



# Factor analysis to investigate genotype and genotype × environment interaction effects on pro-vitamin A content and yield in maize synthetics

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Received: 27 June 2019 / Accepted: 26 September 2019 / Published online: 4 October 2019  
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**Abstract** Vitamin A Deficiency (VAD) is a major public health problem in Sub-Saharan Africa affecting 33 million preschool-age children. Enrichment of maize varieties with provitamin A could provide sustainable and affordable solution to VAD. This study was conducted to understand the extent of GEI effects on both grain yield and provitamin A content in 21 maize synthetics and identify synthetics combining stable performance with high level provitamin A content across diverse environments in West Africa. Combined analysis of variance found significant ( $p < 0.01$ ) GEI effects that prompted further investigation of the GEI magnitude using mixed model with factor analysis. Factors 1 and 2 explained 71% of the total variability. G5, G4, G12, G18, G2 and G14 were broadly adapted to a range of environments and considered the most stable and high yielding. G8, G1, and G10 were specifically adapted to a group of environments. Whereas, G21, G19 and G17 were found to be the worst and unstable genotypes. G4 combined stable performance with high provitamin A

content, whereas G20 and G18 were stable but had low provitamin A contents. Three genotypes, G4, G12 and G14 were found to combine stability with high provitamin A contents. These genotypes can be recommended for production in the low-land tropics of West and Central Africa with similar environments.

**Keywords** Provitamin A carotenoids · GXE interaction · *Zea mays L.* · Stability · Vitamin A deficiency · Grain yield · Factor analysis

## Introduction

Maize (*Zea mays L.*) is a staple food crop for millions of people in sub-Saharan Africa (SSA) and is regarded as a vital crop for global nutrition (IITA 2010; Nuss and Tanumihardjo 2010). Most of the maize that is produced and consumed is white and devoid of provitamin A carotenoids (Menkir et al. 2008; Li et al. 2007). This may partly explain why vitamin A deficiency (VAD) is a major public health problem in SSA (Nuss and Tanumihardjo 2010), affecting approximately 33 million preschool-age children in the continent (West 2002). In South Africa, for example, the number of children with VAD increased from 33% in 1994 to 64% in 2005 (Labadarios et al. 2007; Labadarios and Van Middelkoop 1995). VAD is responsible for several disorders that range from impaired iron mobilization, growth retardation, and

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blindness to a depressed immune response, increased susceptibility to infectious disease and childhood mortality and morbidity affecting 190 million pre-school-age children and 19 million pregnant women, mostly in Africa and South Asia (Sommer and Davidson 2002; Rice et al. 2004; WHO 2009).

Maize is one of the six staple crops that have been targeted for biofortification to combat VAD under the HarvestPlus Challenge Programme (Tanumihardjo 2008; HarvestPlus Brief 2006). Biofortification of maize varieties with provitamin A through conventional breeding is viewed as a potential long-term, sustainable and affordable strategy to alleviate VAD in selected target groups (Howe and Tanumihardjo 2006a, b; Nestel et al. 2006). The current target level set for conventional maize breeding under HarvestPlus is 15 µg/g dry weight (DW) of provitamin A (Ortiz-Monasterio et al. 2007). The high provitamin A content should be combined with high yield potential and consistent performance across a broad range of growing conditions to promote adoption of the biofortified maize cultivars by farmers.

Grain yield and provitamin A content are complex traits that are affected by the environment and genotype × environment interaction (GEI). Environmental variables such as pH, temperature, solar radiation, precipitation, organic matter, and soil texture have the potential to influence nutrient concentration in crops (Cabuslay et al. 2003; Joshi et al. 2010) and must then be taken into consideration while examining the variation for micronutrient content in crop plants. Several studies conducted to assess the nutritional quality of genotypes grown in diverse growing conditions found significant GEI for beta-carotene and provitamin A content in maize (Manjeru 2017; Oikeh et al. 2004; Gregorio 2002; Trethowan 2007), wheat (Gregorio 2002; Trethowan 2007; Hedieh et al. 2013; Bashir et al. 2014), rice (Gregorio 2002; Trethowan 2007), Tomato (Rosales et al. 2006), Irish potato (Haynes et al. 2010), orange fleshed sweet potato (Gurmu et al. 2015; Kathabwalika et al. 2016), and vegetables (Granado et al. 1992). In contrast, other studies did not find significant GEI in maize (Menkir and Maziya-Dixon 2004; Egesel et al. 2003; HarvestPlus Brief 2014), rice (HarvestPlus Brief 2014), cassava (Norbert et al. 2010), orange fleshed sweet potato (Gruneberg et al. 2005; Mbwaga et al. 2008; Tsegaye et al. 2011) and vegetables (Campos et al. 2006).

The maize improvement program at the International Institute of Tropical Agriculture (IITA) has developed synthetics with elevated levels of provitamin A adapted to production conditions in West and Central Africa. Considering the conflicting results of the GEI effects on beta-carotene and provitamin A content reported in the literature, testing these varieties across locations and years is necessary to identify those with high yield potential and provitamin A content as well as minimal GEI. Consequently, the varieties selected for elevated levels of provitamin A have been extensively evaluated in field trials, in which inferior varieties have been constantly replaced with promising new varieties over the years. The resulting unbalanced data from such multi-environment trials is amenable to GEI analysis using mixed models with factor analytic variance and covariance structure (Smith et al. 2001; Kelly et al. 2007; Nuvunga et al. 2015). Nuvunga et al. (2015) used this methodology to assess three levels of data imbalance, reaching up to 50%, and found that the FA (Factor Analysis) methodology is robust in analyzing data recorded in multi-environments, including situations in which the genotypes are not evaluated in all the environments and/or years. The present study was, therefore, conducted to understand the extent of GEI effects on both yield and provitamin A content of maize synthetics and to identify maize varieties with consistently high grain yield and higher levels of provitamin A across diverse environments.

