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Accruing genetic gain in pro-vitamin A enrichment from harnessing diverse maize germplasm

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Abstract Maize has been targeted as one of the major crops for provitamin enrichment and delivery because it is an inexpensive and easily available source of food for millions of people in sub-Saharan Africa. Although tropical-adapted yellow maize contains provitamin-A carotenoids that can be converted into vitamin A in the human body, they represent less than 25% of the total carotenoids in most widely grown and consumed maize cultivars in Africa. Novel genes conditioning high concentration of β-carotene and other carotenoids were then continually introduced from the temperate zone and tropics to boost provitamin A in tropical-adapted maize. Several promising inbred lines developed from backcrosses involving diverse exotic donor lines displayed provitamin A concentrations that match or surpass the current breeding target of 15 μg g^{-1}. Some of these lines attained high provitamin A content by accumulating mainly high β-carotene while others contained high provitamin A by promoting accumulation of high levels of both carotenes and xanthophylls. Several inbred lines with intermediate to high levels of provitamin A have already been used to develop hybrids and synthetics without compromising grain yield and other adaptive traits that are required to profitably cultivate maize by farmers in West and Central Africa.

Keywords Genetic gain · Exotic germlasm · Provitamin A enrichment · Carotenes · Xanthophylls

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Introduction

Millions of people in sub-Saharan Africa thrive primarily on crop-based diets that are deficient in nutrients including vitamin A (World Health Organization 2009). As a consequence, an estimated 42.4% of children younger than 5 years of age and 15.3% of pregnant women in Africa (SSA) are deficient in vitamin A (Aguayo and Baker 2005; WHO 2009). The average prevalence of vitamin A deficiency in children varies from 22.4 to 45.8% across regions in Africa with little improvement in the last twenty years (United Nation Standing Committee on Nutrition 2011). Vitamin A deficiency retards physical growth and cognitive development, reduces mobilization of iron, depresses immune function thereby increasing susceptibility to infectious diseases and diminishing the possibility of survival from serious illness (Sommer 2008; West and Darnton-Hill 2008; Wurtzel et al. 2012; Brown and Noelle, 2015). Deficiency in vitamin A during pregnancy has also been associated with increased maternal mortality (Christian et al. 2000; Black et al. 2008). Conversely, adequate consumption of carotenoid-rich food reduces the risk of developing cancer (Agarwal and Rao 2000) and cardiovascular diseases (McDermott 2000), boosts immune responses (Watzl et al. 2003), prevents night blindness (Granado et al. 2003; Olmedilla et al. 2003; Watzl et al. 2003), neutralizes free radicals and help in maintenance of optimal health (Jerome-Morais et al. 2011; Sen and Chakraborty 2011). Increased dietary intake of lutein and zeaxanthin promotes increased iron absorption (Garcia-Casal 2006), improves vision, provides protection against damage of the macula by the blue light, and lowers the risk of age-related macular degeneration, cataracts, and other degenerative diseases (Mares et al. 2006; Abdel-Aal et al. 2013). The nutritional value and health-promoting properties of carotenoids have prompted flurries of research activities to enhance their concentrations in staple food crops (Nestel et al. 2006; Hefferon 2015). Developing carotenoid-enriched crop varieties can thus contribute not only to alleviating vitamin A deficiencies but also to providing more nutrient rich meals with good antioxidant balance and other health-related benefits.

Maize has been targeted as one of the major staple crops for provitamin A enrichment and delivery under the HarvestPlus Challenge Program because it is an inexpensive and easily available source of food for millions of people in the rural areas including the urban poor who have limited access to animal products and a variety of fruits and vegetables that are required for a healthy diet (Pfeiffer and McClafferty 2007; Bouis and Welch 2010). Maize supplies the largest amount of calories, proteins and other nutrients to millions of people in Africa and its consumption varies from 52 to 328 g^{-1} $person^{-1}$ day across countries (Ranum et al. 2014). Grains of white maize in Eastern and Southern Africa and white and yellow maize in West and Central Africa are converted into well-accepted traditional foods, including gruels, porridges, pastes and infant weaning foods. Also, green cobs of both yellow and white maize are consumed boiled or roasted to mitigate the effects of seasonal food shortages. In addition to its widespread use by the most vulnerable populations, maize is adapted to diverse production environments and has the potential to generate and continually provide nutritious and productive cultivars that are attractive to farmers.

