Provitamin A Maize Biofortification in Sub-Saharan Africa

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Abstract

Maize (*Zea mays* L.) has a great potential of combating food insecurity in sub-Saharan Africa because of its wide production and consumption in this region. However, its role in curbing nutrition insecurity is limited due to lack of key micronutrients such as vitamin A. This negates its capacity to be a suitable solution for both food and nutrition insecurities that have plagued many African countries. This has contributed to high prevalence of "hidden hunger" related conditions in the form of vitamin A deficiency triggered illnesses among others. About fifteen years ago, HarvestPlus and partners introduced provitamin A maize biofortification in Africa to fight Vitamin A deficiency. Provitamin A biofortification is a technology of increasing the provitamin A density in maize kernels through conventional breeding and/or biotechnology. The suitability of any given breeding strategy depends on the genetics and heritability of the provitamin A accumulation as a trait. This review (1) summarises the impacts of vitamin A deficiency in sub-Saharan Africa, pointing out the disparities that exist between rural and urban vitamin A deficiency prevalence in some of the African countries, (2) describes the genetics and molecular science behind maize provitamin A biofortification, (3) narrates the progress made so far in terms of maize cultivars development since the inception of maize biofortification in sub-Saharan Africa and (4) lastly, challenges of maize biofortification and possible solutions are highlighted

KeyWords *biofortification, micronutrients, provitamin A, vitamin A deficiency, maize*

Introduction

Food and nutrition insecurities are the primary challenges in most developing countries especially in sub-Saharan Africa (SSA). Increasing maize productivity has been identified as one of the strategies to curb food insecurity in SSA. This is because maize; (1) is widely produced and consumed in this region, (2) has higher yield potential and (3) is more responsive to management than other cereal crops grown in SSA like sorghum and millet (Badu-Apraku et al., 2011). Maize accounts for 30-60% of total caloric intake in SSA where it is mostly produced by rural households under subsistence farming (Cairns et al., 2013). Therefore, maize is a model crop for productivity and nutritional improvements as efforts to curb food and nutrition insecurities in SSA.

However, white maize which is popularly consumed in many African countries has serious micronutrients deficiencies, which hinders its suitability to be a solution for both food and nutrition insecurities. White maize has a starchy endosperm which provides huge quantities of energy to the human diet but has low micronutrients content (Nuss & Tanumihardjo, 2010). This has been implicated in the prevalence of 'hidden hunger' in maize consuming SSA nations (Muthayya et al., 2013; FAO et al., 2017). White maize by virtue of its white colour has very low and undetectable carotenoids which makes it a poor source of vitamin A (Wurtzel et al., 2012). This, in combination with generally low-provitamin A diets has resulted in high cases of Vitamin A deficiency (VAD) related illnesses in most maize consuming nations in SSA. In contrast to white maize, yellow maize has wider genetic variation in carotenoid content in the endosperm, a character that breeders can exploit through biofortification to develop maize cultivars with high provitamin A content (proVA) (Menkir et al., 2008). Over a decade ago, proVA maize was introduced in SSA through the efforts of HarvestPlus and partners (Bouis et al., 2011). Since then several proVA biofortified maize hybrids and open pollinated varieties (OPVs) have been developed and released in or for several African countries. Both conventional and molecular breeding strategies can be employed in maize biofortification. However, a good understanding of the genetics and biochemical science of proVA synthesis is important for the designing and selection of appropriate breeding strategies. Maize proVA biofortification in SSA faces its portion of challenges in the form of consumer scepticism, technical challenges and the negative stigma of the coloured maize. Therefore, this review seeks to discuss the science and technology of maize proVA biofortification

and its impacts on agriculture-based livelihoods with SSA as a case study. It further gives an estimation of progress in terms of provitamin A cultivars developed and released so far.

