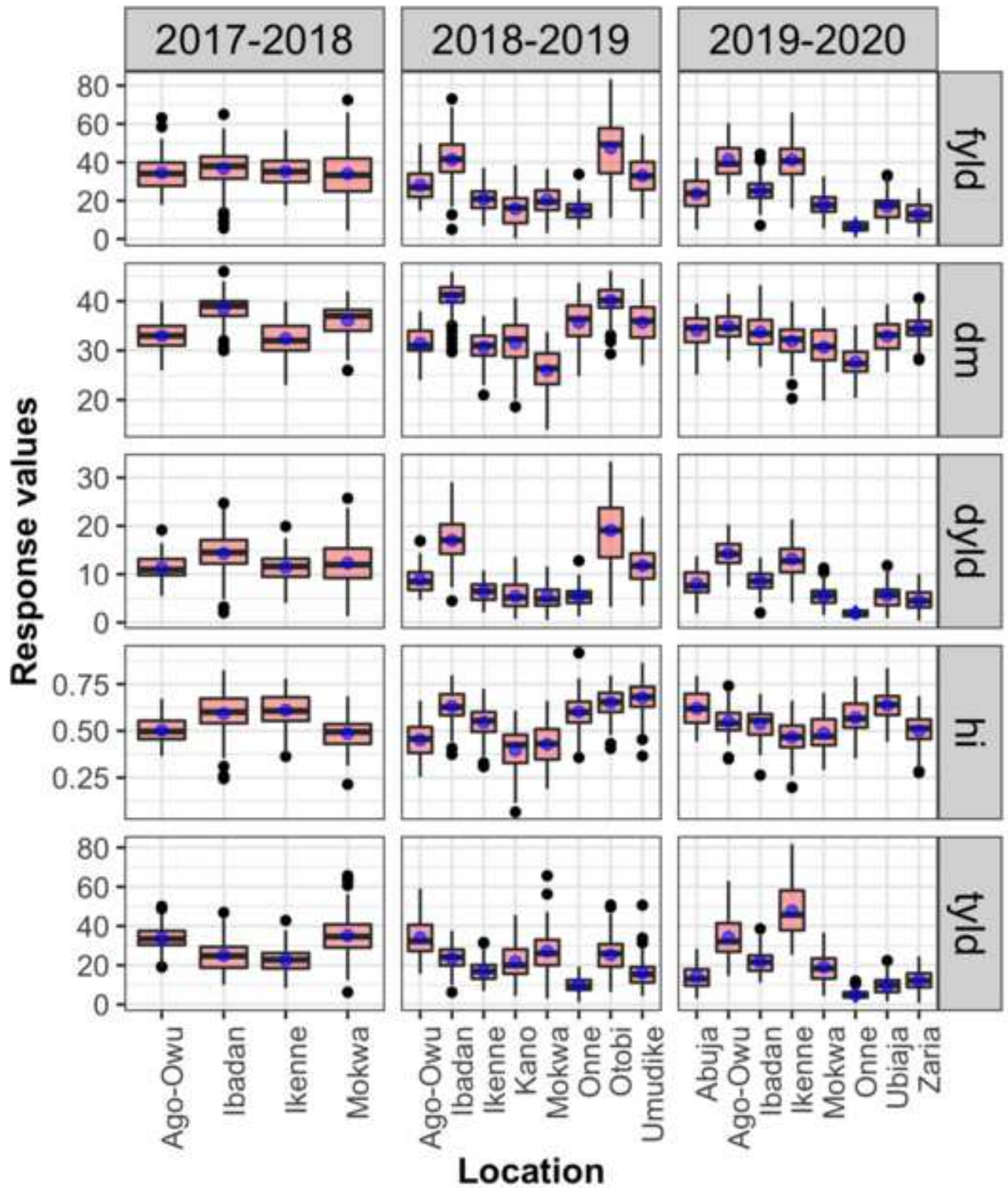
712 **S14 Table.** This is the assessment of stability of genotypes based on cultivar superiority index.