Yellow maize naturally accumulates provitamin A carotenids, including α-carotene, β-carotene and β-cryptoxanthin that are substrates for the synthesis of lutein and zeaxanthin (Cazzonelli and Pogson 2010). The provitamin-A carotenoids are considered to be suitable dietary sources of vitamin A because their conversion by oxidative cleavage in the human body release retinol that is stored in the liver (Combs 2012), which make the crop an ideal target for improving their concentrations in the grain (Harjes et al. 2008). Gannon et al. (2014) demonstrated that the total body reserve of vitamin A in children that consumed provitamin A enriched maize was similar to that obtained through supplementation and fortification. However, the kernels of maize cultivars commonly grown by farmers around the world contain 2 μg g^{-1} of provitamin A (Harjes et al. 2008), which is insufficient to meeting the recommended daily allowance in a diet (Institute of Medicine 2012). As a consequence, considerable efforts have been made to increase the concentration of provitamin-A carotenoids in maize through conventional breeding to reduce the incidence of vitamin A deficiency (Pfeiffer and McClafferty 2007; Harjes et al. 2008). This review summarizes the recent advances in mining novel alleles from diverse exotic germplasm to enhance carotenoid accumulation in tropical maize and the associated improvements in the development, testing, release, and deployment of provitamin A enriched maize varieties and hybrids.

Genetic potential for provitamin A enrichment in maize

Extensive studies were conducted to characterize concentrations of carotenoids in diverse tropical and temperate maize germplasm and found considerable natural genetic variations in accumulation of provitamin A and other carotenoids (Blessin et al. 1963; Kurilich and Juvik 1999; Egesel et al. 2003; Berardo et al. 2004; Islam et al. 2004; Chander et al. 2008; Vallabhaneni and Wurtzel 2009; Burt et al. 2011; Muthusamy et al. 2015). To assess the extent of variation in carotenoid composition and content in adapted germplasm available for breeding, we also evaluated 421 tropical-adapted yellow to orange endosperm maize inbred lines in several independent trials (Menkir et al. 2008). The results of carotenoid analysis of grain samples harvested from these trials found a broad range of genetic variation in lutein, zeaxanthin, σ-carotene, β-cryptoxanthin, and β-carotene content among these maize inbred lines. The best 10 inbred lines identified from these trails had β-carotene content varying from 3.0 to 4.7 μg g^{-1}. The results of our study and others also show that inbred lines consistently maintain their specific carotenoid profiles in different replications and across locations and seasons (Brunson and Quackenbush 1962; Quackenbush et al. 1966; Kurilich and Juvik 1999; Egesel et al. 2003; Menkir et al. 2008; Owens et al. 2014; Suwarno et al. 2015). These findings highlight the possibility of selecting suitable parental lines with diverse carotenoid profiles to improve concentrations of carotenoids in tropical maize. Nonetheless, the levels of σ-carotene, β-cryptoxanthin, and β-carotene in tropical-adapted maize inbred lines were low relative to lutein and zeaxanthin, emphasizing the need for exploitation of novel sources of genetic variation to increase their concentrations to new levels. Carotenoid composition and content in maize are regulated by a complex genetic system and are largely controlled by additive gene effects with strong genotypic component in their inheritance (Egesel et al. 2003; Islam et al. 2004; Wong et al. 2004; Senete et al. 2011; Kandianis et al. 2013; Owens et al. 2014; Suwarno et al. 2015). These critical genetic properties coupled with the capacity of genotypes to accumulate relatively stable amounts of individual carotenoids across diverse growing conditions (Brunson and Quackenbush 1962; Quackenbush et al. 1966; Kurilich and Juvik 1999; Egesel et al. 2003; Menkir et al. 2008; Menkir et al. 2014; Suwarno et al. 2015) as well as the positive correlations among individual carotenoids (Kurilich and Juvik 1999; Menkir et al. 2008; Owens et al. 2014) and between grain yield and provitamin A content (Suwarno et al. 2015; Menkir et al. 2014) demonstrate that simultaneous increases in accumulation of provitamin-A and other carotenoids may be successfully made without compromising yield potential and other desirable agronomic and adaptive traits (Pfeiffer and McClafferty 2007; Bouis and Welch 2010; Menkir et al. 2014).