## Materials and methods

### Genetic materials

The genetic materials used in this study included 21 provitamin A maize synthetics each developed at IITA by intercrossing eight inbred lines selected for intermediate to high level of pro-vitamin A and good combining ability (Table 1). All the provitamin A rich maize synthetics used in this study were grown across nine locations and 4 years, and all were evaluated under rain fed conditions during the main growing season (June–November). Among the synthetics, ten were commonly evaluated across all the 4 years, 19 were commonly evaluated in 2012 and 2013, and 12 were tested in 2014 and 2015. The 21 provitamin A maize synthetics used in this study were evaluated

**Table 1** List of provitamin A maize rich synthetics and other genotypes used in the present study

Code	Name	Year (yield)	Year (PVA)
G1	PVASYN1	2012–2013	2012–2013
G2	PVASYN4	2012–2015	2012–2015
G3	PVA SYN11	2012–2015	2012–2015
G4	PVA SYN13	2012–2015	2012–2015
G5	Pop.66-SR/(Acr. 91 Suwan 1-SR) *2	2012–2013	–
G6	AFLATOXIN R Syn 2-Y	2012–2014	2012–2013
G7	PVA SYN-9	2012–2015	2012–2015
G8	PVA SYN-10	2012–2015	2012–2015
G9	PVA SYN-8	2012–2014	2014–2015
G10	PVA SYN-6	2012–2014	2012–2013
G11	PVA SYN-3	2012–2015	2012–2015
G12	PVA SYN-2	2012–2015	2012–2015
G13	PVA SYN-7	2012–2015	2012–2014
G14	PVA SYN 17	2012–2013	2012–2013
G15	PVA SYN 18	2012–2015	2012–2015
G16	PVA SYN 19	2012–2015	2012–2015
G17	PVA SYN 20	2012–2012	2012–2013
G18	AFLATOXIN SYN-Y2	2012–2015	2012, 13 and 15
G19	Acr. 91 Suwan 1-SR C1	2012–2015	2012–2015
G20	Oba Super 2	2012–2013	2012–2013
G21	Local Check	2012–2015	–

along with a commercial orange single-cross maize hybrid (Oba Super 2), aflatoxin resistant orange maize variety (Aflatoxin syn-Y2) and a commonly cultivated improved orange open pollinated maize variety (Acr.91 Suwan 1-SR C1) used as a reference check as well as a farmers preferred variety as a local check (Table 1). Provitamin A concentrations were measured from seed samples of the 21 provitamin A rich maize synthetics harvested from trials using HPLC. Provitamin A was defined as the sum of b-carotene, b-cryptoxanthin and a-carotene, with a-carotene and b-cryptoxanthin contributing 50% of the value of b-carotene (U.S. Institute of Medicine 2001).

### Experimental design

The trials involving provitamin A maize synthetics and the checks were arranged in  $7 \times 3$  alpha lattice design with three replications in 2012 and 2013, and  $6 \times 3$  alpha lattice design with three replications in 2014 and 2015. The trials were evaluated during the main rainy season across the major maize testing sites in Nigeria, Ghana and Gambia. Each PVA synthetic was planted in two 5-m-long rows with 0.75 m

distance between rows and 0.5 m distance within rows. Three seeds were planted in a hill and thinned to two plants after emergence to attain a population density of 53,333 plants  $\text{ha}^{-1}$  in each location. Standard cultural practices, recommended rates of fertilizer application, and pest and weed control measures were used in all environments. Generally, rainfall distributions were good across test locations and growing seasons during evaluation of these trials (Tables 2, 3).

### Statistical analysis

Each location  $\times$  year combination (environment) was analyzed separately followed by a combined analysis across all the environments. The Combined multi-locational analysis was conducted using multivariate mixed models according to Nuvunga et al. (2015) and proposed by Patterson and Thompson (1971) and described as follows:

$$y = X\beta + Zb + e \quad (1)$$

**Table 2** Test environments for grain yield and PVA content analysis

No.	Locations	Year (yield)	Year (PVA)
1	Ikene	(2012–2015)	(2012, 13 and 15)
2	Saminaka	(2012–2015)	(2013–2015)
3	Zaria	(2012–2015)	(2012–2015)
4	Kadawa	(2012–2015)	(2013–2015)
5	Mokwa	(2012–2015)	(2012–2015)
6	Ejura	(2012–2015)	–
7	Panda	(2012–2015)	–
8	Kudu	(2012–2015)	–
9	Gambia	(2012–2015)	–
10	Ibadan	–	(2012, 13 and 15)
11	Akumadan	–	2013
12	Fumisua	–	2013

where  $y$  is a vector of observations from plots for each variety in each environment.  $\beta$  and  $b$  are the fixed and random effect vectors respectively,  $e$  denotes the random error vector, and  $X$  is the incidence matrix of the fixed effects for the blocks;  $Z$  the incidence matrix of random effects corresponding to the maize varieties.

Considering the mixed model matrix (Santos et al. 2017) below,

$$C = \begin{pmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{pmatrix} = \begin{pmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{pmatrix} \tag{2}$$

The solutions for  $\beta$  and  $b$  from the mixed model equation can be derived as:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{bmatrix} \begin{bmatrix} \beta \\ b \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix} \tag{3}$$

$$\begin{bmatrix} \beta \\ b \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix} \begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{bmatrix}^{-1} \tag{4}$$

The random effect vector  $b$  was classified using Expectation–Maximization (EM) algorithm described by Dempster et al. (1977). The REML solution for the elements of  $G$  matrix is described below:

$$\hat{\sigma}_{b_{ij}} = \left[ b_i^T b_j + tr(C_{ij}^{-1}) \right] / t \tag{5}$$

with

$$\tilde{\sigma}_{b_{ij}} = \begin{cases} \sigma_{b_k}^2 & \text{if } i = j \\ \sigma_{b_{ij}} & \text{if otherwise} \end{cases} \tag{6}$$

The matrix  $C_{ij}^{-1}$  corresponds to the submatrices  $i$  and  $j$  of the inverse matrix  $C^{-1}$ . The error variance estimator is described below:

$$\tilde{\sigma}_{e_{ij}} = \left\{ e_i^T e_j + tr([HC^{-1}H^T]_{ij}) \right\} / n^* \tag{7}$$

$$\tilde{\sigma}_{e_{ij}} = \begin{cases} \sigma_{e_h}^2 & \text{if } i = j \\ \sigma_{e_{ij}} & \text{if otherwise} \end{cases} \tag{8}$$

where  $H$  is  $\{X, Z\}$ . The trace depends on  $i$  and  $j$  submatrix,  $n^*$  is the length of the  $\{j, i\}$  vector.