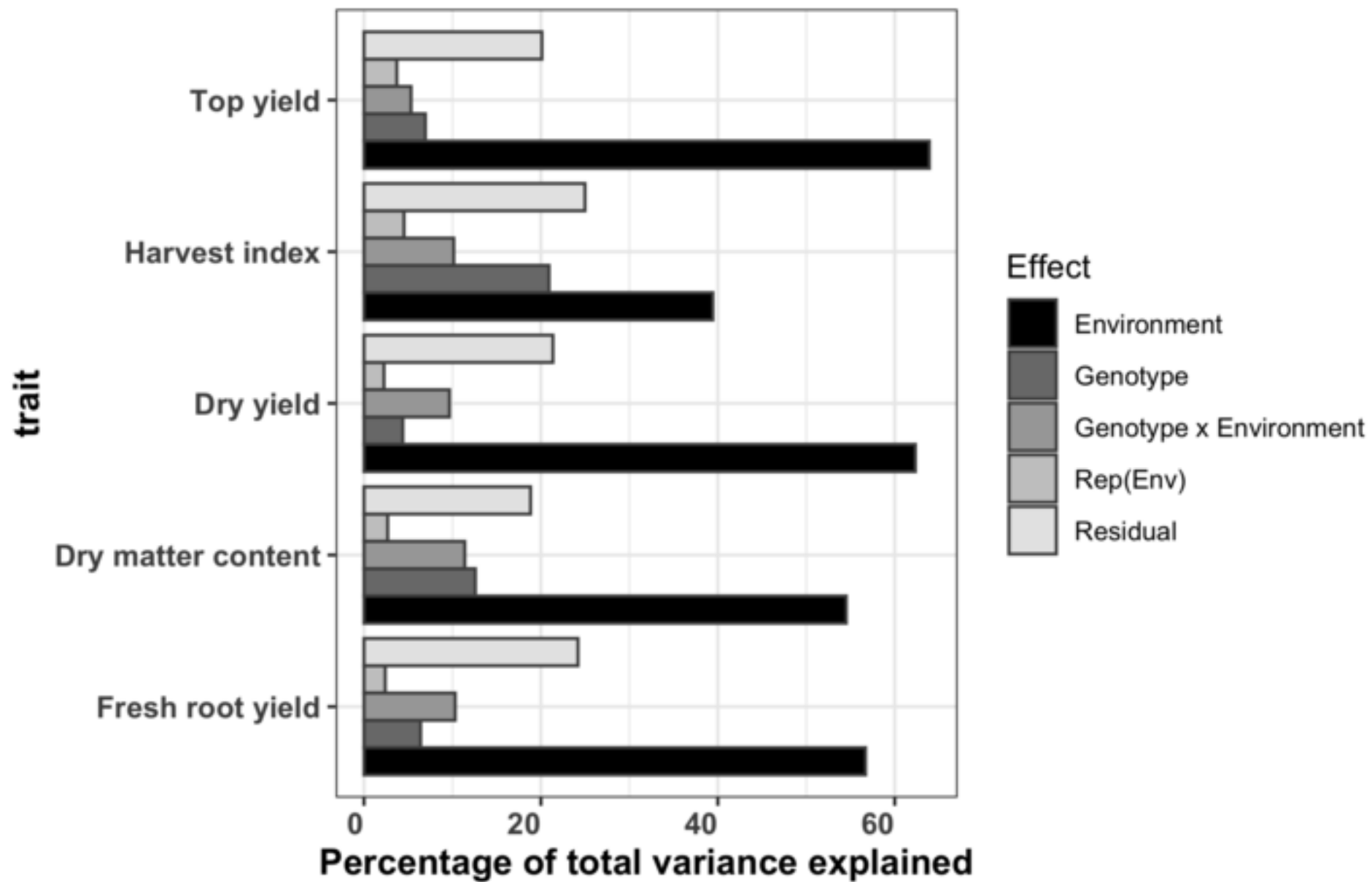
713 **S15 Table.** This is the ranking of testing environments for