Recent advances to increase carotenoid accumulation in tropical maize

Initially, breeders at IITA emphasized on increasing the concentrations of primarily β-carotene in adapted maize because of its highest provitamin A activity, which was found to be low (3.0–4.7 μg g^{-1}) in the best tropical-adapted maize inbred lines selected from trials (Menkir et al. 2008). This prompted us to introduce well characterized maize inbred lines (A619, CI7, DE3, NC323, NC354, and SC55) of temperate non-Stiff Stalk and Stiff Stalk as well as tropical origin (Liu et al. 2003) with β-carotene content ranging from 5.2 to 13.6 μg g^{-1} from the University of Illinois (Islam et al. 2004) to boost provitamin A content in tropical-adapted maize. Several backcross populations involving tropical-adapted maize inbred lines with intermediate levels of provitamin A as recipients and twelve introduced lines as donor parents were developed to recover novel beneficial alleles of β-carotene without reducing the frequency of existing favorable alleles for desirable agronomic and adaptive traits already achieved in tropical maize germplasm (Dudley 1982; Goodman 1999). The breeding scheme used to develop inbred lines from these backcrosses involved the selection of ears harvested from desirable plants and showing bright yellow to orange kernel color and semi-flint to flint kernel texture at the S_1–S_3 inbreeding stages followed by selection based on carotenoid content measured by HPLC at the S_4 and subsequent inbreeding stages (Menkir et al. 2015). As visual score for the intensity of kernel color in maize has moderately high heritability, selection for dark orange kernel color may likely result in high levels of total carotenoids and slightly more provitamin A content (Chandler et al. 2013). Consequently, the use of such simple agronomic traits and kernel properties to select individual plants in early generations has not only been cheaper and more rapid for screening breeding materials than selection based on carotenoids measured using HPLC but also allowed the identification of fairly homozygous inbred lines with high nutrient content and desirable agronomic features for developing hybrids and synthetics.

The HPLC analyses found 23 backcross-derived lines that accumulated 23–313% more β-carotene and 32–190% more provitamin-A than their recurrent parents (Menkir et al. 2015). The best four lines among these had provitamin A concentrations that meet the breeding target level of 15 μg g^{-1}. Several backcross-derived lines carried combinations of favorable alleles of two genes, including lycopene epsilon cyclase (*LCYE*) and β-carotene hydroxylase 1 (*CRTRB1*) encoding key enzymes in the carotenoid biosynthetic pathway (Azmach et al. 2013). The best backcross-derived lines have then been regularly crossed in pairs or with elite tropical yellow and orange endosperm maize inbred lines to generate bi-parental crosses for developing inbred lines with superior agronomic performance and much higher levels of pro-vitamin A. They were also used as recurrent parents to develop new backcross populations containing orange endosperm donor lines of mainly β-cryptoxanthin, zeaxanthin, and lutein derived from six broad-based populations introduced from Thailand to expand the genetic base and enhance the rate of genetic gain in concentrations of both carotenes and xanthophylls of tropical-adapted maize inbred lines. Amongst the 29 S_5 lines with desirable agronomic traits derived from these introductions, nine inbred lines with 7.1–13.8 μg g^{-1} provitamin A, 4.4–8.2 μg g^{-1} β-cryptoxanthin, 23.2–46.4 μg g^{-1} zeaxanthin and 9.0–23.8 μg g^{-1} lutein were selected as non-recurrent parents of the backcross populations.

