Received: 25 July 2023 | Accepted: 25 October 2023

DOI: 10.1002/plr2.20356

Journal of Plant Registrations

REGISTRATION

Germplasm

Registration of provitamin A-enriched tropical maize inbred lines

Abebe Menkir | **Silvestro Meseka** | **Melaku Gedil** | **Tayo Ojo** | **Wende Mengesha**

International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria

Correspondence
Abebe Menkir, IITA, Oyo Road, PMB5320, Ibadan, Nigeria.
Email: a.menkir@cgiar.org

Assigned to Associate Editor Arron Carter.

Registration by CSSA.

Funding information
HarvestPlus Challenge Program, Grant/Award Number: OPP1019962

Abstract

Vitamin A deficiency and its associated disorders are pervasive in sub-Saharan Africa (SSA) including many middle- and low-income countries across the world. Provitamin A-enriched maize (*Zea mays* L.) inbred lines with desirable agronomic and adaptive traits have been developed and used to generate and commercialize maize varieties with medium to high levels of provitamin A in a few countries to curb vitamin A deficiency. Nonetheless, these inbred lines have not been made widely available to the public and private sector breeders in many countries. The main purpose for releasing the 21 provitamin A-enriched tropical maize inbred lines (PI 705424–PI 705444, Reg. nos. GP-624–GP-644) is to supply maize breeders with elite source germplasm for increasing provitamin A and other carotenoids to much higher levels to offset losses during storage, natural degradation, and processing. These inbred lines were developed at the International Institute of Tropical Agriculture (IITA) from backcrosses of high β-carotene temperate lines as donors and elite tropical lines as recipients. These inbred lines were developed through repeated self-pollination with rigorous visual selection among and within lines for plant vigor, synchronous silk emergence and pollen shedding, low ear placement, and resistance to lodging and major tropical diseases, followed by selection for bright yellow to orange kernel color with semi flint to flint kernel texture after harvest. The released maize inbred lines will be diverse sources of favorable alleles to accelerate genetic gain in provitamin A and other beneficial carotenoid enrichment for human health.

1 | INTRODUCTION

Millions of people in rural and urban areas of sub-Saharan Africa (SSA) thrive on white maize (*Zea mays* L.)-based diets that provide inadequate quantity of micronutrients including Zn and vitamin A (Ekpa et al., 2019), exposing many to deficiency disorders (Bailey et al., 2015; Gibson & Anderson, 2009; Prasanna et al., 2020). Vitamin A deficiency alone increases susceptibility of children to infectious diseases and retards their growth, thereby diminishing their overall development and survival (Brown & Noelle, 2015; Sommer, 2008; West & Darnton-Hill, 2008; Wurtzel et al., 2012). Even though yellow maize kernels are packed with carotenoids (Wurtzel et al., 2012), the concentrations of provitamin A carotenoids in commonly grown tropical maize cultivars is too low to meet the daily requirements of consumers (Bouis & Saltzman, 2017). Increasing provitamin A levels in tropical yellow endosperm maize through

Abbreviations: DAPC, discriminant analyses of principal components; GCA, general combining ability; HPLC, High performance liquid chromatography; IITA, International Institute of Tropical Agriculture; KASP, Kompetitive allele-specific polymerase chain reaction; SCA, specific combining ability; SNP, single nucleotide polymorphism; SSA, sub-Saharan Africa.

conventional breeding has therefore attracted considerable attention of breeders who strive to curb vitamin A deficiencies in SSA and other medium- to low-income regions of the world (Bouis & Saltzman, 2017; Gupta et al., 2020).

The HarvestPlus Challenge Program made significant investments in conventional breeding programs to increase the concentrations of provitamin A in tropical maize (Bouis & Saltzman, 2017; Menkir et al., 2017; Pixley et al., 2013). These programs developed diverse tropical maize inbred lines primarily through introduction of diverse sources of favorable alleles from temperate maize and integration into adapted tropical germplasm to enhance accumulation of higher levels of β-carotene (Menkir et al., 2017). Provitamin A-enriched maize inbred lines that meet or exceed the breeding target of 15 μg g^{-1} have thus been developed and used for developing varieties and hybrids with medium to high concentrations of provitamin A (Menkir et al., 2017; Prasanna et al., 2020). These inbred lines also represent as invaluable genetic resources for further improvements in yield potential, provitamin A, and other carotenoids beneficial to human health.

The main purpose for releasing the diverse provitamin A-enriched maize inbred lines is therefore to supply breeders with elite tropical sources for increasing accumulation of both provitamin A and other beneficial carotenoids to much higher levels to offset losses during storage, natural degradation, and processing. This is the first set of provitamin A-enriched tropical maize inbred lines registered and released to public and private sector breeders. The 21 new provitamin A-enriched maize inbred lines (Reg. no. GP-624– GP-644 and PI 705424–PI 705444, see Table 1) were developed at the International Institute of Tropical Agriculture (IITA) from backcrosses of elite tropical lines as recipients and temperate lines as donors of high β-carotene content. The lines have also been selected for desirable agronomic attributes and resistance to the major tropical diseases. These lines will help stack provitamin A and other beneficial carotenoids with other essential nutrients, to accelerate the rate of genetic gain in multiple-nutrient enrichment, and mainstream nutritional quality into climate resilient maize.

Core Ideas

- Vitamin A deficiency and its associated disorders are pervasive in sub-Saharan Africa and other developing countries.
- Provitamin A-enriched inbred lines are important for developing and commercializing maize varieties to curb Vitamin A deficiencies.
- Supplying tropical elite inbred lines to breeders will facilitate increasing provitamin A and other beneficial carotenoids to human health.
- Releasing diverse elite inbred lines promotes access to favorable alleles for further enrichment of beneficial carotenoids.

