ASSESSMENT OF REPEATABILITY AND REPRESENTATIVENESS OF TESTING SITES FOR PROVITAMIN A MAIZE IN SAVANNA AGRO-ECOLOGIES OF NIGERIA

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ABSTRACT

Identification of ideal testing sites for selection of superior maize (Zea mays L.) germplam is vital to the success of a maize breeding programme. Sixteen provitamin A maize genotypes were evaluated at seven locations in savanna agro-ecologies of Nigeria for 3 yr to assess the representativeness, discriminating ability, and repeatability of the testing sites and to identify ideal testing sites for selection of superior maize germplasm. Location, year, and their interaction effects were significant for grain yield and most measured traits while genotype and genotype × year interactive effects were significant for grain yield. The genotype main effects plus genotype × environment interaction (GGE) biplot analysis revealed PVA SYN-18 F₂ as the highest-yielding and most stable genotype across environments. The GGE biplot identified Zaria, Saminaka, and Kaboji as the most discriminating locations. Also, the biplot identified Kaboji, Batsari, Saminaka, and Zaria as the most repeatable locations. Zaria and Saminaka, being among the most discriminating, representative and repeatable locations, were considered as the core testing sites for selection of superior maize genotypes for release and commercialization. The core testing sites identified in this study should facilitate the identification of stable and high-yielding maize germplasm adaptable to the savannas agro-ecologies of Nigeria.

Keywords: Genotype, Genotype × environment interaction, GGE biplot, ideal test location, Zea mays

INTRODUCTION

Maize (Zea mays L.) is an important staple cereal crop and source of energy, protein, vitamins, minerals and lipids for over 200 million people in West Africa (WA). It provides an estimated 15% of the world’s protein and 20% of the world’s calories (Brown et al., 1988). In the sub-region, maize has become increasingly important as staple food and source of income generation for millions of the inhabitants especially women and children who constitute the larger and most vulnerable population of the continent. It has become an important focus for biofortification strategies to combat vitamin A deficiency in the sub-region. Deficiency of vitamin A is referred to as an ‘hidden hunger’ of some essential micronutrients such as carotenoids or vitamin A. It affects over 250 million people worldwide and is one of the most prevalent nutritional deficiencies in developing countries resulting in impaired growth, reproduction, vision, and immunity (WHO, 2008). As a result of this, efforts have been made towards development and deployment of maize germplasm with elevated levels of β-carotene content in maize using conventional breeding strategies. Evaluation of such products in performance trials at multiple locations would enhance the identification of high β-carotene content as well as high-yielding synthetic varieties and hybrids maize for the target region. Multi-environment trials for maize in the sub-region have revealed the existence of genotype × environment interactions (GEI) (Fakorede and Adeyemo, 1986; Badu-Apraku et al., 2007, 2008, 2011a, 2013; Oyekunle et al., 2017). The presence of GEI constitute a problem for identification of superior genotypes for narrow and/or broad adaptation. As a result of this, there is need for extensive testing of genotypes in several locations over years before genotypes could be recommendations for registration and release in the target region. However, due to the limited resources available for variety development and testing in the national maize improvement programs of Nigeria, there is a need to identify few testing sites that could perfectly represent the savanna agro-ecologies
The identification of ideal testing sites for selection of superior maize germplasm is vital to the success of a maize breeding programme in the region. A test location could be considered ideal testing site if it could discriminate well among genotypes; representative of the target environments; and repeatable in assessing the performance of genotypes over years (Yan et al., 2007). The International Institute of Tropical Agricultural (IITA) Ibadan in collaboration with the Institute for Agricultural Research (IAR), Samaru have developed several provitamin A maize synthetic varieties and hybrids. Several of these products have been tested in multi-location trials for several years. Information on the representativeness, discriminating ability, and repeatability of the testing sites utilized for evaluating pro-vitamin A maize genotypes in the savanna agro-ecologies of Nigeria would facilitate a better understanding of the testing sites for effective utilization in the target region.

