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## Genotype by environment interaction effect and fresh root yield stability of cassava genotypes under contrasting nitrogen regimes

Joseph Okpani Mbe<sup>1,2</sup>, Daniel Dzidzienyo<sup>2,4</sup>, Simon Peter Abah<sup>1,2</sup>, Damian Ndubuisi Njoku, Cynhia Idhigu Aghogho<sup>2,5</sup>, Joseph Onyeka<sup>1,5</sup>, Pangirayi Tongoona<sup>2</sup> & Chiedozie Egesi<sup>1,3</sup>✉

Nitrogen (N) is an important nutrient element needed by cassava for optimum yield and it is a vital component of nucleotides (nucleic acids), enzymes, amino acids (proteins), chlorophyll molecules and hormones, among other essential compounds required for growth and development of cassava. Nitrogen stress is a major cassava production constraint, the study aimed to examine genotype by environment interaction (GEI) effects and fresh root yield stability of 203 diverse cassava clones to identify genotypes with stable performance under low and optimum nitrogen regimes across environments using AMMI and GGE biplot analysis. Experiments were conducted using an augmented block design with three replications for two years in three locations in Nigeria. There were significant differences ( $p < 0.001$ ) in the genotype's mean performances as well as significant differences ( $p < 0.001$ ) in the environment's mean performances for all the traits measured in both nitrogen regimes. The AMMI analysis of variance showed significant effects ( $p < 0.001$ ) for genotypes, environments and the interactions for fresh root yield in both nitrogen regimes. The biplot analysis showed that for fresh root yield in the optimum nitrogen regime, the principal component accounted for 81.54% of the G + GE (Genotype plus and Genotype by Environment) variation. The G + GE for fresh root yield in the low nitrogen regime accounted for a total of 71.64% of the variation. Ten genotypes were identified as the best genotypes under the optimum nitrogen regime, while eleven genotypes were the best under the low nitrogen regime. Three genotypes under optimum nitrogen regimes were high-yielding. Still, they were unstable in their fresh root yield performance across the environments and can be recommended as specifically adapted to the environments they performed best. Three other genotypes were high-yielding genotypes under low nitrogen but were highly unstable in their fresh root yield mean performance across the environments. The environments Otobi\_YR1, Igbariam\_YR2, and Umudike\_YR1 were identified as the most discriminatory among the test environments. The environments Umudike\_YR2 and Igbariam\_YR1 were identified as the most representative of the test environments and can represent a mega-environment. The best 21 genotypes that performed above the grand mean for fresh root yield in both nitrogen regimes can be further evaluated on the farmer's field for possible advancement.

In most food-insecure regions of the world, such as sub-Saharan Africa, cassava (*Manihot esculenta* Crantz), which provides more than a quarter of per capita calorie consumption, is a primary source of food for more than a billion people<sup>1</sup>. Nigeria is the world top producer of cassava, and a 12 metric ton shortage is anticipated by 2030<sup>2</sup>. Since the yield of cassava storage roots has not improved much in the majority of regions during the 1990s, there are worries about future cassava yield shortages<sup>3</sup>, with yields declining by 0.024 t ha<sup>-1</sup> per year<sup>4</sup>. Soil nutrient depletion, especially nitrogen, has been identified as the main reason leading to low productivity of cassava. Nitrogen deficiency affects stay green ability, photosynthetic rate, translocation of organic compounds

<sup>1</sup>National Root Crops Research Institute, (NRCRI), Umudike, Nigeria. <sup>2</sup>West Africa Centre for Crop Improvement (WACCI), University of Ghana, Accra, Ghana. <sup>3</sup>Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA. <sup>4</sup>Btechnology Centre, University of Ghana, Accra, Ghana. <sup>5</sup>International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. ✉email: cne22@cornell.edu

from source to sink, and fresh root yield<sup>5</sup>. The challenge of low soil nitrogen is widespread and cuts across sub-Saharan African<sup>6</sup>.

Therefore, a proper understanding about the interactions between cassava, soil, and nutrients is fundamental to improving cassava fresh root yield<sup>7</sup>. Improving nitrogen use efficiency in cassava is economically and environmentally efficient way of increasing cassava productivity<sup>5</sup>.

Over the previous 50 years, the percentage of NUE in cassava output has dropped from 68 to 47% due to the overuse of N fertiliser. In cassava breeding programs, lowering N inputs and increasing N absorption and utilisation efficiency are important objectives<sup>8</sup>.

In sub-Saharan Africa, small-scale farmers who cannot afford the high cost of inorganic fertilizers make up the bulk of the cassava growers<sup>9</sup>. This group of farmers' accounts for over 70% of agricultural production and agriculture generates more than 75% employment in sub-Saharan Africa<sup>10</sup>. Nitrogen is costly to the farmers and the environment<sup>11,12</sup>. Nitrogen is mobile in the soil and crops are able to use a fraction of the applied fertilizers, which is only 30–40%<sup>13</sup>. Therefore, about 60% of fertilizer applied to the cropland is lost to the ecosystem resulting to adverse effect on the environment such as ecological imbalance and environmental pollution<sup>14</sup>. Developing cassava genotypes that respond to low nitrogen soils that are common in cassava-growing regions is one approach to solving these environmental issues. This would simultaneously increase yield and reduce input cost. Therefore, developing varieties of cassava that utilize nitrogen more effectively is a resource-efficient approach that has the potential to have a big impact on livelihoods, food security, and sustainability.

However, because of the variation in the complexity of genotype responses, choosing cassava genotypes that respond to low nitrogen is challenging. These various environmental genotypic expressions are usually described as genotype by environment interaction (GEI)<sup>15</sup>. These types of interactions reduce selection efficiency and the accuracy of genotype recommendation<sup>16</sup>. In varietal release programs, adequate consideration of GEI allows breeders to select genotypes that are superior with better stability and best locations that suits specific genotypes<sup>17</sup>. Stability studies on cassava have been primarily on yield traits such as fresh root yield<sup>18</sup>. The AMMI model integrates principal component analysis (PCA) and analysis of variance (ANOVA) into a single method that may be used to evaluate multi-local trials<sup>19</sup>. In AMMI, principal component analysis and analysis of variance are used to examine the residual multiplicative interaction between genotypes and environments for the main effects of genotypes and environments.

To establish the stability of the genotypes across location and to categorize environments with the best genotypes into mega-environments, PCA and AMMI stability value from AMMI analysis may be employed<sup>20</sup>. The IPCA1 and IPCA2 scores (interaction principal components axis 1 and 2, respectively) for each genotype from the AMMI model serve as the foundation for the AMMI stability value (ASV)<sup>21</sup>. Widely adapted genotypes are those with the least amount of ASV. Stability by itself does not reveal much about the yield level, thus<sup>22</sup> used yield stability index (YSI) which combines high yield performance with stability. Genotype stability index (GSI) and yield stability index (YSI) are determined by adding the performance rating and ranking based on ASV scores. Less stable genotypes with high yield or performance are indicated by lower GSI and YSI values. Plant physiological traits such as stay green ability, leaf retention, photosynthetic active radiation, plant vigor, leaf nitrogen content and chlorophyll content etc. have been reported to promote plants' responses to low nitrogen environments.

Usually, the small scale farmer which forms the major part of cassava production finds it difficult to apply inorganic fertilizers to their cassava crop due to financial constraints. Thus, genotypes with stable and improved expression of these characteristics will increase the effectiveness of resistance to nitrogen stress and raise yield. In order to provide insurance to resource-poor farmers in these situations, there is the need to sustainably breed crop plants that will respond favorable to low nitrogen fertilizers. Since soils are location specific, there is always the need to identify genotypes that can respond to varying amount of nitrogen levels in the soil across different locations. Studies have shown significant  $G \times E$  interaction effects on cassava storage root yield and NUE under different N regimes. Some cassava genotypes exhibit higher NUE and yield stability across varying N conditions compared to others<sup>23</sup>.

This research examined GEI for nitrogen utilization efficiency and associated attributes using multi-location trials and AMMI. The main objective of the study was to assess the performance and stability of cassava genotypes for tolerance to low nitrogen and other yield related agronomic traits.

## Results

### Genotype by environment interaction (GEI) for both treatments

A number of traits were recorded and the following post-harvest data were used for presentation of results and discussion; starch, dry matter content, harvest index, dry root yield, and fresh root yield. The AMMI model included four (4) principal components (PCs) in the ANOVA table and indicates their significance. In all cases, the first two PCs were more than 70% of the total GEI sum of squares and as a result, PC1 and PC2 were retained in the ANOVA table.

The AMMI analysis revealed significant genotype by environment interaction (GEI) for fresh root yield, dry matter content, dry root yield and starch in both nitrogen regimes. The GEI effect for harvest index was not significant across the two nitrogen regimes. More importantly, the percentage contribution of the GEI effect to the treatments sums of squares in both optimum and low nitrogen regimes was the highest (contributing more than 50% for all the traits considered) followed by the genotype and the environment respectively (Tables 1 and 2).