Carotenoid composition and content of the S_4–S_7 lines with desirable agronomic traits and kernel properties developed from these bi-parental crosses and backcross populations in the last 4 years (2013–2016), which were grown under the same growing conditions each year, were measured with HPLC. As shown in Table 1, the inbred lines harvested and sun dried to mimic the common farmers' practice displayed a broad range of genetic variation in accumulating all individual carotenoids in their grains. Mean concentrations of most carotenoids averaged across lines increased from 2013 to 2016. In general, the observed variations in concentrations of individual carotenoids among lines were broader in 2014, 2015 and 2016 than in 2013 (Table 1). The proportion of lines with provitamin A content surpassing 6.0 μg g^{-1} increased from 2013 to 2016, whereas those with less than 6.0 μg g^{-1} provitamin A decreased from 2013 to 2016 (Fig. 1). Amongst these, four lines in 2013, 12 lines each in 2014 and 2015 and 44 lines in 2016 had provitamin A content varying from 15 to 23 μg g^{-1} (data not shown). We calculated the fraction of provitamin-A carotenoids (β-cryptoxanthin, α-carotene, and β-carotene) relative to the total carotenoids to examine the carotenoid composition of the inbred lines evaluated each year (Fig. 2). Lines having 50–100% provitamin-A carotenoids in their grains represented 24, 12, 10 and 16% of all the inbred lines assayed in 2013, 2014, 2015 and 2016, respectively. In contrast, the fraction of lines having 50–100% non-provitamin A carotenoids (lutein and zeaxanthin) in their grains surpassed 75% each year (Fig. 2). Out of the best 72 inbred lines containing 15–23 μg g^{-1} of provitamin A in the last 4 years, 37 also had more than 50% non-provitamin A carotenoids in their grains. In contrast, the remaining 35 best inbred lines attained high provitamin A content by optimizing accumulations of mainly β-carotene while keeping either lutein or zeaxanthin or a combination of both at low levels. The number of lines that accumulated 9–23 μg g^{-1} of provitamin A and 40–110 μg g^{-1} of total carotenoids was 2 for 2013, 9 for 2014, 40 for 2015 and 160 for 2016. Out of these, two lines each in 2014 and 2015 and 28 lines in 2016 had up to or greater than 15 μg g^{-1} provitamin A and 42–110 μg g^{-1} of total carotenoids in their grains.

The results of carotenoid measurements with HPLC in the last four years demonstrated that the breeding strategy used was effective in assembling a large number of favorable alleles of known and unknown genes from diverse sources with profound effect on substrate allocation across the two branches of the biosynthetic pathway, leading to the observed broad range of variation in carotenoid composition and content of the maize inbred lines. Suwarno et al. (2015) identified several genomic regions in diverse tropical, subtropical and temperate germplasm that regulate critical steps in both upstream and downstream of the carotenoid biosynthetic pathway. The fact that several inbred lines had provitamin A concentrations that meet or surpass the current breeding target of 15 μg g^{-1} set under the HarvestPlus Challenge Program (Bouis and Welch 2010) while at the same time accumulating higher levels of lutein or zeaxanthin or a combination of both suggested that conventional breeding was effective in accumulating favorable alleles from diverse sources that affected several steps of the carotenoid biosynthetic pathway to increase the concentrations of both carotenes and xanthophylls simultaneously. Some inbred lines that combined high levels of provitamin A with low concentration of total carotenoids in their kernels may

Table 1 Means and ranges of carotenoids measured in maize inbred lines derived from bi-parental crosses and backcrosses in 2013, 2014, 2015, and 2016

Carotenoids	Number of lines	Mean	Range	Number of lines	Mean	Range
	2013			2014		
Lutin (μg/g)	458	3.2 ± 0.12	0.2–21.9	477	6.3 ± 0.21	0.5–33.5
Zeaxanthin (μg/g)	458	7.3 ± 0.23	0.3–28.7	477	10.0 ± 0.29	0.2–56.6
β-cryptoxanthin (μg/g)	458	3.1 ± 0.08	0.1–8.6	477	3.3 ± 0.09	0.2–10.5
α-carotene (μg/g)	458	0.2 ± 0.01	0.0–1.5	477	0.2 ± 0.02	0.0–2.7
β-carotene (μg/g)	458	3.4 ± 0.11	0.1–19.2	477	4.0 ± 0.14	0.6–21.9
Pro-vitamin A (μg/g)	458	5.1 ± 0.12	0.2–20.5	477	5.8 ± 0.14	0.8–22.3
	2015			2016		
Lutin (μg/g)	231	10.0 ± 0.55	0.0–52.9	496	8.8 ± 0.25	0.0–31.2
Zeaxanthin (μg/g)	231	14.1 ± 0.62	0.0–42.8	496	17.0 ± 0.45	0.1–46.5
β-cryptoxanthin (μg/g)	231	3.6 ± 0.15	0.2–9.6	496	4.0 ± 0.09	0.2–14.4
α-carotene (μg/g)	231	0.8 ± 0.04	0.0–3.0	496	1.2 ± 0.03	0.0–6.1
β-carotene (μg/g)	231	6.1 ± 0.24	1.0–20.0	496	7.0 ± 0.15	0.1–21.5
Pro-vitamin A (μg/g)	231	8.1 ± 0.23	1.6–20.6	496	9.6 ± 0.16	0.9–22.6