Materials and Methods

VAD status in sub-Saharan Africa

Vitamin A is an essential micronutrient that cannot be synthesised by the body and therefore must be provided through the diet. Yellow maize and other plants contain vitamin A precursors (provitamin A) in the form of carotenoids (Wurtzel et al., 2012). Vitamin A is responsible for the normal function of the visual and immunity systems among other key functions in the human body (WHO, 2009). Living on a diet that is chronically deficient of vitamin A is the underlying cause of VAD, a scenario common with most rural communities in SSA who are living on predominantly maize-based diets. VAD can cause xerophthalmia (progressive blindness), increased infant morbidity and mortality, and depressed immunological responses. VAD diagnosis can either be done through clinical assessment of eyes for signs of xerophthalmia and/or biochemical determination of serum or plasma retinol concentration. However, biochemical assessment of retinol concentration is the latest and most commonly used method. VAD is diagnosed when the liver vitamin A content measured in terms of liver retinol is below 0.7 µmol/l (WHO, 2009).

VAD is estimated to affect 190 million preschool children and 19 million pregnant and lactating women worldwide, mainly in Africa and Asia (WHO, 2009; Stevens et al., 2015). Figure 1 graphically illustrates the levels of VAD prevalence among pre-school children (≤ 5years old) and maize consumption in some of the SSA countries (http://www.who.int/nutrition/topics/vad/en//). WHO (2009) declared VAD as one of the threats to human survival and well-being which needs urgent and consistent interventions.

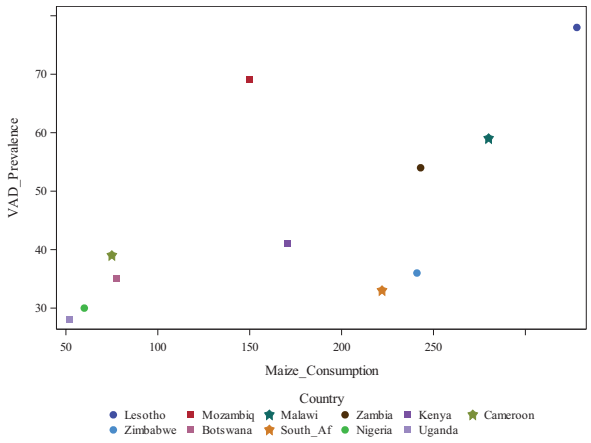

Figure 1 - A dispersion graph showing the level of maize consumption vs VAD prevalence in some of the major maize consuming sub-Saharan Countries. VAD was measured as a percentage of children (≤ 5 years of age) with liver retinol below 0.7µmol/l. Source of data: (http://www.who.int/nutrition/topics/vad/en//; Muthayya et al., 2013; Ranum et al., 2014).

VAD Interventions

Several strategies to curb VAD in vulnerable communities have been put forward. These include dietary diversification, vitamin A supplementation and food fortification (Bouis et al., 2011; Babu et al., 2013). Despite these interventions VAD remains a threat to human survival in SSA especially in rural areas. This could be due to the facts that diet diversification is beyond the financial reach of most poor rural farmers and is greatly affected by crop seasonality. On the other hand, poor infrastructure in developing countries has limited widespread coverage of direct vitamin supplementation programmes with rural areas mostly affected (Nuss & Tanumihardjo, 2010). Mandatory exogenous vitamin A food fortification including maize flour that has been adopted by most countries has a limitation of side-lining rural farmers who do not buy processed fortified maize flour and other maize based products but rather process from their own grown maize. Furthermore, poor enforcement of the mandatory food fortification policy in some of the developing countries is resulting in some of the manufacturers failing to consistently adhering to the policy. A combination of these factors has led to higher VAD prevalence among rural populations in some of the SSA countries than their urban counterparts as illustrated in Figure 2.

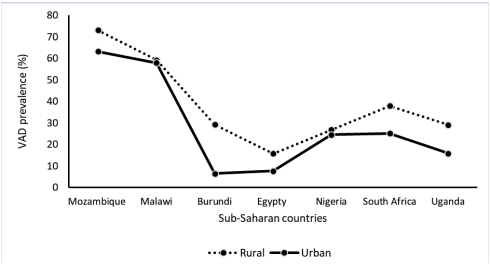

Figure 2 - Figure 2: Disparities between rural and urban VAD prevalence in some of the maize consuming countries of the sub-Saharan Africa. Data source: http://www.who.int/nutrition/topics/vad/en// .