### Performances of genotypes across environments

There were significant differences ( $p < 0.001$ ) in the genotype's mean performances as well as significant differences ( $p < 0.001$ ) in the environment's mean performances for all the traits measured in both nitrogen

| Source                    | Mean square   |              |            |              |              |          |
|---------------------------|---------------|--------------|------------|--------------|--------------|----------|
|                           | df            | Dmc          | Dry        | starch       | Fry          | hi       |
| BLOCK(ENV)                | 45            | 35.47        | 25.51      | 55.81        | 639.06       | 0.10     |
| ENV (E)                   | 4             | 627.73***    | 2676.63*** | 1043.38***   | 23,700.87*** | 0.55***  |
| GEN (G)                   | 198           | 712.46***    | 100.03***  | 1261.55***   | 1108.33***   | 0.04 ns  |
| GEN:ENV (GEI)             | 768           | 290.31***    | 34.25*     | 514.74***    | 377.19***    | 0.014 ns |
| PC1                       | 201           | 10,608.90*** | 702.58***  | 19,158.91*** | 7831.16***   | 0.16**   |
| PC2                       | 199           | 283.63***    | 410.13***  | 529.74***    | 3886.61***   | 0.09 ns  |
| Residuals                 | 160           | 31.39        | 26.49      | 51.63        | 613.86       | 0.11     |
| Total                     | 1967          | 1323.78      | 163.86     | 2384.43      | 1751.87      | 0.05     |
| Source                    | Sum of square |              |            |              |              |          |
|                           | Df            | Dmc          | Dry        | starch       | Fry          | hi       |
| BLOCK(ENV)                | 45            | 1596.04      | 1148.11    | 2511.25      | 28,757.64    | 4.67     |
| ENV (E)                   | 4             | 2510.90      | 10,706.53  | 4173.52      | 94,803.49    | 2.21     |
| GEN (G)                   | 198           | 141,067.24   | 19,806.39  | 249,786.39   | 219,449.80   | 7.14     |
| GEN:ENV (GEI)             | 768           | 222,958.33   | 26,269.83  | 395,319.00   | 289,306.41   | 9.11     |
| PC1                       | 201           | 2,132,388.20 | 141,218.13 | 3,850,940.71 | 1,574,062.84 | 32.82    |
| PC2                       | 199           | 56,441.44    | 81,616.60  | 105,417.88   | 773,435.85   | 18.61    |
| Residuals                 | 160           | 5021.72      | 4132.17    | 8260.39      | 97,604.15    | 17.65    |
| Total                     | 1967          | 2,603,874.72 | 321,483.70 | 4,690,183.17 | 3,442,418.29 | 106.96   |
| % Treatment SS due to E   | 4             | 0.69         | 18.86      | 0.64         | 15.71        | 11.99    |
| % Treatment SS due to G   | 198           | 38.49        | 34.88      | 38.47        | 36.36        | 38.68    |
| % Treatment SS due to GEI | 768           | 60.83        | 46.26      | 60.89        | 47.93        | 49.32    |
| % GEI SS due to PC1       | 201           | 95.60        | 54.40      | 95.60        | 58.00        | 49.60    |
| % GEI SS due to PC2       | 199           | 2.50         | 31.50      | 2.60         | 28.50        | 28.10    |

**Table 1.** AMMI analysis of 199 genotypes including 5 checks evaluated under Optimum nitrogen regime across 5 environments. *Df* degrees of freedom, *fry* fresh root yield, *dry* dry root yield, *dmc* dry matter content, *hi* harvest index, *ns* non-significance \*Significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ , \*\*\*significant at  $p < 0.001$ .

regimes (Table 1 and 2). The AMMI ANOVA detects the presence and absence of genotype by environment interaction effects. It was revealed in this study that the GEI effects for all the traits were significant in both nitrogen regimes except harvest index which was not significant in both nitrogen regimes. The presence of GEI informs non-consistent performances of genotypes for a given trait across the set of environments used in the experiment. These further necessitate a detailed analysis to be able to understand the performances of the genotypes with respect to their environments. Therefore, the traits that had significant GEI effect were used to understand how the genotypes performed, how stable their performances were, which genotype won in which environment, and also how discriminatory and representative these environments were. However, for the presentation of results and discussion purposes this section (Stability Analysis) is limited to fresh root yield since yield is the most important criteria for evaluating the effects of applied treatments for that matter fresh root yield in cassava.

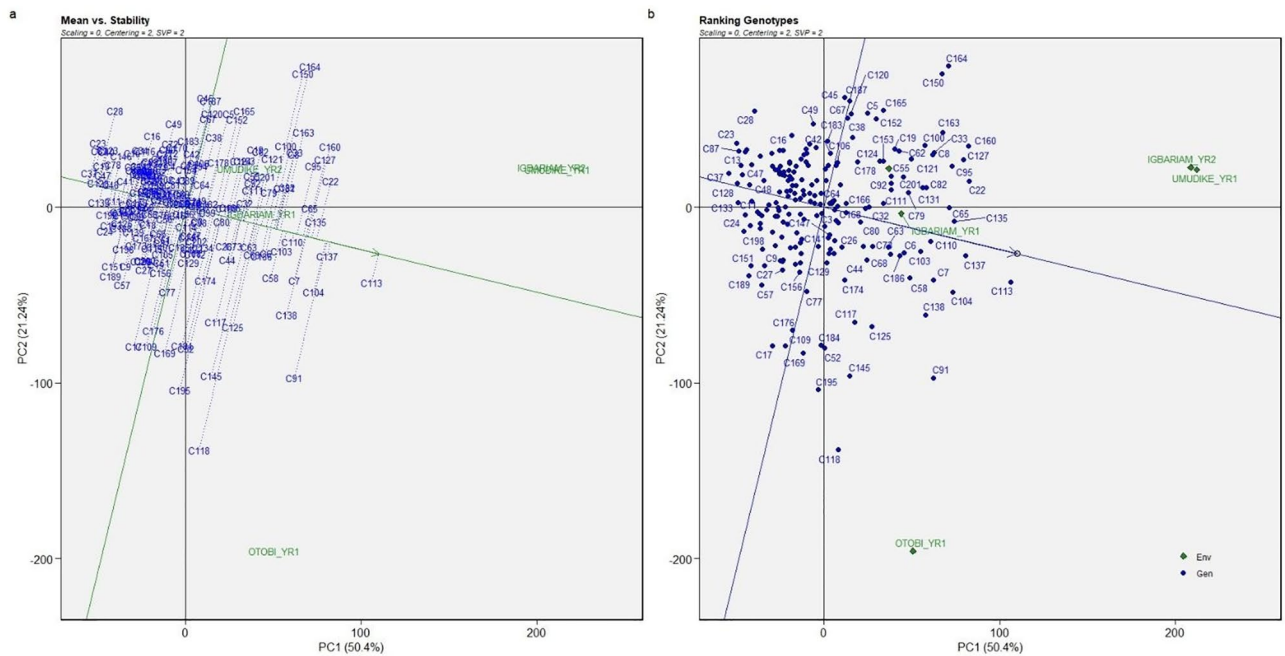
### Genotypes means performance and stability of fresh root yield across the test environments

The genotype-metric preserving (SVP = 1) and environment mean-centered (Centering = 2) GGE biplots study for means versus stability lacked scaling and transformation (Scaling = 0, Transformation = 0). The biplot analysis showed that for fresh root yield in the optimum nitrogen regime, the principal component axis 1 explained 61.88% of the total variation while the principal component axis 2 explained 19.66%, which accounted for 81.54% of the G + GE (Genotype plus and Genotype by Environment) variation (Fig. 1a). For the G + GE for fresh root yield in the low nitrogen regime, axis 1 (50.40%) and axis 2 (21.24) together accounted for a total of 71.64% of the variation (Fig. 2a).

Figures 1 and 2 shows the GGE biplot overview of average yield responses and stability for the one hundred and ninety-nine (199) and two hundred and three (203) cassava clones herein referred to as genotypes including 5 checks evaluated under the optimum and low nitrogen regimes, respectively across the five environments. The average environment coordinate (AEC) abscissa and the average environment coordinate (AEC) ordinate are two essential parts of the biplot<sup>24</sup>. The average environment axis is indicated by a pointer inside a tiny circle on the abscissa line that travels along the GGE biplot origin. On the other hand, the ordinate is a line that also runs parallel to the GGE biplot origin and forms a 90° angle with the abscissa line. The pointer in the circle indicates high mean performances of genotypes. The abscissa line or average environment axis describes the mean performances of genotypes. The direction with the greatest variability and/or least stability in both orientations is also indicated by the average environment coordinate ordinate<sup>25</sup>.