dry matter content based on

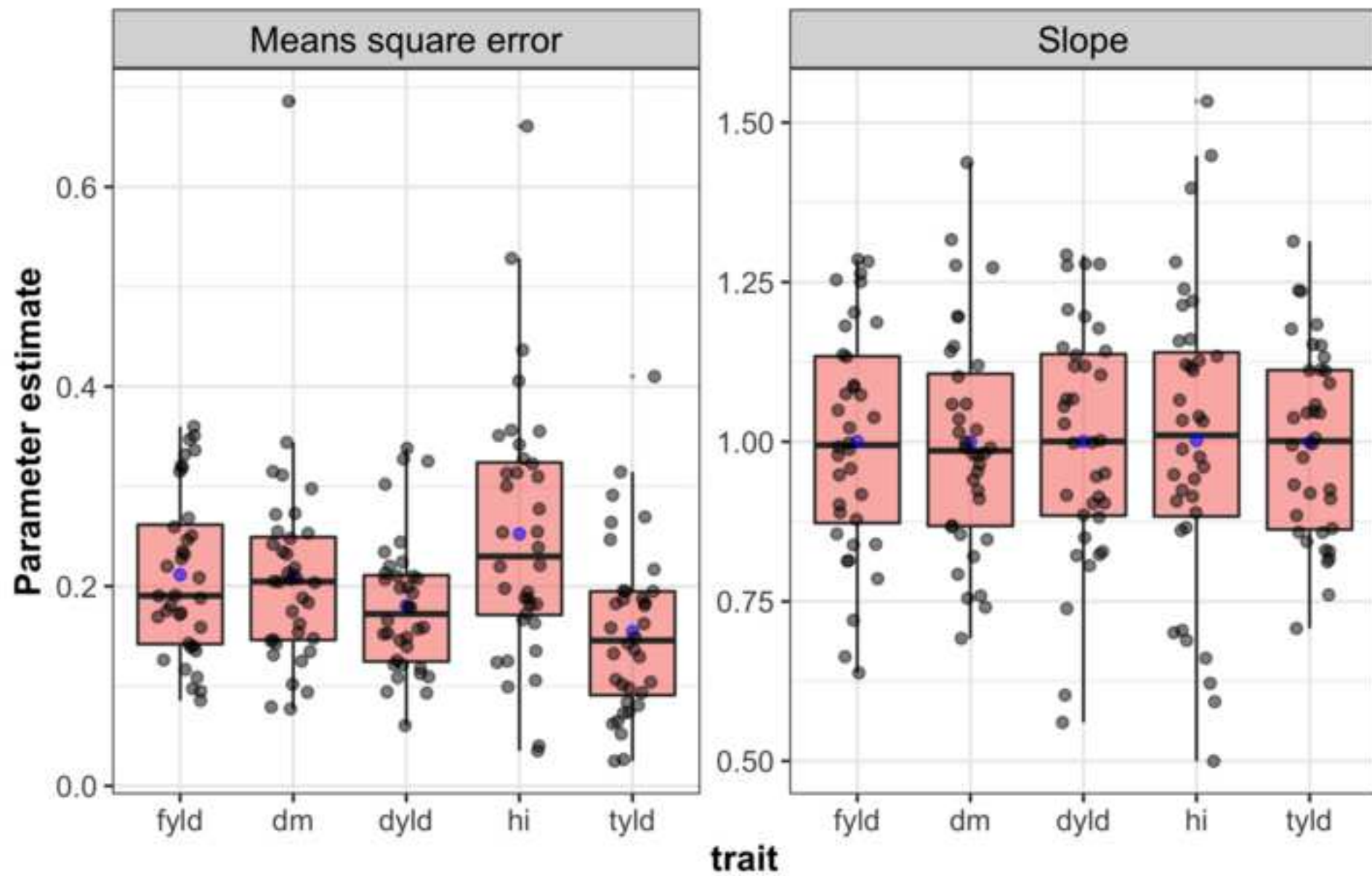
714 correlation and Cullis heritability.