**Table 3** Characteristics of study sites

Place name	Country	Longitude	Latitude	Altitude (m)	Annual rainfall (mm)	Soil type (FAO classification)
Ibadan	Nigeria	3.90	7.38	225	1194	Luvisols, Acrisols, Ferralsols and Lithosols
Ikene	Nigeria	3.43	6.52	60	1636	Luvisols, Acrisols, Ferralsols and Lithosols
Mokwa	Nigeria	5.05	9.30	157	1002	Luvisols
Zaria	Nigeria	7.70	11.06	641	782	Luvisols
Kadawa	Nigeria	8.27	11.39	500	938	Luvisols
Saminaka	Nigeria	6.70	9.10	766	838	Luvisols
Akumadan	Ghana	– 1.95	7.40	400	1264	Luvisols, Acrisols, Ferralsols and Lithosols
Fumesua	Ghana	– 1.50	6.71	289	1484	Luvisols, Acrisols, Ferralsols and Lithosols
Ejura	Ghana	– 1.36	7.40	237	1311	Luvisols, Acrisols, Ferralsols and Lithosols
Kudu	Nigeria	9.28	11.26	172	1002	Luvisols
Gambia	Gambia	– 16.67	13.44	14	1203.5	Ferralsols, Nitosols and Gleysols (Alluvial)

The approach described is synonymous to an unstructured (UN) variance–covariance matrix, hence both variances  $\{\sigma_{ek}, \sigma_{bk}\}$  and covariances  $\{\sigma_{eij}, \sigma_{bij}\}$  are estimated.

Factor analysis approximation in REML

Factor analytic variance–covariance structure was applied to the mixed model equation. Similar to applying factor analysis directly on the matrix of REML estimates (Meyer 2007)

Such that:

$b$  can be written in the form  $I \otimes [Lf + \delta]$ , while  $G$  can also be written as  $(LL' + \Psi)$ .

The factor scores for the effect of  $b$  can be solved by substituting  $I \otimes [Lf + \delta]$  for  $b$  in the equation using the notation of Meyer (2009); such that:

$$y = X + Z[(L \otimes I) f + \delta] + e \tag{9}$$

where  $f \sim N(0, I)$ ,  $\delta \sim N(0, \Psi)$  and  $e \sim N(0, R)$ .  $f$  is the vector of factor scores,  $\delta$  specific factor,  $L$  is the matrix of factor loading and  $\Psi$  represent the matrix of specific variances.

Restricting the factor loadings is an important corrective measure when choosing FA- $n$  models. In order to guarantee the uniqueness of the choice of  $L$ ,  $L'\Psi^{-1}L = \Delta$  was used as restriction; where  $\Delta$  is a diagonal matrix. The very important step to estimating  $L$  and  $\Psi$  is to derive the matrix  $\Psi^{-1/2}(A - \Psi)\Psi^{-1/2}$ . In other to derive  $\hat{L} = \Psi^{1/2}\hat{P}$  the Expectation Maximization algorithm will be used.  $\hat{P}$  is derived by the spectral decomposition of

$$\Psi^{-1/2}(A - \Psi)\Psi^{-1/2} \text{ and } \hat{\Psi} = \text{diag}(A - \hat{L}\hat{L}') \tag{10}$$

The iteration continues until  $L$  and  $\Psi$  matrices converge. Hence,  $L$  can be used to describe the factor loadings for each environment (Nuvunga et al. 2015).

The matrix solution to the derived mixed model equation above (9) by substituting  $ZL$  with  $\theta$ , is given below (Smith et al. 2001):

$$\begin{pmatrix} \hat{\beta} \\ \hat{f} \\ \hat{\delta} \end{pmatrix} = \begin{pmatrix} X'R^{-1}X & X'R^{-1}\theta & X'R^{-1}Z \\ \theta'R^{-1}X & \theta'R^{-1}\theta + I & \theta'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}\theta & Z'R^{-1}Z + \Psi^{-1} \otimes I \end{pmatrix}^{-1} \begin{pmatrix} X'R^{-1}y \\ \theta'R^{-1}y \\ ZR^{-1}\hat{y} \end{pmatrix} \tag{11}$$

The solutions for factor scores, fixed and random effects is estimated below (Smith et al. 2001; Santos et al. 2017):

$$\hat{\beta} = (X'\Sigma^{-1})^{-1}X'\Sigma^{-1}(y - \theta f - Z\delta) \tag{12}$$

$$\hat{f} = (\theta'\Sigma^{-1}\theta + I)^{-1}\theta'\Sigma^{-1}(y - Xb - Z\delta) \tag{13}$$

$$\hat{\delta} = (Z'\Sigma^{-1}Z + \Psi^{-1} \otimes I)^{-1}Z'\Sigma^{-1}(y - Xb - \theta f) \tag{14}$$

Results

The combined analysis of covariance found significant variety, environment and variety  $\times$  environment interaction effects for both grain yield and provitamin A contents ( $p < 0.01$ ) (Tables 4, 5). Environment accounted for most of the total variation for both grain yield (58%) and provitamin A content (39%), indicating the presence of wide variation in testing conditions under which the varieties were evaluated. The repeatability values were 0.90 for grain yield and 0.91 for PVA. The significant variety  $\times$  environment interaction effects prompted further investigation of the magnitude of GEI using mixed model with factor analytic covariance structure. Mean grain yields of the 21 maize synthetics averaged across the environments varied from 3 to 8 tons ha<sup>-1</sup>. All the test synthetics produced higher grain yields in comparison to the local check variety. The maize synthetics included in this study exhibited a broad range in provitamin A concentrations. G3 and G4 consistently showed higher provitamin A content across locations and years, with an average content of 8–11  $\mu\text{g g}^{-1}$ .

Results of factor analysis for yield

In our study, factor 1 and factor 2 together explained 72% of the total variance (Fig. 1). Out of the 36 environments included in this study, 29 environments were placed in quadrant I and IV, which represent the most favorable environments with above average grain yield. In contrast, seven environments fell in quadrant II and III and are considered as unfavorable environments where genotype performance was poor. Environments E11, E12, E23, E35 contributed the