MATERIALS AND METHODS

Methodology

Sixteen medium maturing, provitamin A maize varieties were evaluated at seven locations in the corn belt of savanna agro-ecologies of Nigeria between 2015 and 2017. The trial was evaluated at seven locations (Zaria, Bagauda, Birnin Kudu, Batsari, Panda, Kaboji, and Saminaka) covering the savanna agro-ecologies of the country. The description of the experimental sites is presented in Table 1. The provitamin A maize varieties were emanated from the HarvestPlus project of IITA. The trials were evaluated during the growing seasons when rains at each site had fully established. The 16 varieties were laid out using a randomized complete block design with three replications in each testing site. Each plot consisted of two rows of 5-m-long, with inter-row spacing of 0.75 m and intra-row spacing of 0.5 m. Three seeds were planted per hill, and later thinned to two plants per hill 2 wk after planting (WAP), giving a population density of ~53,333 plants ha\(^{-1}\). A compound fertilizer (N:P:K 15:15:15) was applied at the rate of 60 kg NPK ha\(^{-1}\) 2 WAP at all the testing sites. An additional 60 kg N ha\(^{-1}\) was top-dressed 3 wk later using urea fertilizer. The trial plots were maintained weed free by the application of 5 l ha\(^{-1}\) each of paraquat and atrazine. In addition, manual weeding was done twice to keep the trials weed free.

Data collection and analysis

Observations were made on plot basis on days to 50% anthesis and mid-silk as the number of days from planting to when 50% of the plants produced pollen and silk, respectively. Anthesis-silking interval (ASI) was estimated as the interval between days to 50% anthesis and mid-silk. Plant and ear heights were measured as the distance from the base of the plant to the level of the first tassel branch and the node bearing the first ear, respectively. Husk cover was scored on a scale of 1 to 5 (where 1 = tight husk, extending beyond the ear tip, and 5 = ear tips completely exposed). Plant aspect was scored on a scale of 1 to 5 on the basis of uniformity of plants, ear size, plant and ear heights, resistance to lodging, disease, and insect damage (where 1 = excellent plant type, and 5 = poor plant type). Ear aspect was scored on a scale of 1 to 5 on the basis of cob size, cob uniformity, grain filling, disease and insect damage (where 1 = clean, uniform, large, and well-filled ears, and 5 = ears with undesirable features). Number of ears per plant (EPP) was computed by dividing the total number of ears harvested per plot by the total number of plants harvested. Grain yield was calculated assuming 800 g grain kg\(^{-1}\) ear weight) shelling percentage and adjusted to 150 g kg\(^{-1}\) moisture content. Combined analysis of variance (ANOVA) across locations was performed on plot means for grain yield and other agronomic traits with PROC MIXED procedures in Statistical Analysis System (SAS Institute, 2002). In the combined ANOVA, location, year, interactions, and replication were considered random effects, while genotype was considered fixed effect. The percentage contribution of each source of variation was computed for grain yield and other agronomic traits using sum of squares.
Table 1. Description of testing sites used for evaluation of provitamin A maize varieties in Northern Nigeria, 2015-2017

| Location     | Code | Agro-ecology | Global Position | Year of Evaluation |
|--------------|------|--------------|-----------------|--------------------|
|              |      |              | Latitude | Longitude | Altitude (m asl) | Soil Type | Annual Rainfall | 2015 | 2016 | 2017 |
| Zaria        | ZA   | NGS†         | 12°00'N | 8°22'E    | 640                | Lixisol   | 1150            | x    | x    | x    |
| Bagauda      | BG   | SS           | 12°01'N | 8°19'E    | 520                | Arenosol  | 850             | x    | x    | x    |
| Birnin Kudu  | BK   | SS           | 11°26'N | 3°42'E    | 461                | Leptosol  | 900             | x    | x    | x    |
| Batsari      | BA   | SS           | 12°45'N | 7°49'E    | 605                | Leptosol  | 830             | x    | x    | x    |
| Panda        | PA   | SGS          | 9°15'N  | 7°49'E    | 525                | Nitosol   | 1400            | x    | x    | x    |
| Kaboji       | KB   | SGS          | 11°30'N | 3°30'E    | 580                | Nitosol   | 1200            | -    | x    | x    |
| Saminaka     | SM   | NGS          | 9°05'N  | 6°45'E    | 273                | Lixisol   | 1300            | x    | x    | x    |

*SGS, southern Guinea savanna; NGS, northern Guinea savanna; SS, Sudan savanna.
‡x, year when evaluation was carried out at the location.