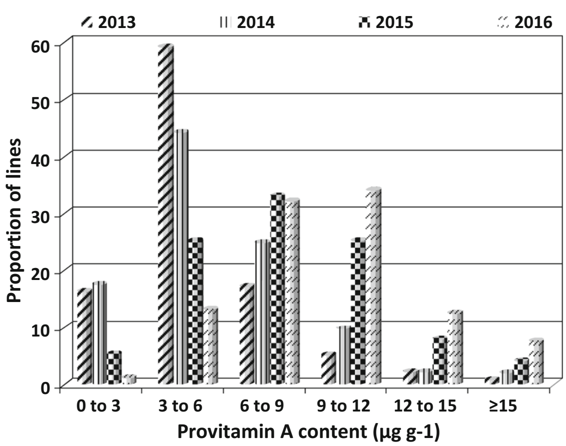

Fig. 1 Frequency distribution of provitamin A content of inbred lines developed in the last 4 years measured using HPLC

carry favorable alleles of LcyE and CrtRB1 to promote accumulation of mainly higher levels of β-carotene at the expense of the synthesis of other carotenoids. Yan et al. (2010) reported that the crtRB1 alleles associated with high β-carotene accumulation concomitantly decreased total carotenoid content in the kernel, resulting in low concentrations of other carotenoids. In contrast, other inbred lines with high concentrations of provitamin A also accumulated greater amounts of total carotenoids possibly because they carried allelic combinations that triggered increased phytone synthase activity, leading to enhanced metabolic flux in lycopene biosynthesis and its subsequent conversion to high concentrations of both carotenes and xanthophylls. It is, therefore, reasonable to conclude that broadening and diversifying the genetic base of tropical-adapted yellow to orange endosperm maize inbred lines through continual infusion of new germplasm has a high probability of achieving or exceeding the current breeding target provitamin A content set under the HarvestPlus Challenge Program with additional genetic gains in concentrations of lutein and zeaxanthin to maximize the health benefits that can be derived from maize.

The best maize inbred lines with intermediate to high levels of provitamin A and desirable agronomic traits and resistance to the major lowland tropical diseases have been regularly evaluated in hybrid combinations with increasing number of testers from an opposite heterotic group and locations through successive stages to develop provitamin A enriched maize hybrids. At each stage of testing, selection of hybrids has been made not only for high provitamin A content but also for high yield potential, good standability, resistance to diseases and other desirable traits that are important to farmers. Summaries of results of the best hybrids selected in five trials each evaluated in multiple locations in 2013, 2014, and 2015 are thus presented in Table 2 to highlight the

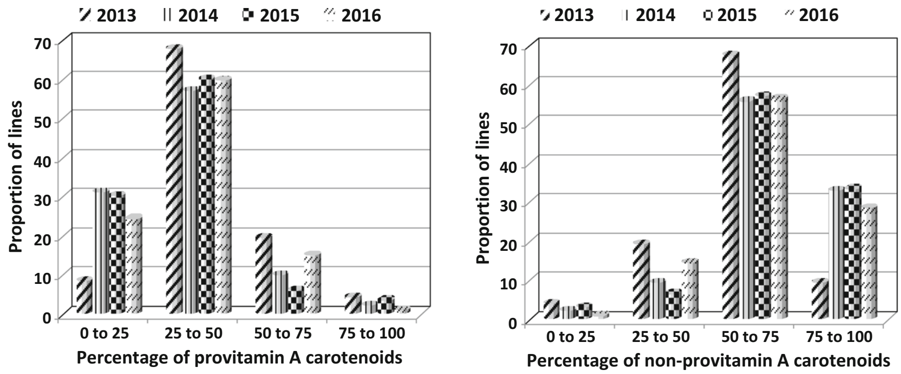

Fig. 2 Frequency distribution of proportion of provitamin A and non-provitamin A carotenoids of inbred lines developed in the last 4 years measured using HPLC

Table 2 Provitamin A content measured from samples collected at two locations and grain yields recorded at four locations for the best hybrids selected from five trials evaluated in 2013, 2014 and 2015