The genotypes were then ranked simultaneously along the average environment axis (abscissa line) and the environment coordinate ordinate for their means and stability, respectively, to demonstrate the genotypes



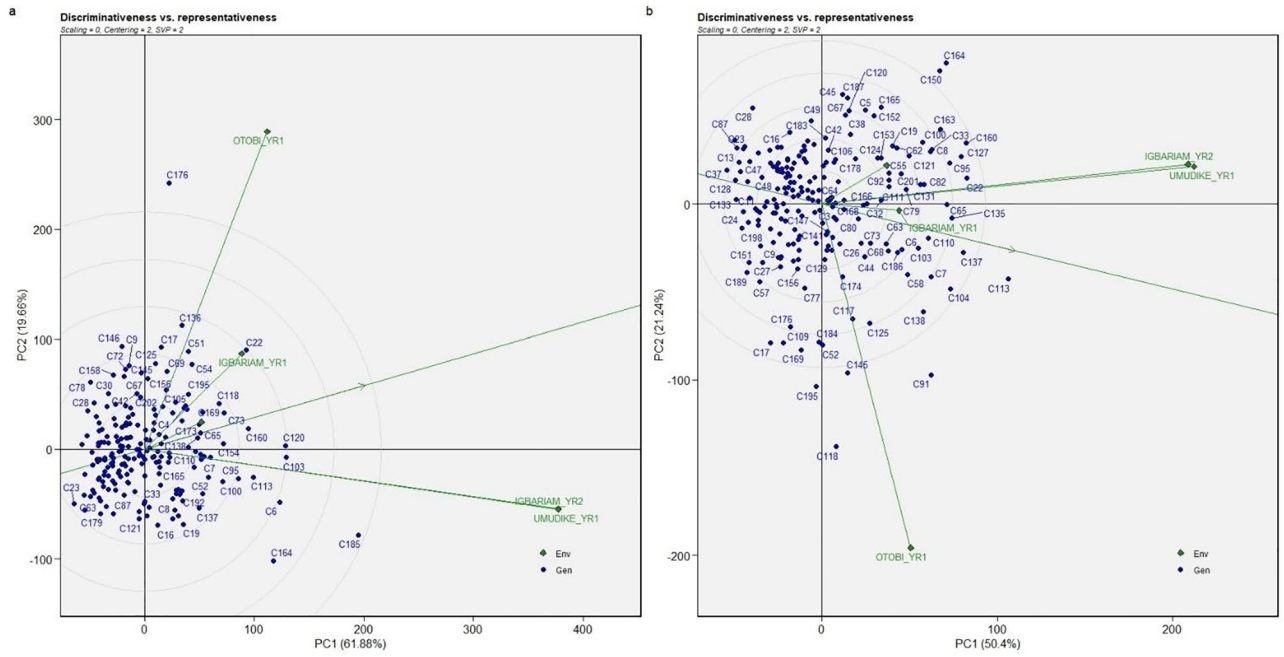


**Fig. 2.** (a) Means versus stability overview, (b) ranking of the genotypes across low nitrogen regime.

mean yield performance and the stability of the test genotypes. Projections were made from the genotypes to the average environment coordinate (abscissa) line in order to be able to see the mean output of genotypes on these lines<sup>26</sup>. They made it simpler by indicating that a genotype did better than the average if it was closer to the average environment axis or farther away from the biplot origin after the projections. Based on that, an attempt was made to identify genotypes that produced mean yields that were above the overall mean. Accordingly, in the order of descending, the following twenty (20) genotypes were identified as high yielding under the optimum nitrogen regime; C185 > C120 > C103 > C22 > C6 > C160 > C176 > C113 > C164 > C75 > C118 > C73 > C95 > C154 > C136 > C51 > C54 > C169 > C100 > C195 (Fig. 4a). Similarly, under the low nitrogen regime, the following genotypes were observed to perform above the average in descending order; C113 > C137 > C91 > C104 > C22 > C135 > C160 > C127 > C7 > C138 > C65 > C95 > C110 > C103 > C58 > C163 > C33 > C182 (Fig. 4b). In terms of stability in the present biplots, it was difficult to visually identify the stable genotypes with respect to their environmental vector length. This difficulty is as a result of the large number of genotypes involved in the study. However, an attempt was made to identify an ideal genotype which is both stable in its performance and has high mean yield performance. The concept of stability is meaningful when the stability is accompanied by high mean yield performance (an ideal genotype)<sup>24</sup>. Figures 1b and 2b for optimum and low nitrogen, respectively, ranks genotypes to the ideal genotype which is found in the centre of the concentric circles. The center of these circles is a point on the average environment axis that is characterized as being completely stable in the positive direction and whose vector length is equal to that of the genotypes with the highest mean performance on the positive side of the average environment axis. Therefore, genotypes located closer to the ideal genotype are more favorable than the others. Thus, under the optimum nitrogen regime, the top 10 genotypes that were more favorable to the other in descending order were C120 > C103 > C160 > C22 > C73 > C118 > C113 > C95 > C6 > C169. Similarly, under the low nitrogen the top 10 genotypes were more favorable to the other in descending order were C113 > C137 > C104 > C135 > C7 > C110 > C103 > C65 > C22 > C58.

### Discriminativeness versus representativeness of the five environments across low nitrogen regime

The GGE biplots analysis for discriminativeness versus representativeness was based on environment-focused singular value partition (SVP) and centered on the means of the environments without scaling and transformation (PCs) (SVP = 2, Centering = 2, Scaling = 0, Transformation = 0). In evaluating genotypes across seasons (environments) or years, one of such objectives is to identify ideal environments that are conducive for the genotypes. Obigbesan and Agboola<sup>27</sup> defined an ideal environment as the one that is both discriminatory and representative. An environment is said to be discriminatory if it's able to identify an adapted and high-yielding genotype whereas representativeness is the ability of the test environment to represent the mega-environment. The discriminatory power of an environment is measured by the relative length of its environmental vectors which is proportional to the standard deviation within the respective environment. The longer the length of the environmental vectors the more its discriminatory ability. Also, the angle between the test environment and the average environmental axis indicates its ability to represent the mega-environment. Therefore, the smaller the angle between them, the more representative it becomes<sup>28</sup>. Based on the above explanations, the discriminatory and representative view of the environments under the optimum and low nitrogen regimes revealed the same environments Otobi\_YR1, Igbariam\_YR2, and Umudike\_YR1 as the ones that had the longest environmental

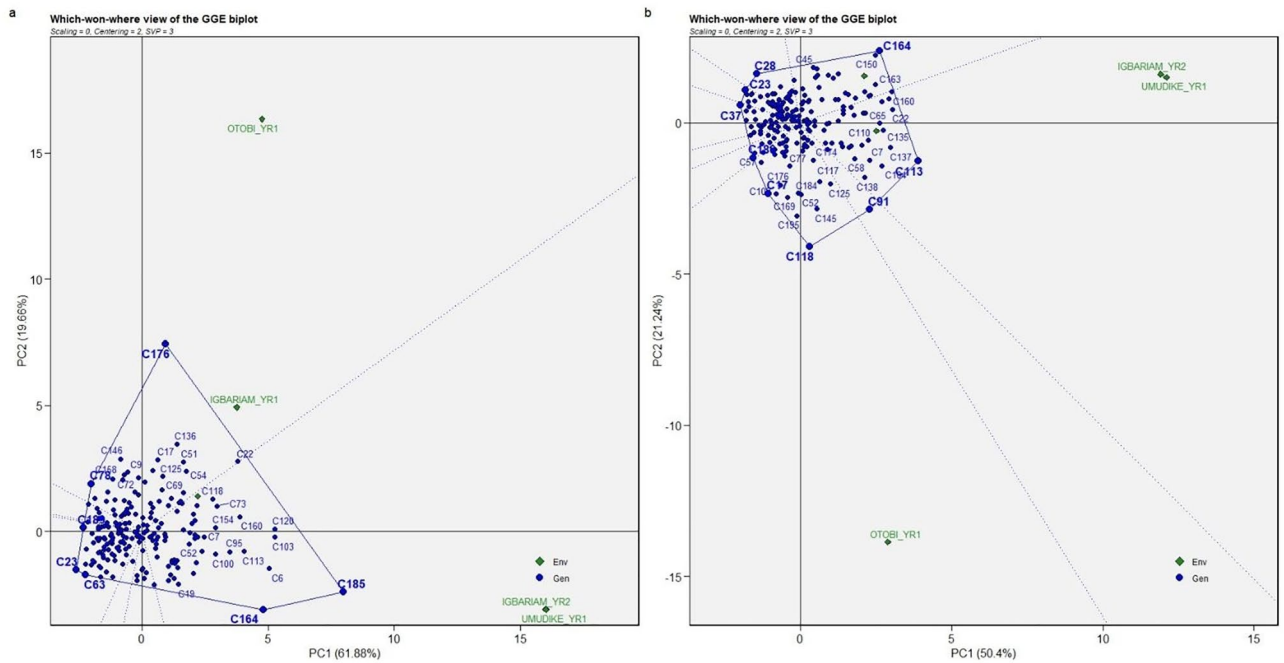


**Fig. 3.** (a) Discriminative and representativeness overview of the test environments under optimum nitrogen regime, (b) Discriminative and representativeness overview of the test environments under low nitrogen regime.

vectors and as such referred to as been the most discriminatory amongst the five environments. However, their respective angle between them and the average environmental axis were not small. The environment that had small angle between the average environmental axis was environment Umudike\_YR2 but also had shorter environmental vector length (Fig. 3a,b).