The data on grain yield in each replication across years and locations were subjected to GGE biplot analysis (Yan, 2001, 2014; Yan et al., 2000, 2010) using genotype x environment analysis with R for window, version 4.0. The following GGE biplot model was used for the analysis (Yan and Kang, 2003):

\[ Y_{ij} - Y_j = \lambda_1 \xi_i \eta_j + \lambda_2 \xi_i \eta_j + e_{ij} \]

where \( Y_{ij} \) is the mean yield of genotype \( i \) in environment \( j \); \( Y_j \) is the mean yield across all genotypes in environment \( j \); \( \lambda_1 \) and \( \lambda_2 \) are the singular values for PC1 and PC2, respectively; \( \xi_i \) and \( \xi_i \eta_j \) are the PC1 and PC2 scores, respectively, for genotype \( i \); \( \eta_j \) and \( \eta_j \) are the PC1 and PC2 scores, respectively, for environment \( j \); and \( e_{ij} \) is the residual of the model associated with genotype \( i \) in environment \( j \).

RESULTS

Genotype and genotype x environment interaction

The results of combined ANOVA across locations and years for the 16 provitamin A genotypes revealed that there were significant (\( P < 0.01 \)) differences among location (L) for all measured traits except EPP (Table 2). Similarly, mean squares for year (Y) and genotype (G) effects were significant for grain yield, days to anthesis and mid-silk, ear height, husk cover, and plant and ear aspects. The mean squares for \( L \times Y \) interaction were significant (\( P < 0.01 \)) for all measured traits. However, \( G \times L \) interaction mean squares were significant for days to anthesis and mid-silk, and husk cover. Also, \( G \times Y \) interaction mean squares were significant for grain yield, anthesis and mid-silk, plant and ear aspects while \( G \times L \times Y \) interaction effects were significant (\( P < 0.01 \)) for days to anthesis and mid-silk (Table 2). The proportion of the total sum of squares contributed by L was highest for ASI, ear height and husk cover while L x Y was the highest for grain yield, days to anthesis and mid-silk, and ear aspect (Table 2).
In contrast, the percentage of the total sum of squares contributed by G was the lowest for days to anthesis and mid-silk, husk cover, and plant and ear aspects whereas Y had the least for grain yield, ASI, plant and ear heights, and EPP. The test environments (location-year combination) contributed 30.6% of the total variation in the sum of squares for grain yield; genotypes accounted for 2.7% whereas GEI accounted for 19.5% of the total variation. The L × Y had the greatest influence on grain yield, accounting for 21.0% of the total variation in grain yield (Table 2), followed by G × L × Y (11.0%), L (9.2%) and the least (0.4%) for Y.

Table 2. Percentage sum of squares from the analysis of variance of grain yield and other agronomic traits of provitamin A maize varieties evaluated at seven locations in Northern Nigeria, 2015-2017.

| Source           | df  | Grain yield | Days to anthesis | Days to silk | ASI† | Plant height | Ear height | Husk cover‡ | Plant aspect§ | Ear aspect¶ | EPP# |
|------------------|-----|-------------|------------------|--------------|------|--------------|------------|-------------|---------------|-------------|------|
| Location, L      | 6   | 9.2**       | 13.4**           | 21.3**       | 36.6**| 10.4**       | 25.0**     | 12.0**      | 5.6**         | 8.8**       | 0.7  |
| Year, Y          | 2   | 0.4*        | 5.3**            | 3.6**        | 0.3  | 0.2          | 1.4**      | 2.3**       | 4.4**         | 4.4**       | 0.4  |
| L × Y            | 11  | 21.0**      | 61.2**           | 58.1**       | 16.8**| 8.7**        | 18.1**     | 9.5**       | 7.9**         | 10.2**      | 4.3**|
| Rep (L × Y)      | 40  | 10.0**      | 3.2**            | 2.3**        | 4.3**| 5.3**        | 7.1**      | 4.0         | 10.6**        | 8.4**       | 4.2  |
| Genotype, G      | 15  | 2.7**       | 0.4*             | 0.3          | 0.5  | 3.0**        | 2.8**      | 1.7         | 2.9**         | 2.1*        | 1.6  |
| G × L            | 90  | 5.6         | 1.9*             | 1.7*         | 3.3  | 7.7          | 4.9        | 10.9**      | 7.9           | 5.8         | 8.9  |
| G × Y            | 30  | 2.9*        | 0.8*             | 0.7*         | 1.0  | 3.0          | 1.9        | 2.8         | 4.6**         | 3.3*        | 3.6  |
| G × L × Y        | 165 | 11.0        | 3.7**            | 3.3**        | 8.0  | 14.9         | 9.7        | 10.1        | 11.2          | 11.9        | 16.0 |
| Error            | 599 | 37.1        | 10.0             | 8.6          | 29.2 | 46.8         | 29.1       | 46.6        | 44.9          | 45.1        | 60.2 |