Hybrid trials	Provitamin A (μg/g)					Grain yield (ton/ha)			
	Number of best lines	Best hybrid range	Commercial hybrid	LSD (0.05)	Hybrid x site interaction	Best hybrid range	Commercial hybrid	LSD (0.05)	Hybrid x site interaction
2013									
A1312	18	6.0–8.8	4.3	1.2	ns	5.9–7.4	5.8	1.0	ns
A1319	10	6.0–9.0	4.4	0.8	ns	5.8–7.1	6.3	1.0	**
A1320	12	6.2–7.7	4.3	1.2	ns	5.6–6.4	5.2	0.8	**
A1321	3	6.9–7.5	3.5	1.1	ns	5.8–7.2	6.2	1.0	*
A1322	5	5.7–7.1	3.6	0.7	ns	6.0–6.3	6.1	0.8	**
2014									
A1404	14	6.0–8.6	3.5	1.1	ns	4.4–6.0	4.4	1.0	*
A1412	15	7.2–9.1	5.5	1.3	ns	5.4–6.1	5.0	1.1	*
A1413	19	9.5–13.5	5.8	2.4	ns	5.3–7.2	5.3	1.1	ns
A1420	26	6.0–9.8	4.6	1.1	ns	4.1–6.7	4.1	0.9	ns
A1432	9	6.1–9.2	4.3	0.7	ns	4.0–6.2	4.8	1.0	ns
2015									
A1507	7	7.2–9.9	5.2	1.0	ns	4.6–5.4	4.6	0.9	ns
A1508	19	7.7–10.7	5.0	1.9	ns	4.8–6.0	4.8	1.0	ns
A1516	20	6.1–9.0	5.0	2.3	ns	4.9–5.7	5.4	1.3	ns
A1517	14	7.1–10.9	6.3	1.8	ns	4.7–6.3	4.9	1.0	ns
A1529	22	7.8–12.8	6.6	2.0	ns	4.2–5.8	4.2	0.8	ns

*, **, ***, **** Means squares significantly different from zero at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ levels, respectively

progress that has been made so far. These hybrids displayed marked differences in pro-vitamin A content varying from 5.7 to 13.5 μg g^{-1}. These hybrids accumulated significantly higher levels of pro-vitamin A and produced comparable or significantly higher grain yields than an orange endosperm commercial maize hybrid marketed in Nigeria. Other studies also showed that selection of provitamin A enriched maize inbred lines as parents was effective in generating high yielding hybrids with enhanced provitamin A content

(Egesel et al. 2003; Senete et al. 2011; Pixley et al. 2013; Menkir et al. 2014; Muthusamy et al. 2014; Suwarno et al. 2015). These results demonstrate that the advances made in the development of provitamin A enriched maize inbred lines can be encapsulated not only in hybrids but also in synthetic varieties to increase the delivery of nutritious maize to farming communities through the informal seed system, which supplies considerable quantity of seeds in rural areas with limited market access (DeVries and Toenniessen 2001).

Channeling provitamin A enriched varieties and hybrids to partners

When promising pro-vitamin A enriched synthetics and hybrids with consistent performance across the different stages of evaluation are identified, they are included in regional trials and an announcement is circulated to the public and private sector partners in several countries to facilitate requests for sets of these trials. The sets of regional trials dispatched to partners for extensive evaluation in 14 African countries varied from 49 in 2010 to 64 in 2016 for the provitamin A enriched synthetics and from 56 in 2010 to 74 in 2016 for provitamin A enriched hybrids. Results of carotenoid analyses and agronomic performance of the best five synthetics and hybrids selected from the two regional trials in 2012–2015 are presented in Table 3 to demonstrate the progress that has been achieved thus far. In the regional trial composed of synthetics (OPV), the OPVs x location interaction mean squares were not significant for provitamin A content but were significant for grain yield in three of the 4 years. Among the best five synthetics selected from these trials, some had significantly higher levels of pro-vitamin A in comparison to an improved orange endosperm open-pollinated maize variety used as a benchmark. The best synthetics were also found to be as high yielding as or higher yielding than the improved orange endosperm maize variety (Table 3). In the second regional trial, the hybrids x location interaction mean squares were significant for both provitamin A content and grain yield in at least three of the four years of evaluation (Table 3). The best five hybrids selected from these trials each year had significantly higher concentrations of provitamin A and produced comparable or significantly higher grain yields than the benchmark orange endosperm commercial maize hybrid (Table 3). These trials have been constant sources of hybrids and synthetics with high pro-vitamin A content, high yield potential and other desirable agronomic and adaptive traits for further testing in national performance and farmer participatory on-farm trials. The involvement of farmers as strategic partners in conducting site specific on-farm trials where farmers' preferred maize varieties and hybrids have been compared with promising provitamin A enriched varieties and hybrids has permitted the selection of suitable candidates for variety registration, release and commercialization. Participatory on-farm trials have thus served as platforms to showcase the potential of these products to the members of the variety release committees as well as to farmers and seed producers. As shown in Table 4, 10 synthetics and nine hybrids having 40–70% of the current breeding target provitamin A content set under the HavestPlus Challenge Program (Bouis and Welch 2010) and producing comparable or better grain yields in comparison to the farmers preferred maize cultivars were identified from the two regional trials and released across four countries.