2 | METHODS

2.1 | Parental backgrounds and inbred line development

IITA introduced yellow and orange endosperm temperate maize inbred lines (A619, CI7, DE3, NC298, NC323, NC354, and SC55) of non-stiff stalk, stiff stalk, and tropical origin (Liu et al., 2003) with high β-carotene content (Islam et al., 2004) from the University of Illinois as potential donor parents to develop maize inbred lines with high provitamin A content. The adapted parents selected as recipients of high β-carotene alleles were elite yellow and orange endosperm tropical maize inbred line with desirable agronomic traits and resistance to the major tropical diseases that had been extensively used as parents for developing inbred lines and hybrids in our breeding program. The elite tropical lines were used as females while the temperate donor lines were used as males for crossing to generate F_1 seeds. Each F_1 was planted adjacent to the corresponding elite female line for crossing with the F_1 to develop a backcross. Each backcross was then planted in a single 5-m-long row with a spacing of 0.75 m between rows and 0.25 m between plants. All identical plants were self-pollinated to produce enough F_2 bulk seeds. Each F_2 bulk seed was planted in 10 5 m rows with a spacing of 0.75 m between rows and 0.25 m between plants. In each backcross population, disease resistant plants were self-pollinated and harvested ears with desirable kernel color and texture were selected and threshed individually for planting ear-to-row in each subsequent inbreeding generation up to the S_4 stage. During each inbreeding generation, rigorous visual selection among and within lines was made for plant vigor, synchronous silk emergence and pollen shedding, low ear placement, resistance to lodging and tropical diseases (*Puccinia polysora* rust, *Bipolaris maydis* blight, and *Curvularia lunata* leaf spot, and *Maize streak virus* disease) followed by selection for well-filled ears with bright yellow to orange kernel color and semi flint to flint kernel texture. Screening lines for carotenoids was delayed to the S_5 inbreeding stage when the number of advanced lines was relatively small due to the high cost of analyzing carotenoid using high-performance liquid chromatography. The selected S_5 lines containing intermediate to high levels of provitamin A were evaluated in hybrid combinations using inbred line or single-cross testers to identify promising lines with superior testcross performance. Promising provitamin A-enriched

TABLE 1 Means of individual carotenoids for maize inbred lines separated into three groups based on canonical discriminant analysis of data recorded in a trial planted at Ibadan in Nigeria in 2021.

Lines	Group	PI no.	Reg. no.	Lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Pro-vitamin A	Total carotenoids
						μg/g				
TZi2142	1	PI 705442	GP-642	19.2	21.4	10.6	2.8	25.4	32.1	79.0
TZi2025	1	PI 705428	GP-628	13.2	38.7	8.4	2.0	15.5	20.7	77.5
TZi1314	1	PI 705427	GP-627	34.8	46.5	13.0	2.0	18.1	25.7	114.0
TZi1304	1	PI 705425	GP-625	10.8	41.0	12.4	1.4	11.8	18.7	77.5
TZi1715	2	PI 705430	GP-630	6.8	4.6	0.7	0.4	45.3	45.8	58.0
TZi1653	2	PI 705429	GP-629	3.0	1.4	0.9	0.6	51.0	51.7	56.5
TZi2071	2	PI 705440	GP-640	26.0	10.2	1.3	0.7	42.4	43.3	80.5
TZi2065	2	PI 705434	GP-634	16.0	8.6	1.1	0.4	35.5	36.2	61.5
TZi2066	2	PI 705435	GP-635	16.5	8.6	1.2	0.5	30.2	31.1	57.0
TZi2067	2	PI 705436	GP-636	16.3	10.6	1.9	0.8	42.0	43.4	72.0
TZi2038	3	PI 705439	GP-639	38.6	10.5	2.9	0.6	18.4	20.2	71.0
TZi2156	3	PI 705444	GP-644	24.6	18.2	1.4	0.4	27.8	28.7	72.5
TZi2012	3	PI 705437	GP-637	27.4	21.0	2.1	0.9	20.8	22.3	72.5
TZi2013	3	PI 705438	GP-638	23.5	16.4	1.5	0.9	26.1	27.3	68.5
TZi2130	3	PI 705441	GP-641	34.5	9.3	3.7	1.4	24.3	26.8	73.0
TZi2005	3	PI 705432	GP-632	19.6	16.2	3.1	1.5	24.8	27.0	65.0
TZi1305	4	PI 705426	GP-626	7.7	39.4	5.0	1.4	26.1	29.3	80.0
TZi2015	4	PI 705433	GP-633	9.4	15.0	6.0	1.5	17.0	20.7	49.0
TZi2143	4	PI 705443	GP-643	10.0	24.2	8.0	1.4	31.0	35.7	74.5
TZi2004	4	PI 705431	GP-631	8.8	13.0	2.2	0.6	21.5	22.9	46.0
TZi1302	4	PI 705424	GP-624	9.3	22.2	7.4	1.2	15.2	19.5	55.5
Mean				15.5	20.1	5.2	1.2	21.4	24.5	63.3
LSD (0.05)				10.2	6.8	3.7	0.7	7.4	7.8	16.9
Repeatability				0.79	0.95	0.87	0.85	0.94	0.93	0.86
CV (%)				33	17	35	31	17	16	13

lines with superior testcross performance were then used as parents to develop source populations of some of the maize inbred lines recommended for release.