* ** Significant at the 0.05 and 0.01 probability levels, respectively.
† ASI, anthesis-silking interval.
‡ Husk cover (scale 1–5), where 1 = husk tightly arranged and extended beyond the ear tip, and 5 = ear tips exposed.
§ Plant aspect (scale 1–5), where 1 = excellent plant type, and 5 = poor plant type.
¶ Ear aspect (scale 1–5), where 1 = clean, uniform, large, and well-filled ears, and 5 = ears with undesirable features.
# EPP, number of ears per plant.

**GGE biplot analysis for genotypes and testing sites**

In the GGE biplot, the PC1 explained 38.87% of total variation, whereas PC2 explained 26.26% of the variation for grain yield. Thus, PC1 and PC2 together accounted for 65.13% of the total variation for grain yield (Figures 1–2).

The polygon view of the biplot showed which genotype performed best in which location (Fig. 1). Yan et al. (2000) reported that the vertex genotypes in each sector represented the highest-yielding genotype in the location that fell within that particular sector.
Base on this information, 4 (PVA SYN-18 F2) and 8 (AFLATOXIN SYN YF2) were the highest-yielding genotypes at Kaboji, Birnin Kudu, and Bagauda; and 1 (PVA SYN-13) was the highest-yielding genotype at Saminaka. No vertex genotype in the sector where Zaria, Batsari and Panda were located. No environment fell into the sector where 6 (PVA SYN-11), 2 (PVA SYN-10), and 3 (PVA SYN3 F2) were the vertex genotypes, indicating that these genotypes were the lowest-yielding genotypes at some or all locations.

The discriminating power vs. representativeness view of the GGE biplot analysis is presented in Fig. 2. According to Yan et al. (2007), test environments that have small angles with the average-environment axis (AEA) are more representative of all environments than those with large angles. The small circle is the average environment and the arrow pointing to it is used to indicate the direction of the AEA. The vector length of a test environment measures the magnitude (discriminating power) of its ability to differentiate genotypes in the test environments (Yan et al., 2007). Birnin Kudu, Bagauda, Batsari and Panda had short-vector locations, suggesting that these locations may be regarded as independent research locations and could be treated as unique locations (Fig. 2). However, the long-vector locations, Zaria, Kaboji, and Saminaka were more powerful in discriminating among the genotypes. Environments or locations with long vectors and small angles with the AEC abscissa are ideal for selecting superior genotypes. Fig. 2 revealed that Zaria had a small angle with the average environment coordinate (AEC) abscissa and was therefore the most representative test location and thus, referred to the ideal testing site.

![Figure 1](image_url)

**Figure 1.** Polygon view of the genotype main effect and genotype by environment interaction (GGE) biplot of 16 provitamin A maize varieties evaluated at seven locations in savanna agro-ecologies of Nigeria between 2015 and 2017.
Repeatability of testing sites

The repeatability and representativeness of the test locations presented in Fig. 3 revealed that PC1 explained 23.9% of the total variation, whereas PC2 explained 15.76% of the total variation for grain yield. Thus, PC1 and PC2 together accounted for 39.66% of the total variation for grain yield. The biplot revealed that Kaboji had the highest repeatability as KB16 and KB17 had the smallest angles between their vectors. Similarly, Batsari had high repeatability as BA15, BA16, and BA17 had angles less than 90° among their vectors followed by SM (Saminaka) and ZA (Zaria). In contrast, Bagauda displayed least repeatability (large angles, > 90° among BG15, BG16 and BG17). Similarly, Birnin Kudu (BK15, BK16 and BK17) and Panda (PA15, PA16 and PA17) had angles greater than 90° among their respective vectors, indicating that they were not repeatable. The biplot view showing the ranking of the test environments (data not shown) revealed Zaria as being the closest to the ideal test environment as ZA15 was located closest to the innermost concentric circle of the biplot. **Performance and stability of provitamin A maize genotypes**