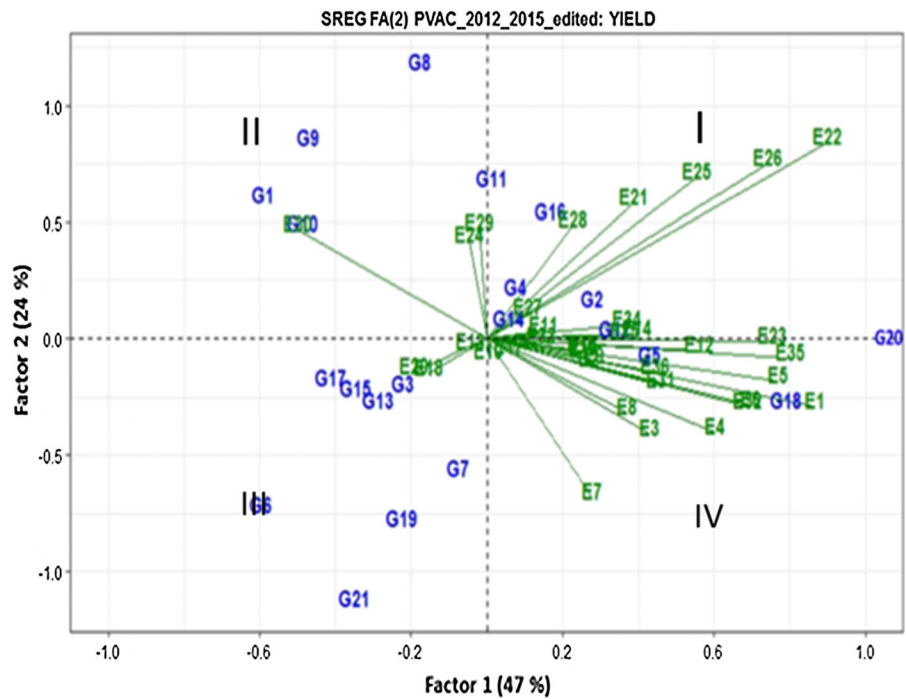
**Table 4** Covariance analysis of grain yield of varieties evaluated across 36 environments

Covariance parameters	Estimate	df	SE	ZValue	ProbZ	%Variation
Env	1,017,034.17	35	259,089	3.93	0.00000	57.43
Rep (Env)	161,089.33	72	30,410.55	5.30	0.00000	9.10
Variety	71,638.03	20	26,472.03	2.71	0.00340	4.04
Env*variety	108,265.07	646	14,708.57	7.36	0.00000	6.11
Residual	413,032.4	1332	16,004.75	25.81	0.00000	

**Table 5** Covariance analysis of provitamin A content of varieties obtained from samples collected from 22 environments

Covariance parameters	Estimate	df	SE	ZValue	ProbZ	%Variation
Env	1.0132	21	0.33	3.04	0.00117	39.12
Rep (ENV)	0.0308	22	0.03	1.20	0.11431	1.19
Variety	0.2164	20	0.09	2.51	0.00603	8.36
Env*variety	0.3703	350	0.07	5.02	0.00000	14.30
Residual	0.959	364	0.07	13.54	0.00000	

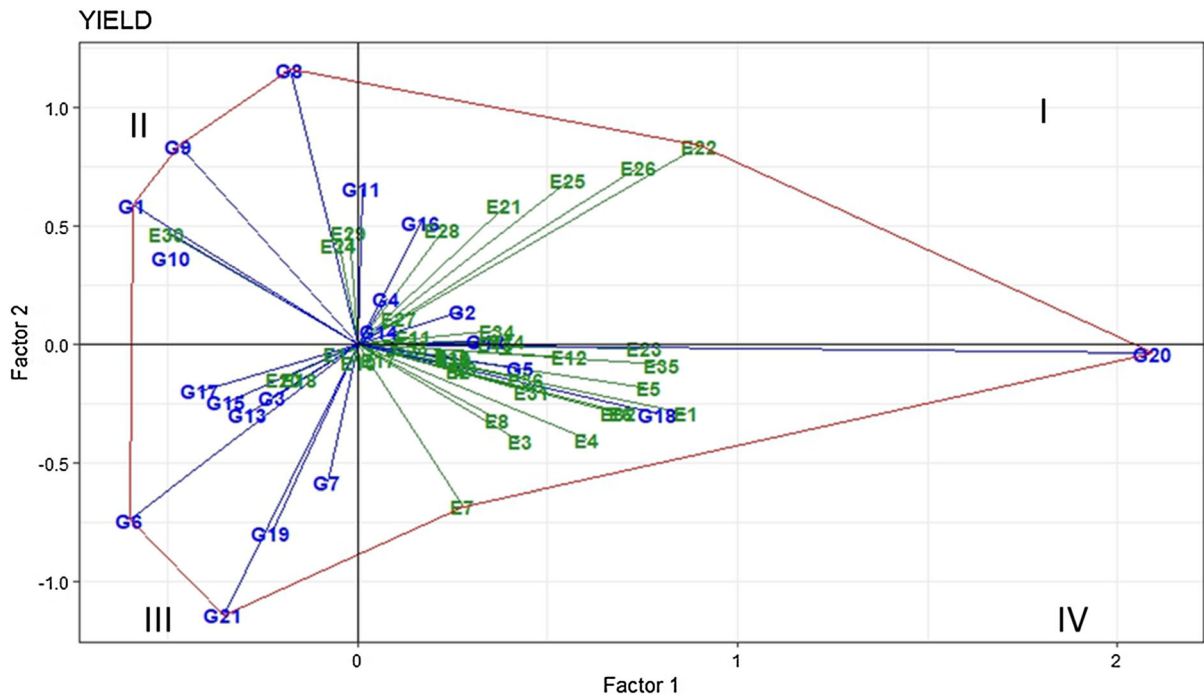
**Fig. 1** Factor loadings and scores of 36 environments (green) and 21 provitamin A maize varieties (blue) for yield. (Color figure online)



least to the GEI (Fig. 1). In this analysis, yield stability can be described by the scores of factors 2. Productive and stable genotypes should thus have high scores for factor 1, but values nearer zero for factor 2. Nine of the 21 maize synthetics were placed in quadrant I and IV and are all better performing varieties with above average yield. Varieties G16, G11, G14 and G4 were specifically adapted to environments E28, E21, E25, E26 and E22. Amongst these varieties G2, G5, G12,

and G20 were found to be stable and high yielding across environments (Fig. 1). On the other hand varieties G1, G6, G9 and G21 were found to be inferior and unstable in terms of grain yield.