2.2 | Pedigrees of the inbred lines

Inbred lines TZi1302, TZi1304, TZi1305, and TZi1314 were derived from a three-way cross of (9450/KI21)-1-5-3-2-2-B-32-B*6 as a recurrent parent, DE3 as donor parent of high β-carotene, and an inbred line (SW5(S)C6)-18-2-1-B derived from an introduced population from Thailand as a source of desirable agronomic attributes (Supplementary Table S1). Inbred line TZi2025 was extracted from a complex cross involving an inbred line derived from a backcross (9450/CM116/9450), an inbred line containing DE3 as a donor parent of high β-carotene and (9450/KI21)−1-5-3-2-2-B-30-B-B-8-B-1-B as a recipient plus an orange maize inbred line (SW5(S)C6)−18-2-1-B derived from an introduced population from Thailand as a source of desirable agronomic features. Two inbred lines (TZi1653 and TZi1715) were derived from a backcross involving DE3 as donor parent of high β-carotene and an orange tropical line (KU1409) as a recipient (Supplementary Table S1). Inbred lines TZi2004, TZi2005, TZi2015, TZi2065, TZi2066, and TZi2067 were derived from biparental crosses of provitamin A-enriched lines containing DE3 and SC55 as donors of high β-carotene and two orange tropical lines (KU1409 and KU1414-SR) as recipients in backcross populations. Likewise, four inbred lines (TZi2012, TZi2013, TZi2038 and TZi2071) were derived from biparental crosses of provitamin A-enriched lines containing DE3, A619, and NC298 as donors of high β-carotene and two orange tropical lines (KU1409 and KU1414-SR) as recurrent parents in backcross populations. The remaining four inbred lines (TZi2130, TZi2142, TZi2143, and TZi2156) were derived from crosses of inbred lines derived from source populations involving two tropical orange lines (KU1409 and KU1414-SR) and temperate lines (KVI3 and M162W), provitamin A-enriched lines containing DE3 as a donor of high β-carotene and KU1409 as a recipient in backcross populations, and an inbred lines ((SW5(S)C6)−18-2-1-B derived from an introduced population from Thailand as a source of desirable agronomic features (Supplementary Table S1).

2.3 | Inbred line performance evaluation and carotenoid analyses

Sixty-two inbred lines and two testers with 10.2 to 39.1 μg g^{-1} of provitamin A in samples harvested from single rows in 2019 were selected for further agronomic performance evaluation and carotenoid analyses in a trial planted at Ibadan (7°29′11.99″N, 3°54′2.88″E, altitude 190 m) in 2020. The 64 inbred lines were arranged in 16 × 4 α-lattice design and planted with two replications. Each inbred line was planted in a single 5-m-long row, with 0.75 m distance between rows and plant-to-plant spacing of 0.25 m within rows. In each plot, only one plant was maintained per hill after thinning to give a population density of 53,000 plants ha^{-1}. A compound fertilizer was applied at the rate of 60 kg Nitrogen (N) ha^{-1}, 60 kg Phosphorus ha^{-1}, and 60 kg Potassium ha^{-1} at planting and an additional 60 kg N ha^{-1} was supplied as Urea 4 weeks later. The trial field was sprayed with premextra and gramazone as herbicides at the rate of 5 L ha^{-1} to control weeds, which were followed by manual weeding to keep the trial weed-free. Days to anthesis and silking were recorded as the number of days from planting to when 50% of the plants shaded pollen and showed emerged silks, respectively. Plant and ear heights were measured after anthesis in cm from the base of the plant to the height of the first tassel branch and the node bearing upper ear, respectively. Plant aspect was rated based on overall phenotypic appeal using a scale of 1 to 5, where 1 = excellent phenotypic appeal and 5 = poor phenotypic appeal. Ear aspect was rated on a scale of 1 to 5, where 1 = clean, uniform, large and well-filled ears and 5 = rotten, variable, and small ears. The severity of southern corn leaf rust (caused by *Puccinia polysora*), *Curvularia* leaf spot (caused by *Curvularia lunata*) and *Maize streak virus* were scored on a scale of 1 to 5, where 1 = minimal leaf infection and 5 = severe leaf infection. The inbred lines were also evaluated in hybrid combinations with two testers at two locations for 2 years (Maazou et al., 2022). The 21 provitamin A-enriched maize inbred lines slated for release were selected from among the 64 inbred lines evaluated in the inbred trial in 2020.

2.4 | Carotenoid analyses

All plants in each plot of the 64 maize inbred lines were self-pollinated to generate kernel samples for carotenoid analysis. Self-pollinated ears in each row were harvested, dried with minimal exposure to direct sunlight, and shelled immediately to minimize loss of provitamin A carotenoids. One hundred kernels were drawn from each sample after shelling and submitted to the Crop Utilization Laboratory at IITA for carotenoid analysis using high-performance liquid chromatography. Carotenoid extraction from grain samples of 64 maize inbred lines and subsequent carotenoid analyses were described in detail by Maazou et al. (2021). Provitamin-A was calculated for each sample as the sum of β-carotene (all-trans plus 13-cis and 9-cis isomers) plus 50% each of α-carotene and β-cryptoxanthin.

2.5 | Genotyping of the inbred lines with allele specific markers

The protocols used to collect leaf samples from each inbred line for deoxyribonucleic acid (DNA) extraction have been

described by Maazou et al. (2021). The 64 provitamin A-enriched maize inbred lines were genotyped with polymerase chain reaction (PCR)-based functional markers of the three genes, namely *LCYE*, *crtRB1*, and *PSY1*, involved in maize endosperm carotenoid biosynthesis (Fu et al., 2013; Harjes et al., 2008; Yan et al., 2010). Furthermore, the inbred lines were genotyped with functional markers of the seven Kompetitive Allele-Specific PCR (KASP) single nucleotide polymorphism (SNP) markers associated with the favorable alleles of crtRB1 gene (Gowda et al., 2017) at the Bioscience Center of IITA in Nigeria.