**Which won where overview of the genotypes across the five environments**

The which-won-where/what pattern consists of an irregular polygon and lines which passes along the biplot origin. A polygon is drawn from the genotypes that are furthest from the biplot origin so that all the remaining genotypes would be contained within the polygon. Then a perpendicular line to each side of the polygon is drawn,



**Fig. 4.** (a) Which won where overview of the genotypes across five environments for fresh root yield for optimum nitrogen regime, (b) which won where overview of the genotypes across five environments for fresh root yield for low nitrogen regime.

|          | Vigour    | staygrn   | leaf_rtn  | chl spad  | leaf_n    | lai       | par       | fry     | Dry     | Hi      | Dmc     | starch |
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|---------|---------|---------|--------|
| Vigour   | 1.00      |           |           |           |           |           |           |         |         |         |         |        |
| Staygrn  | - 0.09**  | 1.00      |           |           |           |           |           |         |         |         |         |        |
| leaf_rtn | 0.13***   | - 0.66*** | 1.00      |           |           |           |           |         |         |         |         |        |
| chl spad | 0.04 ns   | 0.01 ns   | 0.00 ns   | 1.00      |           |           |           |         |         |         |         |        |
| leaf_n   | 0.04 ns   | 0.03 ns   | - 0.03 ns | 0.97***   | 1.00      |           |           |         |         |         |         |        |
| Lai      | 0.00 ns   | 0.18***   | - 0.07*   | 0.00 ns   | 0.00 ns   | 1.00      |           |         |         |         |         |        |
| Par      | 0.13***   | - 0.07*   | 0.02 ns   | - 0.01 ns | 0.00 ns   | 0.42***   | 1.00      |         |         |         |         |        |
| Fry      | 0.09**    | - 0.05 ns | 0.03 ns   | - 0.02 ns | 0.00 ns   | 0.01 ns   | - 0.02 ns | 1.00    |         |         |         |        |
| Dry      | 0.06*     | - 0.09**  | 0.03 ns   | - 0.05 ns | - 0.04 ns | - 0.05 ns | - 0.01 ns | 0.45*** | 1.00    |         |         |        |
| Hi       | - 0.07*   | - 0.01 ns | - 0.03 ns | - 0.03 ns | - 0.03 ns | 0.05 ns   | 0.04 ns   | 0.36*** | 0.16*** | 1.00    |         |        |
| Dmc      | - 0.04 ns | - 0.03 ns | - 0.01 ns | - 0.04 ns | - 0.03 ns | - 0.01 ns | 0.04 ns   | 0.05 ns | 0.75*** | 0.02 ns | 1.00    |        |
| Starch   | - 0.04 ns | - 0.03 ns | - 0.01 ns | - 0.04 ns | - 0.03 ns | - 0.01 ns | 0.04 ns   | 0.05 ns | 0.75**  | 0.02 ns | 1.00*** | 1.00   |

**Table 3.** Pearson phenotypic correlation coefficients amongst traits studied under low nitrogen regime. *Stay\_grn* stay green ability, *leaf\_rtn* leaf retention, *chl spad* chlorophyll spad value, *leaf\_n* leaf nitrogen content, *lai* leaf area index, *par* photosynthetic active radiation, *fry* fresh root yield, *dry* dry root yield, *hi* harvest index, *dmc* dry matter content, *ns* not significant. \*Significant at  $p < 0.005$ , \*\*Significant at  $p < 0.01$ , \*\*\*Significant at  $p < 0.001$ .

|          | Vigour    | staygrn   | leaf_rtn  | chl spad  | leaf_n    | lai     | par       | fry       | Dry       | hi        | Dmc     | Starch |
|----------|-----------|-----------|-----------|-----------|-----------|---------|-----------|-----------|-----------|-----------|---------|--------|
| Vigour   | 1.00      |           |           |           |           |         |           |           |           |           |         |        |
| Staygrn  | - 0.06*   | 1.00      |           |           |           |         |           |           |           |           |         |        |
| leaf_rtn | - 0.04 ns | - 0.50*** | 1.00      |           |           |         |           |           |           |           |         |        |
| chl spad | 0.07*     | 0.03 ns   | - 0.02 ns | 1.00      |           |         |           |           |           |           |         |        |
| leaf_n   | 0.01 ns   | 0.02 ns   | - 0.03 ns | 0.24***   | 1.00      |         |           |           |           |           |         |        |
| Lai      | 0.16***   | - 0.01 ns | 0.07*     | 0.11***   | - 0.06*   | 1.00    |           |           |           |           |         |        |
| Par      | 0.01 ns   | - 0.04 ns | - 0.03 ns | - 0.08*   | - 0.04 ns | 0.27*** | 1.00      |           |           |           |         |        |
| Fry      | 0.06*     | 0.01 ns   | - 0.01 ns | 0.00 ns   | - 0.01 ns | 0.16*** | 0.01 ns   | 1.00      |           |           |         |        |
| Dry      | 0.12***   | - 0.04 ns | - 0.01 ns | - 0.01 ns | - 0.04 ns | 0.20*** | - 0.05 ns | 0.81***   | 1.00      |           |         |        |
| Hi       | - 0.09**  | 0.04 ns   | - 0.07*   | - 0.04 ns | 0.01 ns   | 0.00 ns | 0.10*     | 0.37***   | 0.15***   | 1.00      |         |        |
| Dmc      | - 0.08**  | 0.06*     | - 0.02 ns | 0.02 ns   | 0.02 ns   | 0.05 ns | - 0.01 ns | - 0.07*   | - 0.02 ns | - 0.09**  | 1.00    |        |
| Starch   | - 0.08**  | 0.06*     | - 0.02 ns | 0.02 ns   | 0.02 ns   | 0.05 ns | - 0.01 ns | - 0.05 ns | - 0.01 ns | - 0.04 ns | 1.00*** | 1.00   |

**Table 4.** Pearson phenotypic correlation coefficients amongst traits studied under optimum nitrogen regime. *Stay\_grn* stay green ability, *leaf\_rtn* leaf retention, *chl spad* chlorophyll spad value, *leaf\_n* leaf nitrogen content, *lai* leaf area index, *par* photosynthetic active radiation, *fry* fresh root yield, *dry* dry root yield, *hi* harvest index, *dmc* dry matter content, *ns* not significant. \*Significant at  $p < 0.005$ , \*\*Significant at  $p < 0.01$ , \*\*\*Significant at  $p < 0.001$ .

starting from the biplot origin. The genotypes at the vertex of the polygon are the highest yielding genotype in the location that falls within that particular vector.

The which won where overview biplot presented in Fig. 4a for optimum nitrogen regime identified the vertex genotype C176 (NRCGM0114O48) as the highest yielding genotype in environments Otobi\_YR1 and Igbariam\_YR1 and thus, won in those environments while vertex genotype C185 (NRCGM0414020) also won in environments Igbariam\_YR2 and Umudike\_YR1. In contrast, under the low nitrogen regime, genotype C133 (NR110045) was the winning genotype in environments Igbariam\_YR2 and Umudike\_YR1 since that was the vertex genotype in those environments. Also, genotype C118 (NR100329) won in environment Otobi\_YR1 and Igbariam\_YR1. The vertex genotype C164 (NR110876) won in Umudike\_YR2 environment (Fig. 4b).

### Phenotypic correlation studies among the measured traits

Phenotypic correlation coefficients estimated among the measured traits for the cassava genotypes and the checks evaluated across the test environments are presented in Tables 3 and 4 for both low and optimum nitrogen regimes respectively.

Under the low nitrogen regime evaluation, there was positive and significant ( $p < 0.001$ ) correlation between dry root yield and the following parameters; dry matter content ( $r = 0.75$ ,  $R^2 = 0.56$ ), starch content ( $r = 0.75$ ,  $R^2 = 0.56$ ). There was positive correlation between dry matter content and starch ( $r = 1.00$ ,  $R^2 = 1.00$ ), chlorophyll spad content and leaf number ( $r = 0.97$ ,  $R^2 = 0.94$ ), fresh root yield and dry root yield ( $r = 0.45$ ,  $R^2 = 0.20$ ), fresh

| Trait    | Grand Mean | MSq        | $\sigma_e^2$ | $\sigma_g^2$ | $\sigma_p^2$ | H <sup>2</sup> b | GCV (%) | PCV (%) | GA    | GAM   |
|----------|------------|------------|--------------|--------------|--------------|------------------|---------|---------|-------|-------|
| Vigor    | 4.28       | 3.28***    | 1.50         | 0.36         | 1.85         | 0.19             | 14.02   | 31.78   | 0.54  | 12.62 |
| Staygrn  | 5.32       | 4.47***    | 1.90         | 0.51         | 2.42         | 0.21             | 13.42   | 29.24   | 0.68  | 12.78 |
| Leaf_rtn | 2.16       | 0.89***    | 0.42         | 0.09         | 0.51         | 0.18             | 13.89   | 33.06   | 0.27  | 12.50 |
| Chl spad | 24.77      | 18.51***   | 5.40         | 2.62         | 8.02         | 0.33             | 6.53    | 11.43   | 1.91  | 7.71  |
| Leaf_n   | 8.60       | 1.67***    | 0.49         | 0.24         | 0.73         | 0.33             | 5.70    | 9.93    | 0.57  | 6.63  |
| Lai      | 2.46       | 2.35***    | 0.81         | 0.31         | 1.12         | 0.28             | 22.63   | 43.02   | 0.60  | 24.39 |
| Par      | 158.14     | 56,650***  | 17,535.72    | 7822.87      | 25,358.59    | 0.31             | 55.93   | 100.70  | 101.2 | 63.99 |
| Fry      | 16.82      | 333.80***  | 162.91       | 34.18        | 197.09       | 0.17             | 34.76   | 83.47   | 5.01  | 29.79 |
| Dry      | 4.14       | 153.45***  | 41.78        | 22.33        | 64.12        | 0.35             | 114.14  | 193.42  | 5.75  | 138.8 |
| Hi       | 0.56       | 0.03***    | 0.01         | 0.004        | 0.02         | 0.22             | 11.29   | 25.25   | 0.06  | 10.71 |
| Dmc      | 25.48      | 7127.30**  | 1900.13      | 1045.44      | 2945.57      | 0.35             | 126.90  | 213.00  | 39.68 | 155.7 |
| Starch   | 14.14      | 12,638.80* | 3369.47      | 1853.87      | 5223.34      | 0.35             | 304.50  | 511.12  | 52.84 | 373.6 |