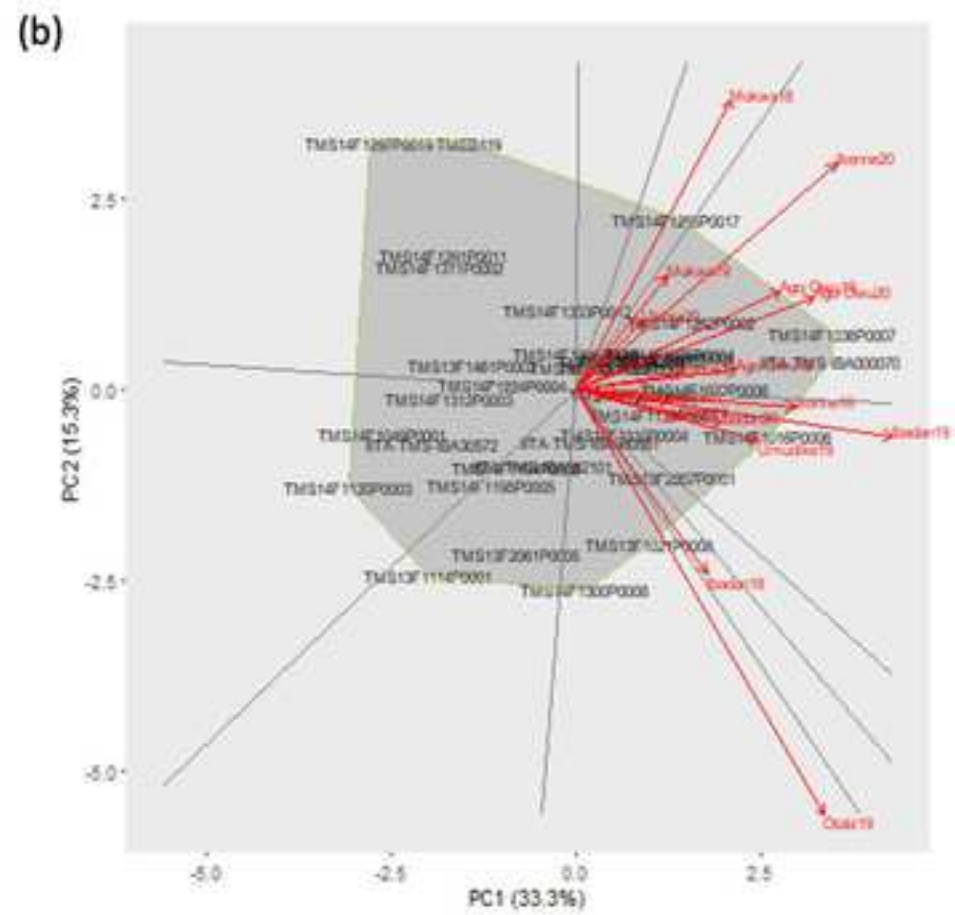
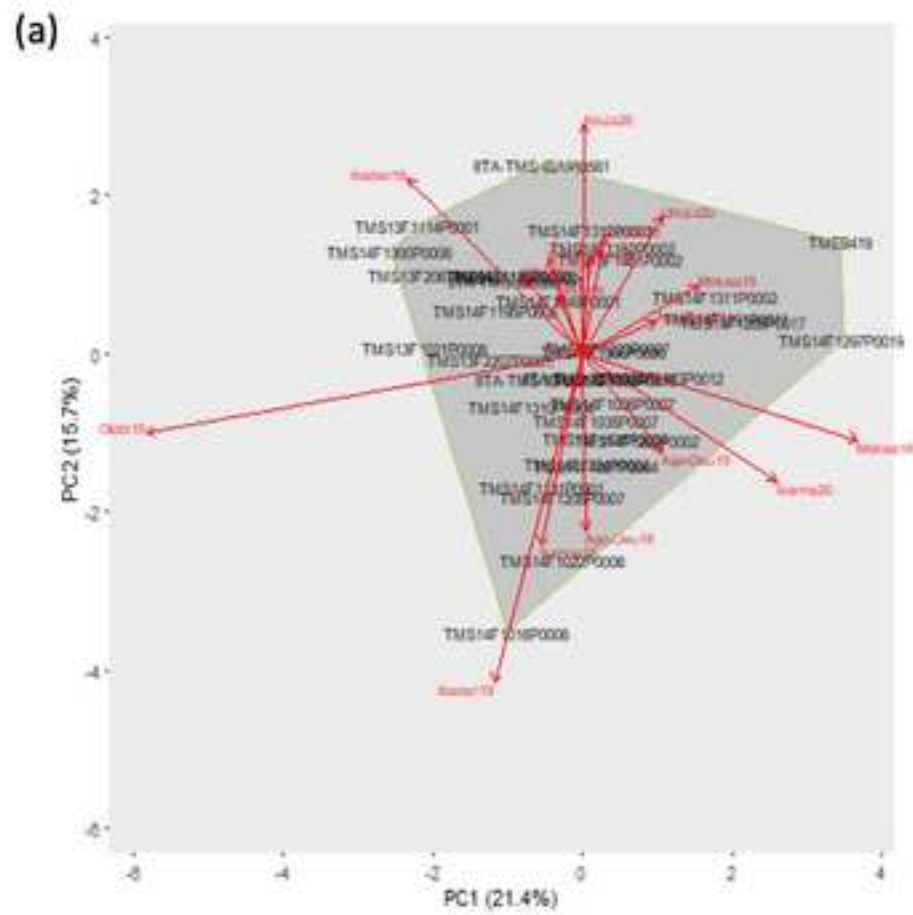
715 **S16 Table.** This is the ranking of testing environments for top yield based on correlation and