2.6 | Statistical analyses

Analysis was computed for carotenoids and agronomic traits using the mixed model analysis in SAS to generate best linear unbiased estimates (BLUEs) for 64 main inbred lines included in the trial (Vargas et al., 2013). BLUEs of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene of 21 provitamin A-enriched maize inbred lines selected from this trial were subjected to principal component analysis (PCA) using the correlation matrix to separate the lines into groups with similar carotenoid composition and content using Wards' clustering method (SAS, 2012). The resulting inbred line groups together with the five principal component axes scores (PC1 to PC5) were then used for canonical discriminant analyses (DAPC) using the CANDISC procedure in SAS (SAS, 2012). This was performed to have a better visual assessment of the structures of carotenoid profiles between inbred line groups (Jambart et al., 2010). Descriptive statistics were computed for individual carotenoids of inbred line groups using the univariate procedure in SAS (SAS, 2012). General combining ability (GCA) and specific combining ability (SCA) effects of the inbred lines for β-carotene, provitamin A, and grain yield were estimated using Analysis of Genetic Design in AGD-R, V.5.0 (Rodríguez et al., 2018).

3 | CHARACTERISTICS

3.1 | Carotenoid profiles of the inbred lines

The 21 maize inbred lines targeted for registration had provitamin a content varying from 11.0 to 28.4 μg g^{-1} of provitamin A in samples harvested from single rows in 2019. These inbred lines also displayed a broad range of variation in concentrations of both provitamin A and non-provitamin A carotenoids (Table 1) in a replicated trial conducted in 2020, with all the lines having provitamin A content exceeding the breeding target of 15 μg g^{-1} set under the HarvestPlus Challenge Program. Rank correlation analyses between concentrations measured in 2019 and 2020 were significant

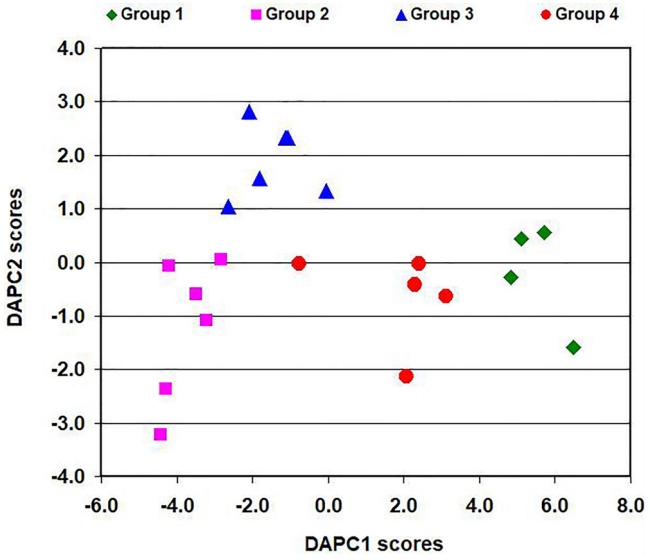

FIGURE 1 Scatter plot of discriminant analyses of principal components (DAPC) 1 and 2 scores of provitamin A-enriched inbred lines obtained from discriminant analysis of the five principal component axes scores.

and strong for both β-carotene ($r = 0.60$, $P = 0.0038$) and provitamin A ($r = 0.61$, $P = 0.0032$). Other studies also reported small year effects on accumulation of carotenoids in tropical and temperate maize inbred lines (Brunson & Quackenbush, 1962; Kurilich & Juvik, 1999; Menkir et al., 2008; Quackenbush et al., 1966). DAPC analysis using BLUEs from the replicated trial summarized the overall variation among the 21 inbred lines into DAPC1 and DAPC2, accounting for 85% and 12% of the total variation, respectively. DAPC1 scores were associated with significant ($P < 0.05$ to 0.0001) increases in concentrations of zeaxanthin ($r = 0.86$), β-cryptoxanthin ($r = 0.95$), α-carotene ($r = 0.84$), and total carotenoids ($r = 0.44$), but with significant ($P < 0.01$ to 0.0001) decreases in β-carotene ($r = 0.75$) and provitamin A content ($r = 0.62$). DAPC2 scores were only associated with significant ($P < 0.0001$) increases in lutein ($r = 0.81$), but again with significant ($P < 0.05$ to 0.01) decreases in β-carotene ($r = 0.52$), and provitamin A ($r = 0.61$).

The scatter plot of the DAPC1 and DAPC2 scores divided the 21 inbred lines into four groups (Figure 1). The four inbred lines belonging to Group 1 (Figure 1) had positive DAPC1 scores and a mixture of positive and negative DAPC2 scores (Figure 1). These inbred lines combined relatively high concentrations of lutein with the highest concentrations of zeaxanthin, β-cryptoxanthin, α-carotene, and total carotenoids, but had moderate levels of β-carotene, and provitamin A. In contrast, the six inbred lines in Group 2 had negative DAPC1 scores but had a mixture of positive and negative DAPC2 scores (Figure 1). These inbred lines combined the lowest concentrations of zeaxanthin, β-cryptoxanthin, and

TABLE 2 Observed alleles of the seven KASP SNP markers for the 24 provitamin A-enriched maize inbred lines.