**Table 5.** Variance components, heritability, and other genetic estimates of the traits studied under low nitrogen regime. *lai* leaf area index, *par* photosynthetic active radiation, *Stay\_grn* stay green ability, *leaf\_rtn* leaf retention, *Chl spad* Chlorophyll content, *fry* fresh root yield, *dry* dry root yield, *hi* harvest index, *dmc* dry matter content, *MSq* genotype means square  $\sigma_g^2$  genotypic variance,  $\sigma_e^2$  error variance,  $\sigma_p^2$  phenotypic variance,  $H^2b$  broad sense heritability, *PCV* phenotypic coefficient of variation, *GCV* genotypic coefficient of variation, *GA* genetic advance, *GAM* genetic advance as percentage of the mean. \*\*\*Significant at  $p < 0.001$ .

| Trait    | Grand Mean | MSq        | $\sigma_e^2$ | $\sigma_g^2$ | $\sigma_p^2$ | H <sup>2</sup> b | GCV (%) | PCV (%) | GA    | GAM   |
|----------|------------|------------|--------------|--------------|--------------|------------------|---------|---------|-------|-------|
| Vigor    | 4.98       | 2.44***    | 1.52         | 0.18         | 1.71         | 0.11             | 8.52    | 26.26   | 0.29  | 5.82  |
| Staygrn  | 5.38       | 4.04***    | 2.08         | 0.40         | 2.47         | 0.16             | 11.76   | 29.21   | 0.51  | 9.48  |
| Leafrtn  | 2.49       | 1.52***    | 0.44         | 0.22         | 0.66         | 0.33             | 18.84   | 32.63   | 0.55  | 22.09 |
| Chl spad | 32.86      | 33.41***   | 8.65         | 4.95         | 13.60        | 0.36             | 6.77    | 11.22   | 2.77  | 8.43  |
| Leaf_n   | 10.72      | 180.77***  | 17.26        | 32.70        | 49.96        | 0.65             | 53.34   | 65.94   | 0.53  | 4.94  |
| Lai      | 3.33       | 2.88***    | 1.22         | 0.33         | 1.56         | 0.21             | 17.25   | 37.51   | 0.55  | 16.52 |
| Par      | 126.65     | 38.29***   | 11,707.95    | 5316.43      | 17,024.39    | 0.31             | 57.57   | 103.02  | 83.94 | 66.28 |
| Fry      | 29.04      | 1106.90*** | 428.08       | 135.77       | 563.85       | 0.24             | 40.12   | 81.77   | 11.78 | 40.56 |
| Dry      | 7.51       | 100.44***  | 32.59        | 13.57        | 46.16        | 0.29             | 49.05   | 90.47   | 4.11  | 54.73 |
| Hi       | 0.56       | 0.04***    | 0.03         | 0.0008       | 0.03         | 0.02             | 5.05    | 30.93   | 0.01  | 1.79  |
| Dmc      | 28.98      | 712.39***  | 235.95       | 95.29        | 331.23       | 0.29             | 33.68   | 62.80   | 10.79 | 37.23 |
| Starch   | 14.84      | 1261.41*** | 417.36       | 168.81       | 586.17       | 0.29             | 87.55   | 163.15  | 14.36 | 96.77 |

**Table 6.** Variance components, heritability, and other genetic estimates of the traits studied under high nitrogen regime. *lai* leaf area index, *par* photosynthetic active radiation, *Stay\_grn* stay green ability, *leaf\_rtn* leaf retention, *Chl spad* Chlorophyll content, *fry* fresh root yield, *dry* dry root yield, *hi* harvest index, *dmc* dry matter content, *MSq* genotype means square  $\sigma_g^2$  genotypic variance,  $\sigma_e^2$  error variance,  $\sigma_p^2$  phenotypic variance,  $H^2b$  broad sense heritability, *PCV* phenotypic coefficient of variation, *GCV* genotypic coefficient of variation, *GA* genetic advance, *GAM* genetic advance as percentage of the mean. \*\*\*Significant at  $p < 0.001$ .

root yield and harvest index ( $r = 0.36$ ,  $R^2 = 0.13$ ). Moreover, a negative and significant association were observed in the association between leaf area index and *par* ( $r = -0.42$ ,  $R^2 = 0.18$ ), stay green and leaf retention ( $r = -0.66$ ,  $R^2 = 0.44$ ), and stay green and vigour ( $r = -0.09$ ,  $R^2 = 0.01$ ) (Table 3).

Positive and significant ( $p < 0.001$ ) correlations were observed for the following traits studied under the optimum nitrogen regime: dry matter content and starch ( $r = 1.00$ ,  $R^2 = 1.00$ ), fresh root yield and dry root yield ( $r = 0.81$ ,  $R^2 = 0.66$ ), fresh root yield and harvest index ( $r = 0.37$ ,  $R^2 = 0.14$ ), chlorophyll spad content and leaf number ( $r = 0.24$ ,  $R^2 = 0.06$ ). Significant ( $p < 0.001$ ) and negative associations were observed in the relationship between leaf retention and stay green ( $r = -0.50$ ,  $R^2 = 0.25$ ), leaf area index and *par* ( $r = -0.27$ ,  $R^2 = 0.07$ ), leaf area index and dry root yield ( $r = -0.20$ ,  $R^2 = 0.04$ ) (Table 4).

### Variance components estimation for the traits studied on the set of genotypes

Tables 5 and 6 presents estimated variance components of traits recorded on the genotypes for both nitrogen regimes. The error variation ( $\sigma_e^2$ ) recorded the highest variation in all the traits under consideration more than the genotypic variances ( $\sigma_g^2$ ) in both nitrogen regimes except under the optimum nitrogen regime which saw the leaf nitrogen content's genotypic variance been higher than the error variance resulting in a higher narrow sense heritability ( $H^2b$ ) of 0.65. These heritability values were utilized to foresee the genetic advancement (*GA*) that can be gotten by applying a selection intensity of 5% selection intensity ( $K = \text{Selection differential}, 2.06$ ) to the genotypes. The ratio of this genetic advancement to the corresponding mean of the variables (*GAM*)

| Trait   | Categorization | Class    | Source                |
|---|----------------|----------|-----------------------|
| Heritability                                      | < 30%          | Low      | Bhateria et al., 2006 |
|   | 30–50%         | Medium   |                       |
|   | > 50%          | High     |                       |
| Genotypic and phenotypic coefficient of variation | < 10%          | Low      | Deshmukh et al., 1986 |
|   | 10–20%         | Moderate |                       |
|   | > 20%          | High     |                       |

**Table 7.** Trait categorizations.

was highest in starch (373.69 and 96.77 for low and optimum nitrogen regimes, respectively) and least in chlorophyll spad content (7.71 for low nitrogen regime) and harvest index (1.79 for optimum nitrogen regime). Genotypic coefficient of variation (GCV) ranged between 5.70% in leaf nitrogen content to as high 304.50% in starch content for low nitrogen regime. This translated to having the least (11.43%) and the highest (511.12%) phenotypic coefficient of variation (PCV). Traits were classified into groups using their PCV, GCV, and broad sense heritability scales presented in Table 7.

## Discussion

It has been noted as the depleted nature of Sub-Saharan African soils in nutrient, particularly in nitrogen accounts for low productivity of cassava crop. However, small scale farmer which forms the major part of cassava production finds it difficult to apply inorganic fertilizers to their cassava crop due to financial constraints. In order to provide insurance to resource-poor farmers in these situations, there is the need to sustainably breed crop plants that will respond favorable to low nitrogen fertilizers.

Yield stability and genotype by environment interaction effects (GEI) are complex traits influenced by multiple genetic and environmental factors. Some potential contributors to these phenomena include: Genetic factors such as genetic diversity, quantitative trait loci (QTLs), interactions between genes influencing yield and environmental responses, gene expression and regulation of genes involved in yield and stress responses<sup>29</sup>. Environmental factors which include the interaction of temperatures with genetic factors on yield, drought or excessive moisture, soil properties, pests and diseases as well as agronomic practices can all influence the yield and GXE effects<sup>7</sup>. Understanding the interplay between these genetic and environmental factors can help breeders and researchers develop crops with improved yield stability and adaptability to diverse environments. Our experiment evaluated a total of two hundred and three (203) cassava clones to low and optimum nitrogen levels.

The fresh root yield was the principal component of the cassava productivity. In effect, the grand mean fresh root yield of genotypes obtained in the low nitrogen regime (16.82 t/ha) was lesser compared to the mean fresh yield obtained under the optimum nitrogen regime (29.04 t/ha) (Table 5). This translated to about 26.65% of yield advantage when nitrogen fertilizer was applied to cassava crop. The differences in mean performance across these two nitrogen regimes suggest genetic variability in their performance to each nitrogen regime. Hence, the genotypes that performed well under the low nitrogen can be identified as been nitrogen use efficient and this warrants a further study into their nitrogen use efficiency. Several researchers around the globe have identified a wide range of genetic variability in cassava under low nitrogen fertilizers<sup>30–32</sup>.