In Fig. 4, the genotypes were ranked along the average-tester axis, with the arrow pointing to a greater value according to their mean performance across all testing environments. The double-arrowed line separated genotypes with below-average grain yield from those with above-average grain yield. The mean grain yield of the genotypes was indicated by the projections of their markers on the average-tester axis. The stability of the genotypes was measured by their projection onto the double-arrow line (average-tester coordinate y-axis). The greater the absolute length of the projection of a genotype, the less stable the genotype (Yan et al., 2007, 2010). Thus, 6 (PVA SYN-11) was the lowest-yielding and most unstable genotype. On the other hand, 4 (PVA SYN-18 F₂), was the highest-yielding and stable genotype and was therefore identified as the ideal genotype across locations.
Figure 3. Vector view of the GGE biplot showing representativeness and repeatability of test environments.

| Location           | Code   |
|--------------------|--------|
| Zaria, 2015        | ZA15   |
| Zaria, 2016        | ZA16   |
| Zaria, 2017        | ZA17   |
| Bagauda, 2015      | BG15   |
| Bagauda, 2016      | BG16   |
| Bagauda, 2017      | BG17   |
| Birnin Kudu, 2015  | BK15   |
| Birnin Kudu, 2016  | BK16   |
| Birnin Kudu, 2017  | BK17   |
| Batsari, 2015      | BA15   |
| Batsari, 2016      | BA16   |
| Batsari, 2017      | BA17   |
| Panda, 2015        | PA15   |
| Panda, 2016        | PA16   |
| Panda, 2017        | PA17   |
| Kaboji, 2015       | KB15   |
| Kaboji, 2016       | KB16   |
| Kaboji, 2017       | KB17   |
| Saminaka, 2015     | SM15   |
| Saminaka, 2016     | SM16   |
| Saminaka, 2017     | SM17   |

| Genotype           | Code |
|--------------------|------|
| PVA SYN-13         | 1    |
| PVA SYN-10         | 2    |
| PVA SYN3FA         | 3    |
| PVA SYN-18F₂       | 4    |
| PVA SYN-2          | 5    |
| PVA SYN-11         | 6    |
| F2A090528          | 7    |
| AFLATOXIN SYN YF₂  | 8    |
| PVA SYN-9          | 9    |
| PVA SYN-8          | 10   |
| ACR91SUWAN1-SRC₁   | 11   |
| PVA SYN-HGA        | 12   |
| PVA SYN-HGB        | 13   |
| PVA SYN-21         | 14   |
| PVA SYN-22         | 15   |
| Local Check        | 16   |
Figure 4. Mean vs. stability view of the GGE biplot showing the performance and stability of the provitamin A maize genotypes.
DISCUSSION
The presence of GEI complicates identification of superior maize cultivars in multi-location trials (Yates and Cochran, 1938; Comstock and Moll, 1963; Fakorede and Adeyemo, 1986; Badu-Apraku et al., 2008; Badu-Apraku et al., 2011a; Oyekunle et al., 2017). The significant differences detected in the present study among genotypes for all traits except days to anthesis and mid-silk, husk cover and EPP, indicated the existence of genetic differences among the genotypes and the possibility of identifying outstanding genotype(s) for narrow and/or wide adaptation. The presence of significant differences among locations for all measured traits except EPP suggested uniqueness of the locations in assessing the performance of the genotypes with respect to the traits. The significant mean squares observed for year for all the traits except grain yield, ASI, plant height, and EPP suggesting the uniqueness of each year in assessing the performance of the genotypes and necessitate the need to evaluate genotypes in multiple years for identification of stable and superior genotype for the target environments. The significant G × Y interaction detected for grain yield, days to anthesis and mid-silk, plant and ear aspects confirmed the need for multiple-year evaluation for identifying superior genotypes. The lack of significant difference observed for G × L × Y interaction for grain yield and other measured traits except days to anthesis and mid-silk indicated that the ranking of the genotypes was consistent in different years and at different locations. This result is in disagreement with the findings of earlier researchers (Moghaddam and Pourdad, 2009; Badu-Apraku et al., 2011a, b, 2015; Oyekunle et al., 2017) who observed significant GEI for grain yield and other agronomic traits.