Lines	Group	ZM0013	ZM0014	ZM0015	ZM0016	ZM0017	ZM0018	ZM0019
TZi2142	1	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi2025	1	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi1314	1	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi1304	1	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi1715	2	**G:G**	**C:C**	**A:A**	**G:G**	**T:T**	**C:C**	**C:C**
TZi1653	2	**G:G**	**C:C**	**A:A**	*G:A*	**T:T**	*C:T*	**C:C**
TZi2071	2	**G:G**	**C:C**	**A:A**	**G:G**	**T:T**	**C:C**	**C:C**
TZi2065	2	**G:G**	**C:C**	**A:A**	NA	**T:T**	*C:T*	**C:C**
TZi2066	2	**G:G**	**C:C**	**A:A**	**G:G**	*G:T*	**C:C**	T:T
TZi2067	2	**G:G**	**C:C**	**A:A**	**G:G**	*G:T*	**C:C**	T:T
TZi2038	3	*C:G*	*C:T*	*G:A*	*G:A*	*G:T*	*C:T*	*C:T*
TZi2156	3	**G:G**	**C:C**	**A:A**	*G:A*	*G:T*	*C:T*	*C:T*
TZi2012	3	C:C	T:T	*G:A*	*G:A*	*G:T*	T:T	T:T
TZi2013	3	C:C	NA	NA	*G:A*	NA	T:T	T:T
TZi2130	3	C:C	T:T	**A:A**	**G:G**	**T:T**	**C:C**	**C:C**
TZi2005	3	*C:G*	*C:T*	**A:A**	*G:A*	*G:T*	*C:T*	*C:T*
TZi1305	4	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi2015	4	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T
TZi2143	4	*C:G*	*C:T*	*G:A*	*G:A*	*G:T*	*C:T*	T:T
TZi2004	4	*C:G*	*C:T*	*G:A*	*G:A*	*G:T*	*C:T*	**C:C**
TZi1302	4	C:C	T:T	G:G	*G:A*	G:G	T:T	T:T

Note: The favorable and heterozygote alleles are provided in Supplementary Table S3. NA = no records of alleles available; bold and underline = presence of favorable alleles; italics = presence of heterozygous alleles; no formatting = absence of favorable alleles.

α-carotene with moderate levels of lutein and total carotenoids but had the highest β-carotene and provitamin A content (Supplementary Table S2). Group 3 consisted of six inbred lines with negative scores for DAPC1 and positive scores for DAPC2 (Figure 1). These lines combined the highest concentrations of lutein with moderate concentrations of zeaxanthin, low levels of β-cryptoxanthin and α-carotene, but had higher levels of β-carotene, provitamin A, and total carotenoids (Supplementary Table S2). In contrast, Group 4 involved five maize inbred lines with positive DAPC1 scores and negative DAPC2 scores (Figure 1). These lines combined the lowest average levels of lutein and total carotenoids with relatively high concentrations of zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and provitamin A (Supplementary Table S2).

3.2 | Allele specific markers of the three genes in inbred line-groups

All inbred lines belonging to the four groups contain the two PSY1 and crtRB1-InDel4 alleles (Table 2). Most of the inbred lines in Group 1 do not carry the favorable homozygote or heterozygote genotypes for six of the seven KASP SNP markers (Table 2). However, at least two inbred lines had favorable homozygote or heterozygote genotypes for all *LCYE* and *crtRB1*genes (Tables 2 and 3). The lines belonging to Group 2 carried favorable homozygote or heterozygote genotypes for almost all KASP SNP markers with most or all also having favorable homozygote or heterozygote genotypes for *crtRB1*-3′TE, *crtRB1*-5′TE and *LCYE-3′*indel markers (Tables 2 and 3). In Group 3, four inbred lines contained favorable homozygote or heterozygote genotypes for the seven KASP SNP markers as well as for *crtRB1-3′*TE, *crtRB1-5′*TE, and *LCYE-3′*indel markers (Tables 2 and 3). Only two lines belonging to Group 4 carried the favorable heterozygote genotypes for six of the seven KASP SNP markers as well as *crtRB1-3′TE*, *crtRB1-5′TE*, *LCYE-3′*indel, and *LCYE-5′TE* markers (Tables 2 and 3).

3.3 | Agronomic traits of the inbred lines

The inbred lines exhibited significant difference for each agronomic trait recorded at Ibadan, except for southern corn leaf blight and *Maize streak virus* diseases. The repeatability values for agronomic traits varied from 0.50 to 0.91. Trait means of the inbred lines included in the four groups defined

TABLE 3 Observed alleles of LCYE and crtRB1 and PSY1 markers in the 21 provitamin A-enriched maize inbred lines.

Line	Group	crtRB1-3′TE	crtRB1-InDel4	crtRB1-5′TE	LCYE-3′indel	LCYE-5′TE	LCYE-SNP (216)	PSY INDEL	PSY SNP7
TZi2142	1	1	0	2	1	2	1	1	1
TZi2025	1	1	0	1	1	1	0	1	1
TZi1314	1	0	0	0	1	1	1	1	1
TZi1304	1	0	0	0	1	1	0	1	1
TZi1715	**2**	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**1**
TZi1653	**2**	**2**	**0**	**2**	**1**	**0**	**0**	**1**	**1**
TZi2071	**2**	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**1**
TZi2065	**2**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**1**
TZi2066	**2**	**1**	**0**	**1**	**1**	**0**	**0**	**1**	**1**
TZi2067	**2**	**1**	**0**	**1**	**1**	**0**	**0**	**1**	**1**
TZi2038	3	0	0	0	1	0	0	1	1
TZi2156	3	0	0	0	0	0	0	1	1
TZi2012	3	2	0	2	1	0	0	1	1
TZi2013	3	2	0	2	1	0	1	1	1
TZi2130	3	1	0	1	1	0	0	1	1
TZi2005	3	1	0	2	0	0	0	1	1
TZi1305	4	0	0	0	1	2	0	1	1
TZi2015	4	1	0	2	0	0	0	1	1
TZi2143	4	0	0	0	0	1	1	1	1
TZi2004	4	2	0	2	0	0	0	1	1
TZi1302	4	0	0	0	1	1	0	1	1