Figure 2 shows the polygon-view of the FA biplot to help in visualizing the pattern of the multi-environment trial results. A polygon was drawn by connecting provitamin A rich maize varieties that were furthest from the biplot origin such that all



**Fig. 2** Factor loadings and scores of 36 environments (green) and 21 provitamin A maize varieties (blue) for yield. (Color figure online)

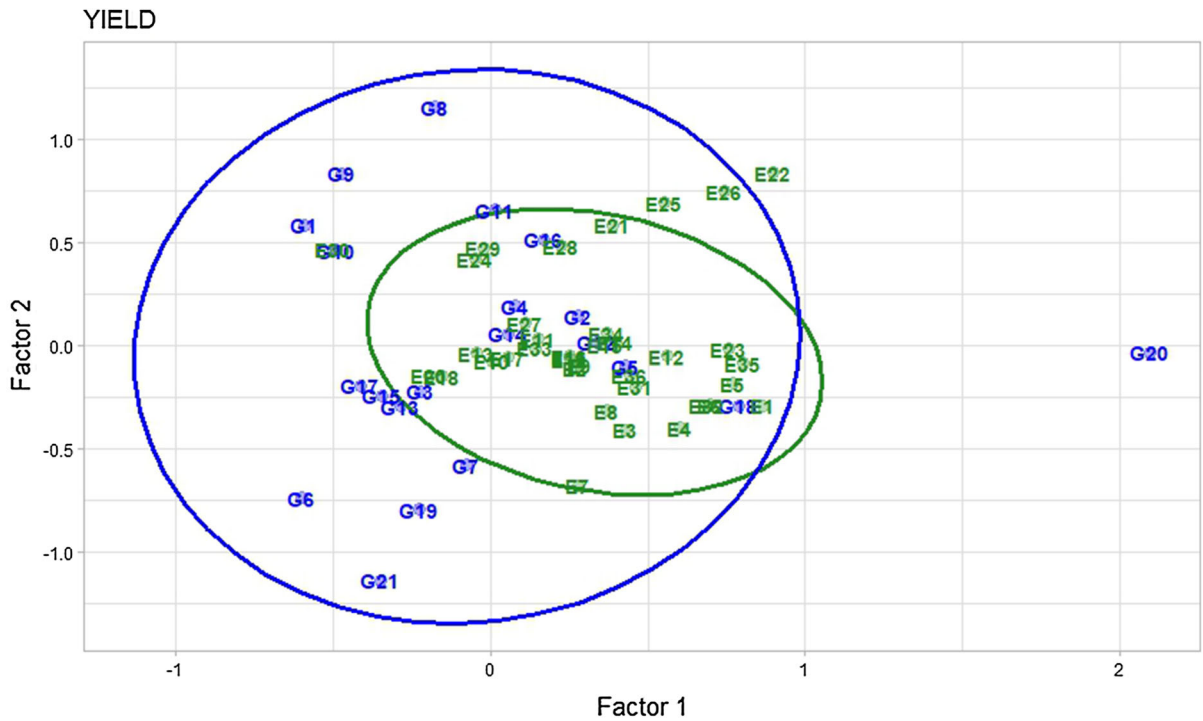
varieties were enclosed within the polygon. An orange commercial hybrid G20 and varieties G8, G9, G1, G21 and G6 located at the corner of the polygon were considered as the vertex varieties (Fig. 2) and they were thus the most responsive varieties to environments compared to others. Amongst these, G20 was a winning and most stable commercial hybrid across 29 environments, whereas, the remaining vertex varieties (G1, G6, G8, G9 and G21) were specifically adapted to certain environment, but were found to be low yielding and unstable (Fig. 2)

As shown in Fig. 2 varieties G5, G4, G12, G18, G2 and G14 were adapted to a broad range of environments and are considered to be the most stable with yield potential of > 6 t/ha. Out of these, G4 and G12 were released in Nigeria and are the most popular PVA synthetics that are currently under production. G8, G1, G9 and G10 were specifically adapted to environments E30 (Zaria 2015), E29 (Saminaka 2015) and E 24 (Ejura 2014). The local check (G21), G19 and G17 were found to be the lowest yielding and unstable varieties.

Figure 3 shows the patterns of distribution of both environments and varieties. Varieties falling within a group of environments having similar patterns of distribution are considered to have common adaptation. While varieties G16, G11, G2, G4, G12, G5 and G18 showed better adaptation to the majority of the environments shown in the confidence ellipses, varieties G8, G9, G1 were found to be specifically adapted to certain environments. Three varieties (G21, G19 and G6) were identified as unstable and low yielding (Fig. 3).

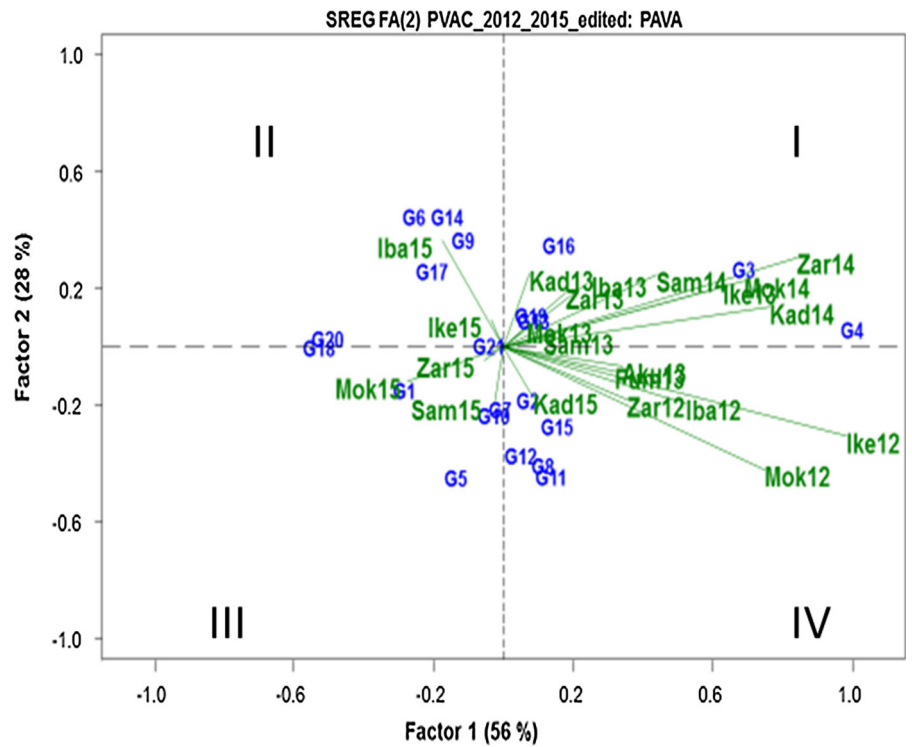
#### Results of factor analysis of pro-vitamin A content

The FA of biplots for provitamin A content of 20 maize varieties are shown in Figs. 4, 5 and 6. As shown in Fig. 4, the two factors together explained 84% of the total variance among the varieties. Among all the environments used under this study, only five environments were placed in quadrant II and III which were considered as unfavorable environments where low level of provitamin A content was recorded (Fig. 4). The majority of the environments were