GEI has been broadly characterized as the failure of genotypes to realize the same relative performance in different environments. GEI happens when two or more genotypes are evaluated across contrasting environments and their comparative achievements are found not to be the same. That's genotype A may perform best in one environment A and perform worse in environment B. This can be as a result from differential response of the cultivar to different climatic conditions. This phenomenon impedes advance in selection made in one environment to correspond to selection made in another environment. The term environment depicts the setting, circumstances, habitat or atmosphere under which plants grow and this includes locations, years and management practices adopted. There are two environmental variables that challenge plant breeders during genotype evaluation, namely; predictable and unpredictable factors. The predictable environmental factors are those that can be controlled and manipulated by the breeder whereas the unpredictable environmental factors are those that vary erratically like weather which the breeder has no control over<sup>33</sup>. Genotype on the other hand is the biological or genetic make-up of an individual.

The observable characteristics and performance of cultivar of a plant, is its phenotype, which is the result of the cultivar's genotype, as influenced by the environment<sup>34,35</sup>. GEI is a universal occurrence when different genotypes (cultivars) are ranked across diverse environments<sup>36,37</sup>. Many advanced plant breeding programs have a major goal of determining the suitability of individual crop genotypes for agricultural purposes under a variety of agro-ecological conditions. Breeders conduct multi-environment trials for this purpose. A multi-environment trial evaluates a set of genotypes across several environments, attempting to depict the environmental range across which the genotypes should perform well, either partially (specific adaptation) or completely (wide adaptation)<sup>38</sup>. GEI has exacerbated the selection of superior genotypes in many environments because a breeder's selection of superior performers in one environment may not hold true in another. Most agronomically and economically important quantitative traits, such as grain yield, frequently encounter GEI.

The AMMI analysis of variance showed significant effect ( $p < 0.001$ ) for genotypes, environments and the interactions for fresh root yield (which is the principal component of cassava yield) in both nitrogen regimes (Tables 5 and 6). The significant differences observed amongst the genotypes were as expected due to the diverse

nature of genotypes. Significant GEI effect for fresh root yield suggested a substantial differential genotypic response to the five varying environmental conditions captured in this study. As a result, genotypes were further assessed for their stable performances, which of the genotypes won in which environment, and how the test environments were most informative and truly represent a mega environment.

One of the relevant objectives in breeding program is to detect genotypes with distinguished production prospect, broad adaptability, and steadiness. This is why, before releasing varieties and recommending to farmers; genotypes are evaluated under many locations. However, cultivars evaluated in different environments may routinely exhibit dissimilar behavior due to environmental variations, which would characterize the interaction between genotype and environment<sup>39</sup>. The term stability refers to the capability of the genotypes to be invariable, or persistent, or steady or unfluctuating in terms of having high or low yields in various environments<sup>17</sup>.

It is then on the account of the inherent ability of the genotype to produce excellent production under a perfect environment, and is ought to give out satisfactory or appreciable achievement under lesser environment. Most often, breeders overlook the concepts of stability when the variety has excellent performance throughout different environments and years. This instance presents the best opportunity to recognize the excellent genotype<sup>40</sup>, but forasmuch as there is an interaction between the genotype and the environment, this situation becomes unattainable. From agricultural production perspective, two scenarios of stability evolve which can be desired or not desired. For instance, a genotype that has stable performance across environment is not desired if the genotype is not responsive to increase or decrease level of inputs. However, genotype that has a stable performance, but gives response to environment is desired. Thus, producing better yields at high performing environments and producing appreciable level at low performing environments.

The means versus stability biplot revealed top 20 genotypes that performed above the grand mean. The 5 checks [C43 (CW3514), C44 (CW5036), C46 (CW48216), C66 (IITA-TMS-IBA30572), C191 (TME419)] included in the study were not amongst these top 20 genotypes indicating the high performance of the genotypes than the checks. This was the same instances under both nitrogen regimes. These top 20 performing genotypes in both nitrogen regimes were not the same genotypes that won in both nitrogen regimes. For instance, genotype C185 (NRCGM0414020) was the highest performing genotype under the optimum nitrogen followed by genotype C120 (NR100401). In the low nitrogen regime, it was genotype C113 (NR100265) which had the highest fresh root yield followed by genotype C137 (NR110160).

The large number of genotypes in the study hampered detection of stable genotypes so ranking of genotypes were ensued. This helped detect genotypes that were ideal, thus they both has high yielding performance and stable performance for fresh root yield. Different sets of ideal genotypes were identified for optimum and low nitrogen regimes. These ideal genotypes may however be regarded as broad adaptability to the 5 sets of environments in this study. Furthermore, some of the genotypes were having high yield performance but had unstable performances like genotypes C185, C164 and C176 under the optimum nitrogen can be treated as specifically adapted genotypes and they can be a target genotype for a target environment (s). Additionally, under the low nitrogen regime, the genotypes C91 (NR090088) C160 (NR110489) and C138 (NR110165) were seen as high yielding but having unstable performances across the test environments. They can also be specifically adapted to the environments in which they performed best.

In order to identify which of the genotype won in which environment, the GGE biplot overviews of which won where were used (Fig. 4a,b). Each vertex cultivar in the polygon represents the highest yielding cultivar in the location that falls within that sector. When two or more environments are present within a sector, it indicates that a single genotype has the highest yield in those environments. Different genotypes won for environments in various sectors<sup>41</sup>. C176 (NRCGM0114048) was the highest yielding genotype in environments Otobi\_YR1 and Igbariam\_YR1 and thus, won in those environments while vertex genotype C185 (NRCGM0414020) also won in environments Igbariam\_YR2 and Umudike\_YR1. It is apparent that no environment fell into the sector where the following vertex genotypes were C164 (NR110876), C63 (IITA-TMS-IBA070602) and C58 (IITA-TMS-IBA050540). This implies, these three genotypes did not perform well in any of the test environments. Moreover, the genotypes within the polygon including those who were closer to the biplot origin indicate their less responsiveness to the environment than the vertex genotypes.

The “which genotype won in what environment” aggregated the five (5) test environments into two (2) mega environments. Environments Otobi\_YR1 and Igbariam\_YR1 fell in one place, environments Igbariam\_YR2, Umudike\_YR1, and Umudike\_YR2 fell at one place (Fig. 4a).

Different genotype C133 (NR110045) was the winning genotype in environments Igbariam\_YR2 and Umudike\_YR1 since that was the vertex genotype in those environments. Also, genotype C118 (NR100329) won in environment Otobi\_YR1 and Igbariam\_YR1. The vertex genotype C164 (NR110876) won in Umudike\_YR2 environment under the low nitrogen regime (Fig. 4b).

The biplots overview for discriminatory and representative for fresh root yield in both nitrogen regimes were the same. In these scenarios, Otobi\_YR1, Igbariam\_YR2, and Umudike\_YR1 were described as most discriminative (informative). They were informative but not representative of a mega environment. The discriminativeness of Otobi environment could be as a result of the fact that genotypes were only evaluated in that environment for only one year and as such, it will only treat any variations from that environment as highly diverse when it comes to the mean performances of the genotypes in the environment for that year without comparison. In this case, this study cannot precisely say the environment Otobi was truly informative.

However, environments Igbariam and Umudike were repeated in both years and as a result can be guaranteed as most discriminatory. Though, these environments are discriminatory, they were seen not to be representative. This is because the angle between them and the average environmental axis were wider. The environments that had small angle with the average environmental axis were Umudike\_YR2 and Igbariam\_YR1 and were referred to as most representative. Nonetheless, they were also non-discriminatory because they had short environmental vector lengths. Discriminatory but non-representative has their merits as well as representative

but non-discriminatory. An ideal environment that possesses both discriminatory and representative would have been the one that should be sought for, but in our text environments that is lacking. The advantage of the text environments that were captured in this study as discriminatory but unrepresentative can be used to identify a specifically adapted genotype (s). Moreover, environments that were uninformative can be dropped from further studies with respect to cassava fresh root yield to save scarce resources.

The possibility of indirect selection based on correlated traits is quantified by trait correlation, which is an important estimate in genetic studies. This is so, because some traits are difficult to be improved upon, some has low heritability estimate to select for, so when these traits are in strong and significant association with traits can be otherwise improved upon and has high heritability estimate, then an improvement in the easy to improve and select will consequently lead to the improvement of the difficult to select. A strong positive and significant correlation implies traits in such association can be improved at the same time during breeding activities while a strong negative and significant association between two traits indicates if one of the traits is not improved, the productivity of the other trait can be weakened.

These associations are an important aspect in practical breeding. However, correlation coefficients ( $r$ ) values only help us to appreciate the kind of relationships between two traits and their level of significance without telling the strength of the association. Therefore, to be able to determine the strength of associations, coefficient of determination ( $R^2$ ) values helps to predict and examines how differences in one of the variables can be explained by the differences in the second variable. This is able to predict the outcome of a given event<sup>42</sup>. Coefficient of determination values ranges between 0 and 1. The closer the value to 1, the more robust the outcome can be predicted. Coefficient of determination between the following associations were very high; between dry root yield and dry matter content ( $R^2 = 0.56$ , i.e. 56%), dry root yield and starch ( $R^2 = 0.56$ , i.e. 56%), chlorophyll content and leaf nitrogen content ( $R^2 = 0.97$ , i.e. 97%), fresh root yield and dry root yield ( $R^2 = 0.66$ , i.e. 66%).