Note: The favorable alleles are bolded and underlined; The best favorable alleles of the functional DNA markers are provided in Supplementary Table S4.

based on carotenoid composition and content are presented in Table 4. The lines in Group 1 showed considerable variation in plant height, ear placement, anthesis and silking days, and had desirable plant aspect, ear aspect, and disease scores. Although the testing location for the inbred trial was a low yielding environment due to low solar insolation, the four inbred lines in Group 1 produced mean grain yields exceeding 1000 kg ha^{-1}. The six inbred lines belonging to Group 2 also showed marked differences in plant height, ear placement, and flowering days with four lines exhibiting undesirable plant and ear aspect scores and producing grain yields of less than 600 kg ha^{-1} (Table 4). All the inbred lines included in Group 3 displayed considerable differences in plant height, ear placement, flowering days, desirable plant aspect, ear aspect, and leaf disease scores, and yielded more than 1000 kg ha^{-1}. The remaining five inbred lines included in Group 4 showed desirable plant aspect, ear aspect, and disease scores (Table 4). Three inbred lines in this group also produced grain yields of more than 1200 kg ha^{-1}. DAPC1 scores were significantly ($P < 0.05$) correlated with plant aspect ($r = -0.45$), ear aspect ($r = -0.45$), and grain yield ($r = 0.44$) but not with other traits. In contrast, DAPC2 scores were not significantly correlated with agronomic traits recorded in the inbred trial.

3.4 | Combining ability estimates for β-carotene, provitamin A and grain yield

GCA effects of the 21 provitamin A-enriched maize inbred lines estimated for β-carotene, provitamin A, and grain yield by Maazou et al. (2022) are presented in Supplementary Tables S5 and S6. Three inbred lines belonging to Group 1, five inbred lines included in Group 2 and four inbred lines included in Group 3 had positive GCA effects for both β-carotene and provitamin A content (Supplementary Table S5). Similarly, a set of inbred lines crossed to either Tester-1 or Tester-2 had positive SCA effects for both β-carotene and provitamin A content. Amongst the 11 inbred lines with positive GCA effects for grain yield (Supplementary Table S6), four also had positive GCA effects for both β-carotene and provitamin A content (Supplementary Table S5). Four inbred lines in crosses with Tester-1 and five inbred lines in crosses with Tester 2 had positive SCA effects for grain yield, β-carotene, and provitamin A content. Inbred lines with positive GCA effects for β-carotene, provitamin A, and grain yield can thus be used as desirable sources of favorable alleles for increasing productivity and nutrient enrichment in tropical maize. The positive SCA effects for PVA accumulation and grain yield recorded in testcrosses with Tester-1 or Tester-2

TABLE 4 Means of agronomic traits of maize inbred lines recorded in a trial planted at Ibadan in Nigeria in 2021.

Lines	Group	Anthesis	Silking	Plant height	Ear height	Plant aspect	Ear aspect	*Curvularia* leaf spot	Leaf rust	Grain yield
		days		cm		1–5[a]		1–5[b]		kg ha^{-1}
TZi2142	1	54	55	206	83	2.4	1.6	2.0	2.3	2912
TZi2025	1	59	60	136	57	2.3	2.5	3.0	1.4	1043
TZi1314	1	57	58	160	65	2.5	2.5	1.5	2.1	1575
TZi1304	1	60	58	132	60	2.0	2.5	1.5	1.7	1125
TZi1715	2	62	66	165	57	3.3	3.5	1.7	2.7	199
TZi1653	2	62	62	134	70	2.5	3.3	1.5	2.1	403
TZi2071	2	58	58	176	72	2.3	2.2	1.6	2.0	1665
TZi2065	2	59	57	191	88	2.3	2.2	1.7	1.9	1303
TZi2066	2	61	60	153	87	2.7	3.0	1.5	2.2	437
TZi2067	2	61	60	160	88	3.5	3.0	1.5	1.7	525
TZi2038	3	58	58	184	85	2.7	2.5	1.8	2.0	1024
TZi2156	3	57	61	184	86	2.4	3.2	1.5	1.9	1159
TZi2012	3	57	56	168	83	3.0	2.5	1.5	2.5	1348
TZi2013	3	57	59	173	71	2.0	2.2	1.5	1.9	1500
TZi2130	3	61	60	166	84	2.2	2.8	2.4	1.5	1391
TZi2005	3	62	61	186	97	2.7	2.3	2.0	1.8	1076
TZi1305	4	60	61	167	82	2.0	2.8	1.5	1.6	1290
TZi2015	4	59	59	134	61	2.3	2.2	1.5	1.4	1858
TZi2143	4	54	54	174	79	2.0	2.0	1.6	2.0	1823
TZi2004	4	62	61	175	92	3.1	2.2	2.4	1.5	915
TZi1302	4	62	60	131	61	2.2	2.7	1.7	1.7	446
Mean		60	60	167	79	2.5	2.6	1.8	1.9	1103
LSD		2	3	25	20	0.9	0.9	0.7	0.6	905
Heritability		0.88	0.91	0.84	0.72	0.57	0.50	0.68	0.77	0.67
CV (%)		2	3	8	14	17	17	20	17	41

[a]Plant aspect (1–5): a Plant aspect (1–5): where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Ear aspect (1–5): 1 = clean, uniform, large, and well-filled ears and 9 = rotten, variable, and small ears.