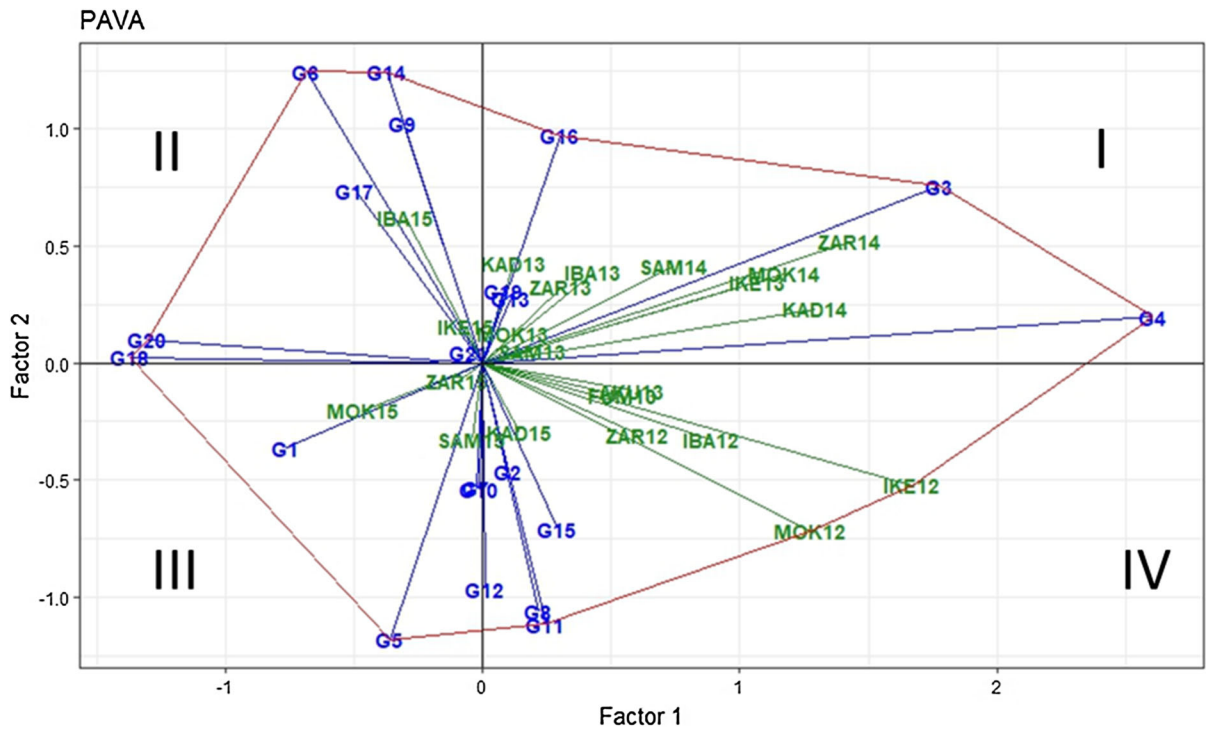


**Fig. 3** Confidence ellipses for the factor scores of several genotypes

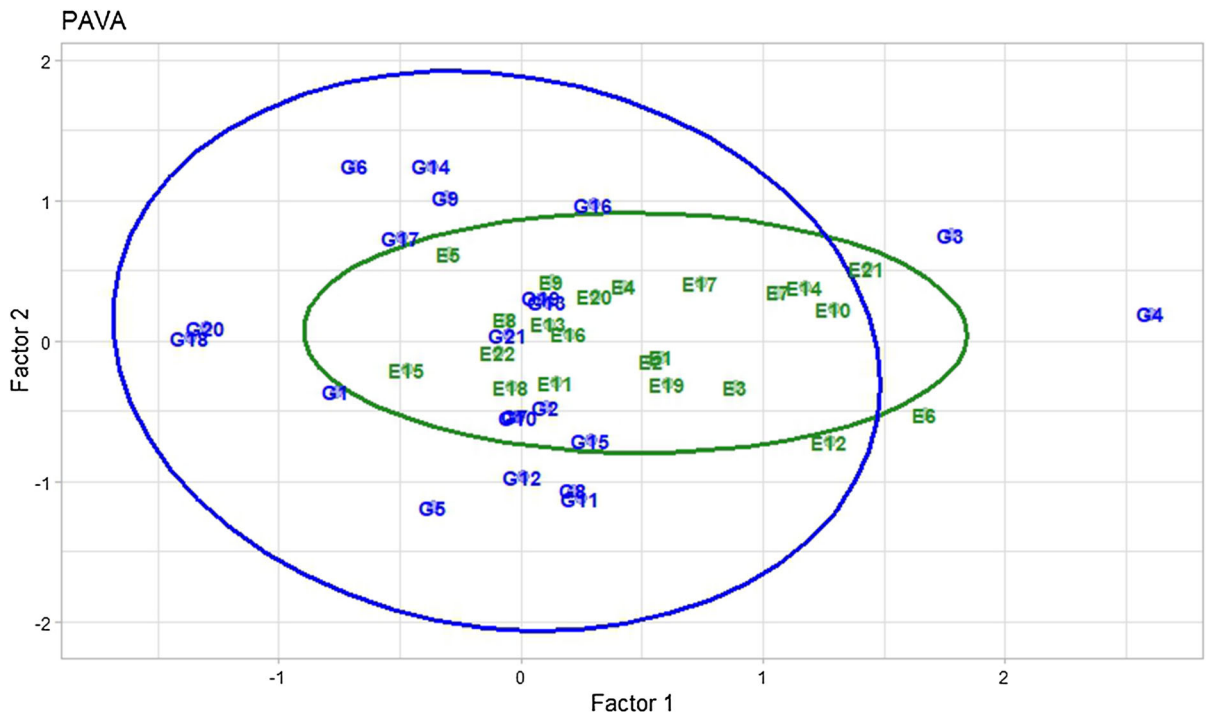
**Fig. 4** Factor loadings and scores of 22 environments (green) and 20 provitamin A rich maize varieties (blue) for Provitamin A contents. (Color figure online)







**Fig. 5** Factor loadings and scores of 22 environments (green) and 20 provitamin A rich maize varieties (blue) for Provitamin A contents. (Color figure online)



**Fig. 6** Confidence ellipses for the factor scores of several genotypes. Cluster analysis for grain yield and provitamin A content

placed under quadrant I and IV representing the most favorable environments with above average provitamin A content. Environments that least contributed to the GEI were environments E11, E12, E23, E35 (Fig. 4). Ten of the 20 provitamin A maize varieties were placed in quadrant I and IV and are all better performing varieties with above average provitamin A content. The remaining 10 varieties were placed in quadrant II and III with below average performance in provitamin A content, with G18 and G20 being the worst performer. E14 (Mok 2013) contributed less to GEI for provitamin A content in the maize varieties (Fig. 4). Three varieties (G4, G3 and G16) showed better adaptation to Zar 2014, Kad 2014, Mok 2014 and Sam 2014. G2, G11, G15, G8 and G12 showed better adaptation to Kad 2015, Zar 2012, and Mok 2012 (Fig. 4). While environments Sam 13 and Mok 13 contributed less to the GEI of provitamin A content (Fig. 4), Zar14, Ike12 and Mok12 contributed the most to the interaction. Two provitamin A varieties (G4 and G3) were better performing in environments Kad14, Zar14 and Mok14 and were also found to be the most stable across locations and years.