Promoting commercialization of provitamin A enriched varieties and hybrids

To stimulate uptake and commercialization of the released provitamin A enriched maize synthetic varieties and hybrids, the necessary partnerships with the national agricultural research systems (NARS), private seed companies and community-based seed producers have been established. A total of 1145 kg seed of provitamin A enriched hybrids were supplied to the NARS and the private seed companies to support on-farm demonstrations to create awareness among farmers, policy makers, and other partners. In addition, 2118 kg seed of parental lines of the released hybrids were supplied to seed companies since 2012 to stimulate initiation of production, promotion, and marketing of the provitamin A enriched hybrids to farmers. At the same time, 24027 kg seeds of the released provitamin A enriched synthetic varieties was provided to community-based and other seed producers as well as startup local seed companies to help accelerate multiplication and commercialization of these products to farming communities in rural areas with limited market access. The breeder seeds provided to the public and private sector partners

Table 3 Provitamin A contenet and grain yields of the best five synthetic varieties (OPV) and hybrids selected from each of the two regional trials evaluated at four to six locations in 2013, 2014 and 2015

Regional trial	Number of testing sites		Provitamin A (µg/g)			OPV x site interaction	Grain yield (ton/ha)			Hybrid x site interaction
	Provitamin A	Grain yield	Best 5 entries range	Common improved orange OPV	LSD (0.5)		Best 5 entries range	Commercial orange hybrid	LSD (0.5)	
2012										
OPV Trial	4	28	6.8–9.3	6.0	0.9	ns	2.4–2.8	2.4	0.2	*
Hybrid Trial	5	41	8.9–11.3	4.6	0.7	****	3.6–4.7	4.0	0.4	***
2013										
OPV Trial	4	13	6.0–6.4	5.5	0.7	ns	3.4–4.1	3.9	0.5	***
Hybrid Trial	5	14	7.0–8.6	5.7	1.1	*	4.8–5.7	4.6	0.5	***
2014										
OPV Trial	4	13	6.8–9.1	5.6	1.2	ns	3.1–3.4	3.2	0.6	**
Hybrid Trial	5	19	6.3–7.2	4.0	1.1	ns	3.6–5.0	3.5	0.5	**
2015										
OPV Trial	6	4	7.2–7.7	6.3	1.1	ns	3.8–4.7	4.4	0.8	ns
Hybrid Trial	6	19	10.1–11.1	5.8	1.1	*	4.1–4.7	3.8	0.5	***

contributed to the production of 1,586,320 kg certified seeds of provitamin A enriched maize synthetics and hybrids in 2015 and 2016, which was sold to farmers for planting an estimated 79316 hectares in three countries (Table 5). Considering the current favorable market demand for orange maize in Nigeria and other countries, the prospect for production and marketing of more seeds of provitamin A enriched maize varieties and hybrids is good.

Conclusions and future prospects

In conclusion, continual infusion of diverse temperate and topical exotic maize germplasm into elite tropical inbred lines followed by repeated visual selection for desirable kernel properties and agronomic features during the early stages of inbreeding was effective in generating maize inbred lines with diverse carotenoid composition and content. Several promising inbred lines had provitamain A concentrations that match or surpass the present breeding target of 15 µg g^{-1} under the HarvestPlus Challenge Program. Some of the promising lines had high provitamin A content by accumulating mainly higher levels of β-carotene at the expense of the synthesis of other carotenoids while others contained high provitamin A by accumulating high levels of both carotenes and xanthophylls. These lines will be useful not only for the development of hybrids and synthetics with high concentration of carotens and xanthopylls but also for elucidating the mechanisms that regulate the synthesis of more substrates upstream in the carotenoid biosynthetic pathway and the resulting increased flux downstream

Table 4 Summary of the number of provitamin A enriched maize varieties and hybrids selected from regional trials and released in four countries from 2012 to 2016