[b]*Curvularia* leaf spot (1–5): 1 = slight leaf infection and 5 = severe leaf infection. Leaf rust (1–5): 1 = slight leaf infection and 5 = severe leaf infection.

highlights the possibility of developing hybrids using specific pairs of inbred parents to enhance agronomic performance and provitamin A accumulation.

4 | CONCLUSIONS

The 21 provitamin A-enriched maize inbred lines developed through conventional breeding exhibited four distinct profiles for carotenoid composition and content. As these inbred lines involved parents containing diverse tropical and temperate germplasm in their backgrounds, they are likely to harbor novel combinations of complimentary alleles that promote accumulations of provitamin A and other beneficial carotenoids. These lines can be utilized as sources of diverse favorable alleles to accelerate the rate of genetic gain in enriching maize with provitamin A and other carotenoids beneficial to human health. Although increasing accumulation of β-carotene in maize is presently accomplished mainly through selection of allele specific markers of three key genes involved in maize endosperm carotenoid biosynthesis, *PSY1*, *crtRB1*, and *LCYE*, the diverse released provitamin A-enriched maize inbred lines can be used as desirable genetic resources to unravel regulatory mechanisms controlled by many genes with large effects on the synthesis and flow of substrates in the carotenoid biosynthetic pathway. This can facilitate the development of novel functional markers for use to accelerate selection for much higher levels of provitamin A and other carotenoids with health benefits. The lines may also be used directly as parents to develop provitamin A-biofortified maize hybrids and synthetic varieties for commercialization in tropical areas affected by vitamin A deficiency.

5 | AVAILABILITY

IITA will multiply and maintain breeder seeds of these inbred lines. Small quantities of seed of these lines can be obtained from the leader of the maize breeding unit at IITA, PMB 5320, Ibadan, Nigeria for breeding and research use. Seeds of these lines will also be maintained in the National Plant Germplasm System, where they will be available immediately upon publication for research purposes, including development and commercialization of new varieties. Recipients of seeds are required to make appropriate recognition of the original seed source when these germplasm lines contribute to research or the development of new lines, hybrids, or synthetics.

AUTHOR CONTRIBUTIONS

Abebe Menkir: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; rervision; validation; visualization; writing—original draft; writing—review and editing. **Silvestro Meseka**: Data curation; investigation; methodology; supervision. **Melaku Gedil**: Data curation; formal analysis; supervision; validation. **Wende Mengesha**: Data curation; investigation; methodology; supervision. **Tayo Ojo**: Data curation; methodology; supervision.

ACKNOWLEDGMENTS

This research was conducted at IITA with funding from the HarvestPlus Challenge Program. The authors express their appreciation with thanks to Torbert Rocheford for supplying the high β-carotene donor temperate inbred lines used in our breeding program and his constant support and sharing of information. The authors are also grateful to all staffs that participated during planting, data recording, harvesting, and management of trials conducted at various locations as well as staffs that ran carotenoid analyses in the IITA crop utilization laboratory.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Abebe Menkir https://orcid.org/0000-0002-5907-9177
Silvestro Meseka https://orcid.org/0000-0003-1004-2450

REFERENCES

Bailey, R. L., West, K. P., & Black, R. E., Jr. (2015). The epidemiology of global micronutrient deficiencies. *Annual Nutrition Metabolism*, *66*, 22–33. https://doi.org/10.1159/000371618

Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: A review of evidence from HarvestPlus, through 2016. *Global Food Security*, *12*, 49–58. https://doi.org/10.1016/j.gfs.2017.01.009

Brown, C. C., & Noelle, R. J. (2015). Seeing through the dark: New insights into the immune regulatory functions of vitamin A. *European Journal Immunology*, *45*, 1287–1295. https://doi.org/10.1002/eji.201344398

Brunson, A. M., & Quackenbush, F. W. (1962). Breeding corn with high provitamin A in the grain. *Crop Science*, *2*, 344–347. https://doi.org/10.2135/cropsci1962.0011183X000200040020x

Ekpa, O., Palacios-Rojas, N., Kruseman, G., Fogliano, V., & Linnemann, A. (2019). Sub-Saharan African maize-based foods: Processing practices, challenges and opportunities. *Food Reviews International*, *35*, 609–639. https://doi.org/10.1080/87559129.2019.1588290

Fu, Z., Chai, Y., Zhou, Y., Yang, X., Warburton, M. L., Xu, S., Cai, Y., Zhang, D., Li, J., & Yan, J. (2013). Natural variation in the sequence of PSY1 and frequency of favorable polymorphisms among tropical and temperate maize germplasm. *Theoretical and Applied Genetics*, *126*(4), 923–935. https://doi.org/10.1007/s00122-012-2026-0

Gibson, R. S., & Anderson, V. P. (2009). A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc. *Food and Nutrition Bulletin*, *30*, S108–S143. https://doi.org/10.1177/15648/265090301s107

Gowda, M., Worku, M., Nair, S. K., Palacios-Rojas, N., & Prasanna, B. M. (2017). *Quality assurance/quality control (QA/QC) in maize breeding and seed production: Theory and practice*. CIMMYT.

Gupta, S., Brazier, A. K., & Lowe, N. M. (2020). Zinc deficiency in low- and middle- income countries: Prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics*, *33*, 624–643. https://doi.org/10.1111/jhn.12791

Harjes, C. E., Rocheford, T. R., Bai, L., Brutnell, T. P., Kandianis, C. B., Sowinski, S. G., Stapleton, A. E., Vallabhaneni, R., Williams, M., Wurtzel, E. T., Yan, J., & Buckler, E. S. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science*, *319*(5861), 330–333. https://doi.org/10.1126/science.1150255

Islam, S. N., Paul, C., Buckler, E. S., & Rochford, T. (2004). Genetic and diversity studies on carotenoids (provitamin A) in maize. In *Proceedings of the Fortieth Annual Illinois Corn breeders' School* (pp. 77–107). University of Illinois at Urban-Champaign.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94.