Because early phenotypic evaluations are based on non-replicated trials, cassava, like all clonally propagated crops, is bred using phenotypic recurrent selection. As a result, breeders make critical decisions to try to reduce the large number of genotypes in the early stages of selection while keeping in mind that selection based on trials without replication is prone to large experimental errors<sup>43</sup>. Meanwhile, successful varieties necessitate the combination of multiple traits. Many traits may be negatively or positively associated with one another. Knowing the type of associations of characters to combine in a breeding program is of practical importance. For instance, fresh root yield has been reported to have low heritability estimates across several studies, as a result, selection for fresh root yield is difficult and as an alternative indirect selection for harvest index has been practiced all over by breeders because higher heritability estimates for harvest index is high and there are acceptable correlations with fresh root yield<sup>44,45</sup>. Interestingly, the association between dry matter content and starch in both nitrogen regimes had 1.00 coefficient of determination, thus, 100% of the chances that their association can be predicted.

This means, their association can be predicted accurately, then simultaneous improvement in one will automatically improves the other one which brings a lot of progress in breeding programs and management. Starch in cassava contains the main nutritional component (carbohydrates) of the crop which has several uses just to mention the ethanol production industries. Any breeding procedures that improve dry matter content will improve that of the starch content to target the demands of ethanol industries. On the other hand, where traits are in negative association, it suggests an improvement in one will not weaken the other.

Take for instance, yield and disease incidence, the higher the disease incidence, the lower the yield. This association is believed to be of negative association and significant. So, effort to curtail disease incidences will not weaken yield but rather increase it. In effect, as one goes down, the other goes up. Our study result adds up to existing literature on the possibility of using our correlation values amongst characters to set up an indirect selection index criteria for breeding of cassava and also makes progress in simultaneously improving more than one trait at a time.

The Variance components estimated for all the traits revealed a higher proportion of their phenotypic variance ( $\sigma^2_p$ ) being accounted for by the environmental variance than genetic variance ( $\sigma^2_g$ ) except with leaf nitrogen content which had had a greater (65.45%) contribution of genotypic variance to their environmental variance (Table 5). The low genotypic variance signifies the considerable environmental influence on these traits. Thus, the apparent variations in these traits are not solely of genotypic but also due to environmental influence. The extent of environmental influence on any trait indicates the size of the difference between PCV and GCV values, with a large difference reflecting a large environmental influence, while a smaller difference indicates a large genetic influence.

The high values observed for the traits show the immense contribution of the environment in expression of these traits. Several studies on cassava and other crops have discovered similar findings<sup>46–48</sup>. Values of Genotypic and phenotypic coefficient of variation below 10% are considered low, 10–20% medium and above 20% high (Table 6). Two (2) of the traits had low GCV, four (4) had intermediate GCV and six (6) had high GCV in the low nitrogen regime. Also, PCV was generally higher than their corresponding GCV for all the traits (Table 5). Similar trend was observed in these GCV and PCV values for the traits measured under the optimum nitrogen regime. Here, three (3) of the traits had low GCV, three (3) had intermediate GCV while six (6) had high GCV.

Broad sense heritability ( $H^2_b$ ) was utilized in order to ascertain the reliability of trait performance in subsequent generations<sup>49</sup>. Heritability and genetic advance (GA) help to determine the extent of genetic variations and the effectiveness of trait selected<sup>48</sup>.  $H^2_b$  of six (6) traits (vigour, stay green, leaf retention, leaf area index, fresh root yield, harvest index), in the low nitrogen regime were classified as having low broad sense heritability which presumes the huge influence of environment on the expression of these traits. Six of the remaining traits were classified as having an intermediate or medium heritability (Table 5). Eight traits under the optimum nitrogen regime were grouped as having low heritability while 3 were grouped as having low heritability, and only one trait (Leaf nitrogen content) was classified as having a high heritability (Table 6). High broad sense heritability

for leaf nitrogen content suggests the presence of heritable portion of variation, this portion can be exploited by plant breeders and that would translate to rapid progress in selection, even with simple selection procedures.

Notwithstanding this, Hossain et al.<sup>50</sup> concluded that  $H^2b$  on its own doesn't give a broad understanding of the genetic gain (GA) that can be obtained. Thereby, if there is a high genetic advance, heritability estimates will be reliable. A high heritability accompanied by a high genetic advance is primarily due to the additive gene effect; however, if the heritability is primarily due to dominance and epistasis, the genetic gain would be low<sup>51</sup>. Three traits (photosynthetic active radiation, dry matter content, starch) had a genetic advance of more than 20%. This finding is consistent with that of<sup>52</sup>, who noted high estimates of genetic advance for the number of cassava storage roots studied.

## Conclusion

The study revealed that the cassava genotypes responded favorably to the optimum nitrogen fertilizer application. However, further research needs to be constituted to test the optimal rate for maximum productivity. The genotypes C120, C103, C160, C22, C73, C118, C113, C95, C6, and C169 were identified as the ideal genotypes under the optimum nitrogen fertilizer application. Moreover, genotypes C113, C137, C104, C135, C7, C110, C103, C65, C22, C58, and C136 were the ideal genotypes under the low nitrogen fertilizer application. This calls for further investigation to validate their nitrogen use efficiency. The genotypes C185, C164, and C176 under the optimum nitrogen were high-yielding but were unstable in their fresh root yield performance across the environments and can be recommended as specifically adapted to the environments they performed best. The genotypes C160, C113, and C138 were similarly high-yielding genotypes under the low nitrogen but were highly unstable in their fresh root yield mean performance across the five environments.

The top 20 genotypes that performed above the grand mean for fresh root yield in both nitrogen regimes can be further evaluated on farmer's field for possible advancement. The environments Otobi\_YR1, Igbariam\_YR2, and Umudike\_YR1 were identified as the most discriminatory amongst the five test environments. The environments Umudike\_YR2 and Igbariam\_YR1 were identified as the most representative of the five test environments and can represent a mega-environment. The current study identified Umudike and Igbariam as mega environment and the most representative of the test environments and most suitable for multiplication of improved NUE planting materials.

## Materials and methods

### Study sites, soil test and land preparation

Experimental trials were conducted over two cropping seasons (2020/2021 and 2021/2022, April–May) at three locations: Umudike (5° 29' N, 7° 24' E; mean annual rainfall of 2200 mm; altitude of 120 m; mean annual temperature of 22–31 °C; Dystric Luvisol soils; humid forest zone), Otobi (7° 20' N, 8° 41' E; mean annual rainfall of 1500 mm; altitude of 319 m; mean annual temperature of 24–35 °C; Ferric Luvisol soils; southern Guinea savannah zone) and Igbariam (5° 56' N, 7° 31' E; mean annual rainfall of 1800 mm; altitude of 150 m; mean annual temperature of 24–32 °C; Dystric Luvisol soils; forest savannah transition zone and Umudike (5° 29' N, 7° 24' E; mean annual rainfall of 2200 mm; altitude of 120 m; mean annual temperature of 22–31 °C; Dystric Luvisol soils; humid forest zone) in Nigeria. These locations represent the major cassava-growing regions in Nigeria. In the present study, each year in each location constituted a single environment. This gave a total of six test environments, Umudike\_Y1 (2020–2021), Umudike\_Y2 (2021–2022), Otobi\_Y1 (2020–2021), Otobi\_Y2 (2021–2022) and Igbariam\_Y1 (2020–2021), Igbariam\_Y2 (2021–2022). Prior to the land preparation, a composite auger soil samples were taken from the experimental sites at soil depths ranging from 0 to 20 cm in both years for determination of nitrogen, phosphorus, and potassium (N.K.P.) in the soil across the location (Table 8). The soils were air-dried crushed thoroughly, mixed together to passed through a 2-mm sieve before subjected to physical and chemical analysis using standard analytical methods as described by<sup>53</sup>. Land preparation in each year, across the experimental site was manually cleared of debris and mechanically slashed, ploughed, harrowed, and ridged with a tractor.

### Experimental materials

A panel of two hundred and three (203) diverse cassava genotypes was used in this study. The planting materials were obtained from the National Root Crops Research Institute Umudike (NRCRI) in Nigeria under the NEXTGEN cassava breeding project with other partners including the International Institute for tropical Agriculture (IITA) and the International Center for Tropical Agriculture (CIAT). The genotypes were genetically diverse to capture a range of responses to different environmental factors. Selected genotypes showed varying

| Location | Nitrogen N (%) |      | Potassium P (pmm) |      | Potassium K (me/mg) |      |
|----------|----------------|------|-------------------|------|---------------------|------|
|          | 2020           | 2021 | 2020              | 2021 | 2020                | 2021 |
| Umudike  | 0.14           | 0.12 | 27                | 25   | 0.40                | 0.37 |
| Igbariam | 0.07           | 0.10 | 16                | 15   | 0.05                | 0.08 |
| Otobi    | 0.15           | 0.13 | 22                | 24   | 0.30                | 0.17 |

**Table 8.** Soil fertility status (NPK) in Umudike, Igbariam and Otobi.

| Nutrients           | Low       | Medium    | High     | Reference                  |
|---------------------|-----------|-----------|----------|----------------------------|
| Nitrogen N (%)      | < 0.15    | 0.15–0.20 | > 0.20   | Chude et al. <sup>54</sup> |
| Recommendation      | 196 kg/ha | 98 kg/ha  | 49 kg/ha |                            |
| Potassium P (pmm)   | < 15      | 15– 25    | > 25     | Chude et al. <sup>54</sup> |
| Recommendation      | 288 kg/ha | 144 kg/ha | 72 kg/ha |                            |
| Potassium K (me/mg) | < 0.15    | 0.15–0.25 | > 0.25   | Chude et al. <sup>54</sup> |
| Recommendation      | 90 kg/ha  | 45 kg/ha  | 0 kg/ha  |                            |

**Table 9.** Soil fertility classes and cassava NPK recommendation for each class in Nigeria.

phenotypes such as architecture, plant height, petiole colour, leaf shape and flowering habit etc.), to allow for assessment of how different traits respond to environmental factors. This is imperative to ensure contrasting responses to specific environmental factors is captured within a range of genetic architectures. These genotypes were evaluated under low nitrogen and optimum nitrogen regimes. The two hundred and three (203) genotypes were evaluated under the low nitrogen but the number was reduced to one hundred and ninety-nine (199) under the optimum nitrogen regime.