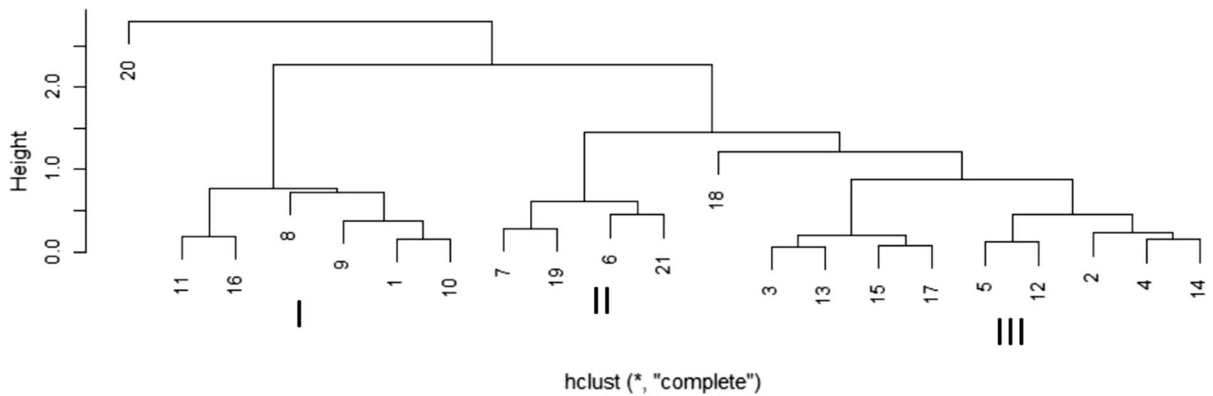
Variety G4 was the most adapted with high and stable PVA content. The varieties that are most distant from the biplot origin, including G15, G14, G6 and G11 contributed most to GEI (Fig. 5). The local check G20 and synthetic variety G18 were stable but had low provitamin A content compared to other varieties under investigation. Figure 5 shows the polygon-view of the FA biplot to help in visualizing the pattern of the multi-environment trial dataset. G11, G4 and G3, G14, G6, G18 and G5 located at the corner of the polygon were the vertex varieties. Among these G11, G4 and G3 had high pro-vitamin A content. G4 was found to be a variety with the highest provitamin A content and also the most stable across environments. The other vertex varieties (G6, G14, G5 and G18) were specifically adapted to IBA15 and MOK15 but found to be low in provitamin A content (Fig. 5). G3 had the highest provitamin A content, but specifically adapted to environments with less stable provitamin A concentration as compared to G4 (Fig. 5). The local check (G21) had the lowest but stable provitamin A content and also recorded the lowest grain yield across all the test locations. G4, G15 and G16 were stable with high PVA content across environments. The FA bi-plot analysis identified G16, G4, G12 and G2 as stable with high PVA content. These varieties can be

recommended for release in Nigeria, Ghana and Gambia. G4 (PVA SYN 13) was already released in Nigeria, Ghana and Cameroon. Generally, this study identified G4, G2, G15, G16, G11 and G8 as varieties with broad adaptation and consistent provitamin A content across environments. G18, G20, G5, G1 and G17 were identified as poorly adapted varieties with low and inconsistent provitamin A expression.

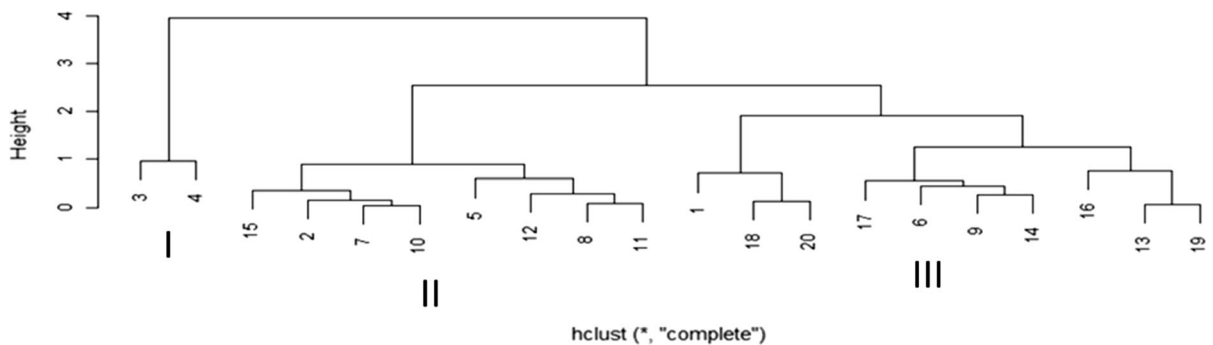
Clustering can help in identifying varieties having similar performance across environments and provides valuable information for future varietal recommendation. The varieties were clustered based on their grain yields across test environments (Fig. 7). There were three major groups of PVA maize varieties, with the commercial hybrid check (Oba Super 2) being separated from the three groups. Cluster I had six varieties (G11, G16, G8, G9, G1 and G10) with four of them having higher grain yield and two varieties (G1 and G10) having lower grain yields. Cluster II had four varieties (G6, G7, G19 and G21), all of them having lower grain yield. Cluster III had 12 varieties (G3, G19, G6, G18, G13, G15, G17, G5, G12, G2, G4 and G14) with most of them having similar grain yields. Cluster III is comprised of the most promising varieties for release, two of the most popular provitamin A maize varieties released in Nigeria were also included in this cluster. Clustering of the varieties was also done for provitamin A content, which resulted in three major groups (Fig. 8). Cluster I comprised of two varieties (G3 and G4), which had higher pro-vitamin A content compared to others. Cluster II had seven varieties (G15, G7, G12, G2, G10, G8 and G11) all with intermediate provitamin A content. Cluster III consisted of nine varieties (G1, G18, G17, G6, G14, G16, G13, G19 and G20), containing lower levels of provitamin A. Cluster analysis found these varieties (G2, G3, G4 and G12) that combined high grain yield with high provitamin A content. In contrast G1 and G16 were found to be low yielding with low provitamin A content.