Country	Year of release	Number	Provitamin A (μg g^{-1})	Yield advantage over farmers' preferred variety (%)
Synthetics				
Democratic Republic of Congo	2016	3	6–10	14–35
Ghana	2012 and 2015	2	6–10	29–42
Mali	2012 and 2015	3	6–10	0–60
Nigeria	2012	2	6–8	47–48
Hybrids				
Ghana	2015	2	7–10	40–45
Mali	2016	2	6–9	60–80
Nigeria	2015 and 2016	5	6–11	10–29

Table 5 The quantity of certified seeds of provitamin A enriched varieties and hybrids produced and marketed in the various countries in 2015 and 2016

Country	Quantity of certified seed produced and marketed (kg)		Total	Estimated number of hectares plated with certified seeds		Total
	2015	2016		2015	2016	
Ghana	0	10,000	10,000	0	500	500
Mali	35,000	162,420	197,420	1750	8121	9871
Nigeria	281,300	1,097,600	1,378,900	14,065	54,880	68,945
Total	316,300	1,270,020	1,586,320	15,815	63,501	79,316

in the pathway. Several inbred lines with intermediate to high levels of provitamin A have already been used to develop hybrids and synthetics without compromising grain yield and other adaptive traits that are required to profitably cultivate maize by farmers in West and Central Africa.

Even though significant progress has been made in boosting provitamin A to higher levels in tropical maize, studies have demonstrated that the loss in provitamin A during storage, milling, and preparation of diverse traditional foods may reach up to 70% (Muzhingi et al. 2008; Mugode et al. 2014; Pillay et al. 2014; De Moura et al. 2015). Also, the extent of loss in provitamin A carotenoids exhibits a broad range of variation among maize genotypes in these studies. These findings underscore the need to further increase the concentration of provitamin A to much higher levels to offset the losses resulting from the diversity of storage practices and methods of preparations of traditional foods. Furthermore, evaluation of promising provitamin A enriched maize varieties at an advanced stage of testing for nutrient retention during storage, processing and cooking may permit selection of suitable maize varieties to maximize the potential health benefits derived from nutritious maize cultivars.

Wurtzel et al. (2012) considers the need for assembling favorable alleles of numerous genes that maximize the synthesis of total carotenoids, optimizing accumulation and retention of β-carotene, minimizing degradation of beneficial endosperm carotenoids and controlling carotenoid sequestration as significant challenges to breed cereals for high provitamin A content. Also, the genetic regulation of carotenoid profile and content in maize grain are coordinated through several loci distributed across the three metabolic pathways with most of them exhibiting additive and pleiotrophic effects (Kandianis et al.

2013). Considering the complex nature of carotenoid biosynthesis and the involvement of numerous genes, a breeding strategy that permits stacking favorable alleles of these genes may lead to simultaneous improvement of multiple carotenoids to maximize the potential health benefits obtained from maize. Perhaps the formation of broad-based synthetics from tropical-adapted maize inbred lines displaying different carotenoid composition and content that also contain diverse exotic germplasm in their genome and subjecting them to recurrent selection can be a viable strategy to increase accumulation of favorable alleles of the different genes in the populations. Additional infusion of orange flint germplasm from Argentina and other countries with known potential to contribute high levels of zeaxanthin and lutein (Burt et al. 2011) into these broad-based synthetics can further diversity and expand their genetic base and increase the probability of generating lines combining carotenes and xanthophylls to much higher levels. During each selection cycle, visual assessment for desirable kernel properties and agronomic features coupled with screening for favorable allele-specific markers of LcyE, CrtRB1 and other genes (Harjes et al. 2008; Yan et al. 2010; Wurtzel et al. 2012; Owens et al. 2014) may allow efficient selection of progenies with combination of alleles of these genes for recombination to further improve the populations. This approach may facilitate not only the increase in accumulating favorable alleles with large effects on concentrations of carotenes and xanthophylls in maize kernels but also in the placement of the alleles in diverse favorable genetic backgrounds that optimize carotenoid synthesis. This enhances the probability of producing an array of plants with combinations of desirable alleles that exhibit diverse carotenoid composition and content in their kernels. The advanced selection cycles of these synthetics may then serve as sources of inbred lines with unique allelic combinations that encourage the synthesis of high levels of carotenoids across the two branches of the biosynthetic pathway.

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