Assessment of the total sum of squares revealed that the environments (L and Y) accounted 30.6% of the total variation in the sum of squares for grain yield; genotypes accounted for 2.7% and GEI accounted for 19.5% of the total variation, indicating a high magnitude of environmental effects over genotypic effects. Similar findings have also been reported by several researchers (Badu-Apraku et al., 2011a, 2011b, 2013; Oyekunle et al., 2017). The low proportion (2.7%) observed for genotypic main effects to the total variation for grain yield in the present study could be due to comparable yield levels of the varieties evaluated in the multi-location trials.

Identification of ideal testing sites is an important breeding strategy for effective selection of superior genotypes for commercialization. According to Yan and Kang (2003), an ideal test environment was described as an environment with the most discriminating of the genotypes and representative of all testing environments. Yan et al. (2007) defined the discriminating power of an environment or location as its ability to differentiate among genotypes, whereas representativeness refers to the ability of a test location to be representative of other test locations. In the present study, Zaria, Kaboji, and Saminaka had long-vector and were therefore more powerful in discriminating among the genotypes. However, Zaria had a long vector and a relatively small angle with the AEC abscissa and was therefore the most discriminating and representative test location, thus identified as the most ideal testing site for the identification of superior genotypes for registration and release of maize adaptable to the savanna agro-ecologies of Nigeria. The identification of Zaria as the idea testing site in the present study corroborating the findings of previous study reported by Oyekunle et al. (2017); Badu-Apraku et al. (2011a, b, 2013).

An important objective of the study was to assess the repeatability of the testing sites used in evaluating maize genotypes for registration and release in the northern part of the country. The low proportion of the total sum of squares accounted for by PCI and PC2 reflected the complexity in the analysis of the 16 provitamin A maize genotypes evaluated at seven locations for three years. Assessment of repeatability is importance to determine the representativeness of the testing sites over years. Yan et al. (2011) classified test locations into four categories based on repeatability analysis. In the present study, Zaria and Saminaka may be classified as Type I (core testing site) according to Yan et al. (2011) for the region due to their relatively high repeatability and proximity to the average environment axis. Batsari could be classified as Type II due to their relatively high representativeness and repeatability. These locations should be included in the multi-location trials for the identification of superior genotypes for commercialization in the region. Panda, Birnin Kudu, and Bagauda could be classified as Type IV due to their low representativeness and repeatability. These locations should be avoided in the multi-location testing of advanced maize germplasm. However, the low repeatability and representativeness of Panda, Birnin Kudu, and Bagauda could be due to the nature of germplasm utilized in the present study. This is in agreement with the findings of Yan et al. (2011) who reported that repeatability of a given location may vary with the set of genotypes involved.
The low repeatability observed for some locations in the present study could also be due to low levels of variation in the genotypes tested as measured by the magnitude of percentage sum of squares accounted for by genotypic effects as compared to environmental effects. It can be concluded that the type of cultivars (hybrids, open-pollinated cultivars, landrace, or populations) evaluated determined the most suitable locations for multi-location testing.

CONCLUSIONS
In conclusion, the results of this study revealed the existence of significant differences among locations, years, genotypes and their interactions for grain yield and most other traits. The GGE biplot analysis identified PVA SYN-18 F$_2$ as the highest-yielding and most stable genotype across testing sites. Zaria, Saminaka, and Kaboji were highly discriminating and repeatable. Zaria and Saminaka were identified as core testing sites for selection of outstanding genotypes for commercialization. The core testing sites identified in this study could facilitate the identification of stable and high-yielding maize germplasm adaptable to the savannas agro-ecologies of Nigeria.

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Contribution of authors
Oyekunle M.: The author contributed substantially in conception, experimental design, acquisition of data, analysis and interpretation of data, revising the manuscript, and final approval of the manuscript. Ado S.G and Usman I.S.: The authors contributed in experimental design, acquisition of data, and final approval of the version to be published.

Conflict of interest:
The authors declare no conflict of interest.

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