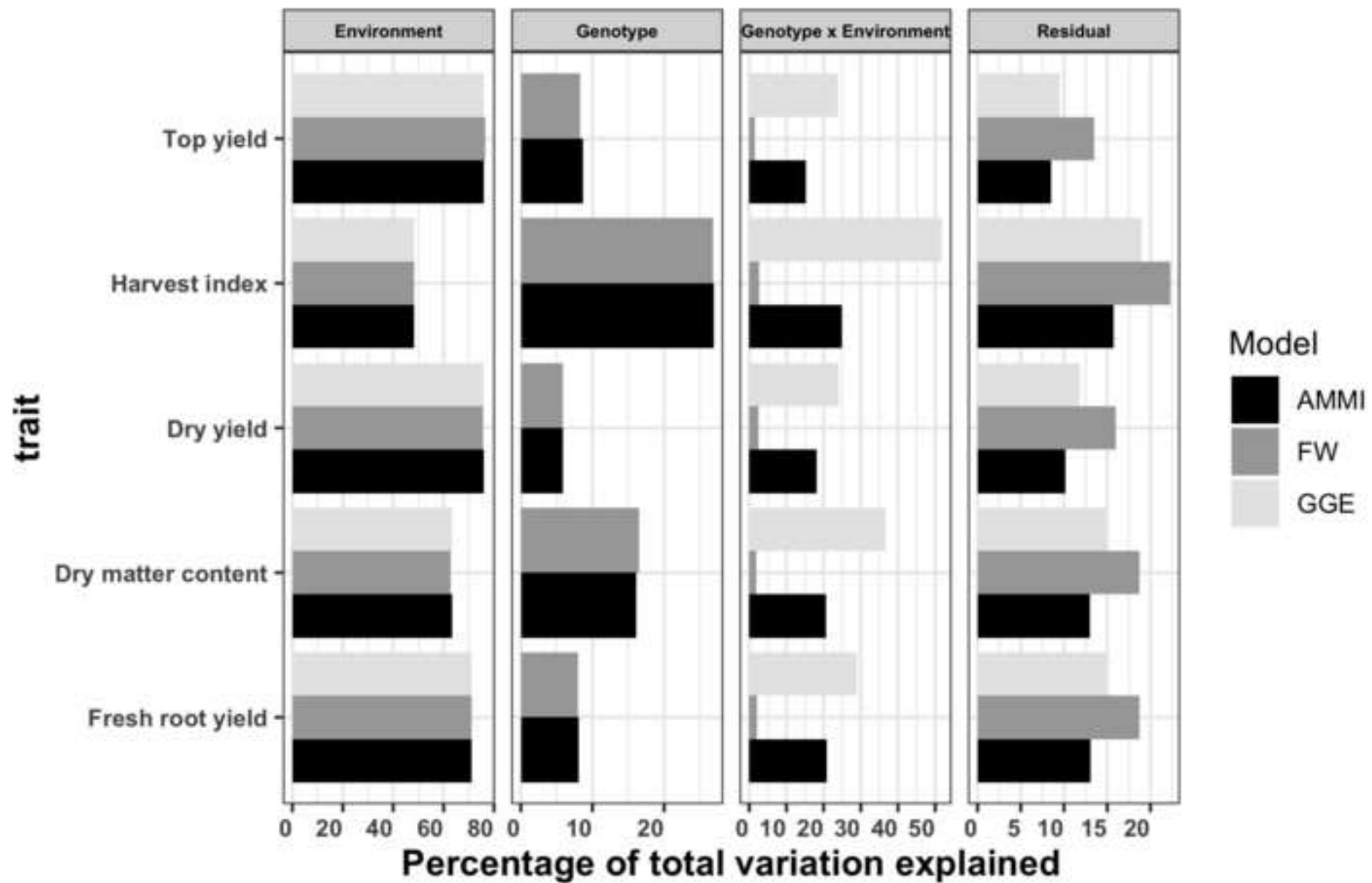
716 Cullis heritability.

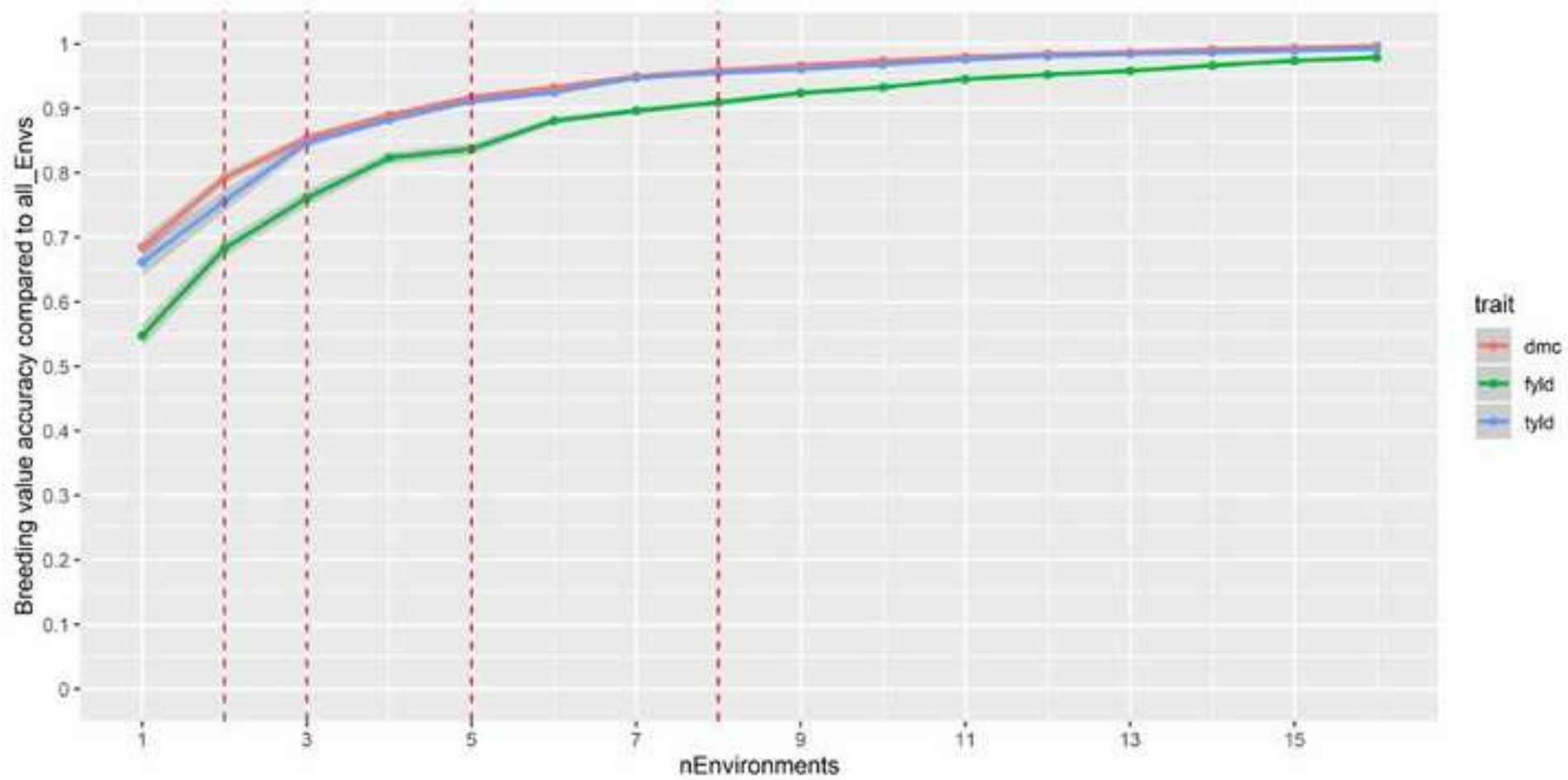














Click here to access/download

Supporting Information - Compressed/ZIP File Archive
supporting_information.zip





Click here to download Data Review URL
https://github.com/mab658/classical_analysis_GxE