Kolawole, A. O., Menkir, A., Blay, E., Ofori, K., & Kling, J. (2018). Genetic advance in grain yield and other traits in two tropical maize composites developed via reciprocal recurrent selection. *Crop Science*, *58*, 2360–2369. https://doi.org/10.2135/cropsci2018.02.0099

Kurilich, A. C., & Juvik, J. A. (1999). Quantification of carotenoid and tocopherol antioxidants in *Zea mays*. *Journal of Agricultural and Food Chemistry*, *47*, 1948–1955.

Li, J., Dellapenna, D., Brutnell, T., Buckler, E. S., & Rocheford, T. (2010). Rare genetic variation at *Zea mays* crtRB1 increases β-carotene in maize grain. *Nature Genetics*, *42*(4), 322–327. https://doi.org/10.1038/ng.551

Liu, K., Goodman, M., Muse, S. J., Smith, J. S., Buckler, E., & Doebley, J. (2003). Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics*, *165*, 2117–2128.

Maazou, A. R. S., Adetimirin, V., Gedil, M., Meseka, S. K., Mengesha Abera, W., & Menkir, A. (2022). Suitability of testers to characterize

provitamin a content and agronomic performance of tropical maize inbred lines. *Frontiers in Genetics*, *13*(955420), 1–11.

Maazou, A. R. S., Gedil, M., Adetimirin, V., Meseka, S. K., Mengesha, W., Babalola, D., Offornedo, Q. N., & Menkir, A. (2021). Comparative assessment of effectiveness of alternative genotyping assays for characterizing carotenoids accumulation in tropical maize inbred lines. *Agronomy*, *2*, 1–17.

Menkir, A., Liu, W., White, W. S., Maziya-Dixon, B., & Rocheford, T. (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chemistry*, *109*, 521–529.

Menkir, A., Maziya-Dixon, B., Mengesha, W., Rocheford, T., & Alamu, E. O. (2017). Accruing genetic gain in pro-vitamin A enrichment from harnessing diverse maize germplasm. *Euphytica*, *213*, 105. https://doi.org/10.1007/s10681-017-1890-8

Menkir, A., Rocheford, T., Maziya-Dixon, B., & Tanumihardjo, S. (2015). Exploiting natural variation in exotic germplasm for increasing provitamin-A carotenoids in tropical maize. *Euphytica*, *205*(1), 203–217.

Pixley, K., Palacios-Rojas, N., Babu, R., Mutale, R., Surles, R. L., & Simpungwe, E. (2013). Biofortification of maize with provitamin A carotenoids. In S. A. Tanumihardjo (Ed.), *Carotenoids and human health* (pp. 271–292). Springer Science.

Prasanna, B. M., Palacios-Rojas, N., Hossain, F., Muthusamy, V., Menkir, A., Dhliwayo, T., Ndhlela, T., San Vicente, F., Nair, S. K., Vivek, B. S., Zhang, X., Olsen, M., & Fan, X. (2020). Molecular breeding for nutritionally enriched maize: Status and prospects. *Frontiers in Genetics*, *10*, 1–16.

Quackenbush, F. W., Firch, J. G., Brunson, A. M., & House, L. R. (1966). Carotenoid, oil, and tocopherol content of corn inbreds. *Cereal Chemistry*, *40*, 251–259.

Rodríguez, F. J., Alvarado, G., Pacheco, A., Crossa, J., & Burgueño, J. (2018). *AGD-R (Analysis of genetic designs in R)* (Version 5.0) [Computer software]. International Maize and Wheat Improvement Center (CIMMYT).

SAS Institute. (2012). *SAS System for Windows* (Release 9.4) [Computer software]. SAS Institute.

Sommer, A. (2008). Vitamin A deficiency and clinical disease: An historical overview. *Journal of Nutrition*, *138*, 1835–1839.

Vargas, M., Combs, E., Alvarado, G., Atlin, G., Mathews, K., & Crossa, J. (2013). META: A Suite of SAS programs to analyze multi-environment breeding trials. *Agronomy Journal*, *105*, 11–19.

West, K. P., Jr., & Darnton-Hill, I., (2008). Vitamin A deficiency. In R. D. Semba, M. W. Bloem, & P. Piot (Eds.), *Nutrition and health in developing countries* (2nd ed., pp. 377–433). Humana Press. https://doi.org/10.1007/978-1-59745-464-3_13

Wurtzel, E. T., Cuttriss, A., & Vallabhaneni, R. (2012). Maize provitamin A carotenoids, current resources, and future metabolic engineering challenges. *Frontiers in Plant Science*, *3*, 1–12.

Yan, J., Kandianis, C. B., Harjes, C. E., Bai, L., Kim, E. H., Yang, X., Skinner, D. J., Fu, Z., Mitchell, S., Li, Q., Fernandez, M. G. S., Zaharieva, M., Babu, R., Fu, Y., Palacios, N., Li, J., Dellapenna, D., Brutnell, T., Edward, S., … Rocheford, T. (2010). Rare genetic variation at Zea mays crtRB1 increases B-carotene in maize grain. *Plant and Microbial Biology*, *42*(4), 322–327.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Menkir, A., Meseka, S., Gedil, M., Ojo, T., & Mengesha, W. (2024). Registration of provitamin A-enriched tropical maize inbred lines. *Journal of Plant Registrations*, 1–10. https://doi.org/10.1002/plr2.20356