## Discussion

Most crop-breeding programs require flexibility when dealing with the addition and removal of genotypes which results in generating unbalanced data that can be analyzed using mixed models to study GEI (Piepho 1998; Crossa et al. 2006). In the present study



**Fig. 7** Grain yield-based clustering of the 21 PVA synthetics planted across 36 environments



**Fig. 8** Pro-vitamin A content-based clustering of 20 PVA synthetic varieties evaluated across 22 environments

involving unbalanced data significant GEI was found for both grain yield and provitamin A content, consistent with the results in other studies (Granado et al. 1992; Oikeh et al. 2004; Bashir et al. 2014; Gurmu et al. 2015; Manjeru 2017). Changes in genotype raking for PVA from environment to environment were detected in the present study, which is in agreement with high environmental variation reported for maize in other regions (Ali et al. 2010; Beyene et al. 2013; Makumbi et al. 2015). Such rank changes could arise from differences in altitude, latitude, rainfall pattern, soil PH, temperature, solar radiation, acidic condition and soil textures affecting grain yield of the varieties in this study. Changes in environmental factors could also result in differential accumulation of provitamin A carotenoids in varieties included in the present study, which were contrary to the results reported in other studies (Brunson and Quackenbush 1962; Egesel et al. 2003; Kurilich and Juvik 1999; Menkir et al. 2014).

Stability estimates of promising genotypes must be repeatable across years in order to detect and recommend superior genotypes (Annicchiarico 2002). To detect the relative stability and GEI of varieties, the applicability of factor-analysis associated with mixed models for MET analysis of unbalanced data is of paramount importance (Figueiredo et al. 2014; Santos et al. 2017). The current study showed that the FA models was a powerful statistical tool for discriminating the provitamin A maize synthetics, providing strong basis for making inferences regarding their adaptability and stability in yield and provitamin A contents, despite the complex nature of the data (Burgueño et al. 2008, 2011). Varieties showing positive interaction with the environments could be exploited for specific agro-ecological conditions of the target environments and are therefore best suited to those environments (Kandus et al. 2010; Gurmu et al. 2015). Most of the synthetics in our study had broad adaptability to the environments under which the trials were conducted, with only few synthetics still showing

relatively narrow adaptation. There were also synthetics with specific adaptation to certain environments, consistent with the results in other studies (Yan and Kang 2003). Furthermore, environments which are positioned closer in the bi-plot are considered to reveal strong positive association, which in turn shows their similarity in discriminating genotypes (Gauch and Zobel 1997; Yan et al. 2007). In the present study, environments E28, E21, E25, E26 and E22 were positioned closer to each other in the factor bi-plot, these environments showed a positive association in discriminating the synthetics.

The probability of success in adoption of high provitamin A maize varieties by producers, processors and consumers depends on high yield potential and other desirable agronomic and end-use quality traits (Menkir et al. 2008). In the present study, several synthetics competitive to a commercial hybrid in grain yield potential and had high provitamin A content were identified, selected and recommended. Carotenoid levels throughout the plant are influenced by developmental stages and various biotic and abiotic stresses (Cuttriss et al. 2011). Therefore, understanding carotenoid biosynthesis in relation to environmental factors and the GEI are important in selection and identification of provitamin A rich varieties with high and stable carotenoid levels (Suwarno et al. 2015). Genetic analyses conducted with populations segregating in seed color (Chandler et al. 2013) and carotenoid profiles (Chander et al. 2008) have demonstrated that differences in composition and content of seed carotenoids are quantitatively inherited and highly influenced by GEI, which is in agreement with our study, where there was significant GEI for carotenoid content. Provitamin A carotenoids are much more sensitive to degradation due to environmental factors (Gregory 1996; Kimura et al. 2007; De Moura et al. 2015), which might be the potential factor behind the fluctuation in PVA content and rank changes in the present study. Several carotenoid cleavage dioxygenases (CCDs) and carotenoid cleavage genes, whose activity are mainly influenced by environments are known to catalyze degradation of carotenoids to apocarotenoids (Vallabhaneni et al. 2010).

The development and dissemination of high yielding and nutritionally enhanced maize varieties in Nigeria and other similar environments in SSA is crucial as the economic conditions of the subsistence

farmers in SSA does not allow them to buy hybrid seeds every year (Pixley and Bjarnason 2002). Identification of varieties combining high yield potential with better nutritional quality is an important step towards shaping future breeding because varieties can be used as variety per se and as sources of outstanding inbred parents for hybrid development (Pixley and Bjarnason 2002). Our study demonstrated the possibility of developing maize varieties that combine elevated levels of provitamin-A with high grain yields and broad adaptation across environments. In the present study, synthetics placed in one cluster had both high grain yields and high provitamin A content, indicating that they had gone through a rigorous selection process for the two traits. This is an important milestone towards achieving the goals of biofortification to increase the availability of nutritionally enhanced maize varieties associated with high yield potentials. Further testing of these provitamin A maize varieties and generating promising inbred lines for developing provitamin A rich maize hybrids with much higher levels of carotenoids and enhanced performance and wide adaptation can improve food and nutritional security of farmers in SSA. As breeding for nutritional quality in maize is at an early stage compared to breeding for other traits in white maize, more focus and funding on breeding, selection, development and deployment of better performing and stable provitamin A rich maize varieties are important to curb the wide spread VAD in SSA in a sustainable and affordable manner.

In conclusion, four PVA synthetics G4, G2, G16, and G12 were identified as stable and high yielding with significantly enhanced levels of provitamin A across test environments. These synthetics were developed from provitamin A rich elite inbred lines adapted to low-land tropical environments and are tolerant to most biotic and abiotic constraints in the target production zones. G4 was released in Nigeria, DR Congo, Ghana and Cameroon whereas G12 was released in Nigeria. Also, G2 and G16 can be recommended for release in Nigeria, Ghana and Gambia. Varieties identified in this study can also be registered in other African countries with similar agro-ecological zones, through the regional seed harmonization policy currently being implemented in most African countries.

**Acknowledgements** This research was conducted at the International Institute of Tropical Agriculture and financed by the HarvestPlus Challenge Program (Grant Number: HP-2018). The author expresses their appreciation to all staff that participated during planting, data recording, harvesting and management of the trial at all the locations and the staff who conducted the carotenoid analyses.

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