### Field layout and experimental design

The experimental field was divided into two blocks, viz.,

- i. Optimum nitrogen regime (196 kg N/ha)
- ii. Low nitrogen regime (0 kg N/ha).

The two blocks received recommended doses of Phosphorus (P) and Potassium (K) for cassava production in each region<sup>54</sup> (Table 9). Fertilizer was applied at eight weeks after planting using the ring method, where a round small channel is made around the plants to input the fertilizers after planting. The rationale for ring method of fertilizer application is that it increases the fertilizer use efficiency and efficient nutrient uptake as fertilizer is placed in a ring around the plant, allowing roots to absorb nutrients more efficiently, this way, nutrient loss through erosion or leaching is minimized, reducing waste and environmental impact<sup>55</sup>.

The trials were laid out following an augmented block design with 5 checks. Each plot consisted of 5 plants per row on ridges. Spacing between ridges was 1 m within row and 0.8 m between plants making a total plant population of 12, 000 plants/ha. The fields were hand weeded using hoes three times during the first four months.

### Data collection

Pre-harvest data for individual genotypes were collected at 3 and 6 months after planting, and these include: disease score, plant vigour, stay green ability, leaf retention, photosynthetic active radiation, leaf area index, chlorophyll and leaf nitrogen content. Harvest data collected at 12 MAP at harvest was fresh root yield and other derived traits which include specific gravity, dry root yield, starch content, dry matter content and harvest index were computed using standard procedures following cassava-base ontology.

### Data analysis

Data analysis was done using R statistical software (Version 4.2.1)<sup>56</sup>. The locations and years were modeled as an environment to get the different environments for each treatment (Low nitrogen and Optimum nitrogen) to study the interaction effect of the clones (herein referred as genotypes) and environments. Data was first analyzed as augmented RCBD and then means were extracted for AMMI and GGEBIPLOTS analysis. Statistical equation for augmented design is as follows,

$$Y_{ij} = \mu + \beta_i + C_j + \tau_k(i) + \epsilon_{ij}$$

where;  $Y_{ij}$  is the response variable measured for  $i$ -th genotype in the  $j$ -th environment,  $\mu$  is the overall mean,  $\beta_i$  is the effect of the blocks,  $C_j$  is the effect of the checks,  $\tau_k(i)$  is the effect of the new entries, and  $\epsilon_{ij}$  is the error term.

Data was subjected to combined analysis of variance (ANOVA) for the treatments separately. The package “statgenGxE” was used to estimate the variance and heritability components<sup>57</sup>. Estimation of genetic variances was done using the package “variability”<sup>58</sup>. Additive main effect and multiplicative interaction (AMMI), GGE biplots and correlation analyses were done using “metan” package<sup>59</sup>.

A single model with additive and multiplicative parameters was created using the AMMI model, which combines principal component analysis of the genotype-environment interaction with analysis of variance for the genotype and environment main effects<sup>60</sup>. AMMI model is supported by the equation:

$$Y_{geb} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \epsilon_{geb} \quad (1)$$

where;  $Y_{geb}$  = the experimental yield for genotype  $g$  in environment  $e$  for block  $b$ ,  $\mu$  = grand mean,  $\alpha_g$  = the difference of genotype  $g$  from the overall mean, and  $\beta_e$  = difference of environment  $e$ ,  $\lambda_n$  = the singular value for interaction principal component axis (IPCA)  $n$ ,  $\gamma_{gn}$  = the genotype eigenvector for axis  $n$ , and  $\delta_{en}$  = the environment eigenvector. The eigenvectors are scaled as unit vectors and are unit-less, whereas  $\lambda$  has the units

of yield. A convenient scaling for the multiplicative parameters is  $\lambda 0.5\gamma g$  and  $\lambda 0.5\delta e$ , termed the “genotype IPCA scores” and “environment IPCA scores”. A reduced model leaves residual,  $\rho$  ge, and for the block, there is also error term  $\epsilon$  geb.

Burgueno et al.<sup>61</sup> described the GGE biplot model as a graphical representation of two causes of variation based on the SREG model. A biplot is a diffuse diagram that estimates and clearly displays findings in a pictorial view for quick identification and comprehension of any associations surrounded by the factors and their fundamental relationship to one another<sup>24</sup>. The Sites Regression (SREG) linear-bilinear model is the foundation for the GGE biplot, which divides the GGE into multiplicative components<sup>35,24</sup>. The statistical model for the GGE biplot based on the singular value decomposition (SV) of the first two main components was described by<sup>62</sup> as follows:

$$Y_{ij} - \beta_j = \lambda_1 \sum_i i_1 \eta_{ij} + \lambda_2 \sum_i i_2 \eta_{2j} + \epsilon_{ij} \quad (2)$$

where;  $Y_{ij}$  = the measured yield of genotype  $i$  in environment  $j$ ,  $\beta_j$  = the mean yield in environment  $j$ ;  $\lambda_1$  and  $\lambda_2$  represent the singular values (SV) of principal component one and two (PC1 and PC2), respectively;  $\epsilon_{i1}$  and  $\epsilon_{i2}$  denote the eigenvectors of genotype  $i$  for PC1 and PC2, respectively;  $\eta_{i1}$  and  $\eta_{i2}$  are the eigenvectors of environment for principal component one and two respectively;  $\epsilon_{ij}$  is the residual associated with genotype  $i$  and environment  $j$ .

According to the methods described by<sup>63</sup>, the variance components for the different quantitative characters were calculated from their mean squares. Following the steps described by<sup>63</sup> the genotypic and phenotypic coefficient of variation were also determined. Following the techniques outlined below, heritability in a broad sense and genetic progress were also estimated. Variance due to the environment,

$$\sigma^2 e = \text{error mean square (MSE)} \quad (3)$$

Genotypic variance,

$$\sigma^2 g = \frac{MSG - MSE}{r} \quad (4)$$

where,  $\sigma^2 g$  = variance due to the genotypic,  $MSG$  = genotype mean square,  $r$  = number of replications. Phenotypic variance,

$$\sigma^2 p = \sigma^2 g + \sigma^2 e \quad (5)$$

where,  $\sigma^2 g$  = is genotypic variance,  $\sigma^2 e$  = Environmental variance.

Genotypic Coefficient of variation:

$$GCV = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100 \quad (6)$$

Phenotypic Coefficient of Variation:

$$PCV = \frac{\sqrt{\sigma^2 p}}{\bar{X}} \times 100 \quad (7)$$

where;  $PCV$  = phenotypic coefficient of variation,  $GCV$  = genotypic coefficient of variation,  $\sigma^2 g$  = the genetic variance,  $\sigma^2 p$  = phenotypic variance and  $\bar{X}$  = mean.

The heritability in broad sense was estimated as;

$$H^2 b = \frac{\sigma^2 g}{\sigma^2 p} \times 100 \quad (8)$$

where,  $H^2 b$  = Broad sense heritability,  $\sigma^2 g$  = Genotypic variance,  $\sigma^2 p$  = Phenotypic variance. Genetic Advance; Genetic advance was calculated as,

$$GA = K \cdot \sigma p \cdot H^2 b \quad (9)$$

where,  $\sigma p$  = phenotypic standard deviation,  $H^2 b$  = broad sense heritability,  $K$  = selection intensity. Calculating genetic advancement as a proportion of the mean

$$(GAM) = \frac{GA}{\bar{X}} \times 100 \quad (10)$$

where,  $GAM$  = Genetic advance as proportion of mean,  $GA$  = Genetic advance under selection and  $\bar{X}$  = Grand Mean of the trait.

The variance and covariance components as described by<sup>64</sup> were used to compute phenotypic correlation coefficients as

$$P(xy) = \frac{Pcov.x \cdot y}{\sqrt{\sigma^2 px \cdot \sigma^2 py}} \quad (11)$$

where;  $P(xy)$  = phenotypic correlation coefficient between the characters  $x$  and  $y$ ,  $pcovx.y$  = covariance between  $x$  and  $y$ ,  $\sigma^2_{px}$  = variance of  $x$ ,  $\sigma^2_{py}$  = Variance of  $y$ .

## Data availability

The datasets collected from the field trials and analyzed during the present study can be found in the Cassavabase repository (<https://www.cassavabase.org/>).

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## Author contributions

J.O.M, J.O and C.E. conceived, designed, and conducted the study. D.N.N. provided the plant material used in the study. J.O.M. and S.P.A. collected all data and analysed the data sets. J.O.M. wrote the original draft of the manuscript. D.D., P.T. and C.E. supervised J.O.M. All authors reviewed and approved the final version of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to C.E.

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