The advent of endogenous maize fortification, which is also known as biofortification can be a complimentary solution to the above-mentioned strategies in curbing VAD challenges in rural Africa. Biofortification which is the genetic enhancement of vitamin A through crop breeding and biotechnology has been reported to be a more sustainable, cost effective and practical solution for VAD in chronically malnourished rural populations that have limited access to diverse diets and oth-

er micronutrient interventions (Nuss & Tanumihardjo, 2010; Bouis & Saltzman, 2017).

Developing agronomically competitive maize cultivars that are biofortified with high concentrations of vitamin A precursors has been regarded as a key approach towards alleviating VAD in maize consuming regions of SSA and Asia (Bouis & Saltzman, 2017). ProVA refers to the carotenoids that can be converted into physiologically activated vitamin A in the human body and these are α-carotene, β-carotene, and β-cryptoxanthin. HarvestPlus and its partners through the global challenge programme are credited for championing biofortification of maize and other crops for enhanced vitamin A and other micronutrients content in SSA (Andersson et al., 2017).

The carotenoid biosynthetic pathway

Molecular and biochemical aspects of the carotenoid biosynthetic pathway have been studied comprehensively in many crops including maize (Harjes et al., 2008; Yan et al., 2010; Wurtzel et al., 2012). Carotenoids are categorised into proVA and non-proVA carotenoids. ProVA carotenoids which are α-carotene, β-carotene and β-cryptoxanthine serve as dietary sources of vitamin A. On the other hand, non-proVA carotenoids which are lutein and zeaxanthin have been reported to act as antioxidants in the human body (Chander et al., 2008). Lutein and zeaxanthin are the primary products of the biosynthetic pathway so are normally found in greater quantities in the maize endosperm than their proVA counterparts which are the intermediates of the biosynthetic pathway (Nuss & Tanumihardjo, 2010). Among the three vitamin A precursors, β-carotene has higher proVA activity because of its unique double ring molecular structure (Harrison, 2015). Figure 3 shows the outline of the key steps of the biosynthetic pathway and the key genes that are responsible for the catalysis of relevant biochemical stages.

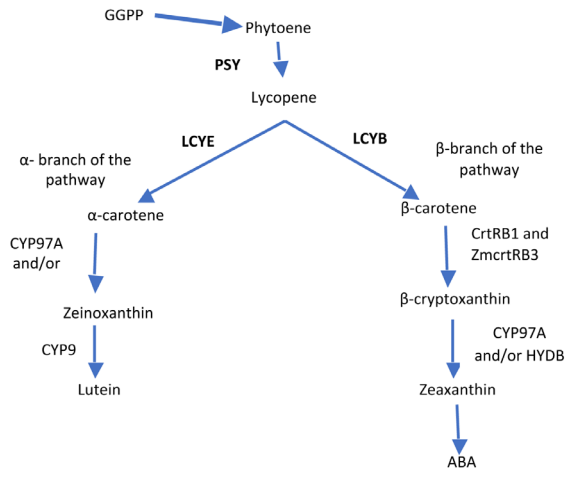

Figure 3 - Carotenoid biosynthetic pathway and the major genes. GGPP: genanylgeranyl diphosphate, PSY: phytoene synthase, LCYB: β- cyclase, LCYE: E cyclase, ABA: abscisic acid. Adopted from Babu et al. (2012).

Genetics of Provitamin A

Understanding the heritability and gene action controlling the trait of interest is crucial in choosing a breeding strategy and designing a breeding programme. ProVA content is influenced by additive gene action and has been reported to have moderate to high heritability (Babu et al., 2013; Suwarno et al., 2014). In maize, proVA accumulation has been reported to be affected by three key enzymes in the carotenoid biosynthetic pathway, namely phytoene synthase (PSY1), lycopene epsilon cyclase (LCYE) and β-carotene hydroxylase 1 (CRTRB1) (Messias et al., 2014). PSY1 gene encodes for phytoene synthase, an enzyme that is responsible for the shift from white to yellow grain colour by catalysing the conversion of genanylgeranyl (GGPP) to phytoene (Babu et al., 2013). LCYE encodes for the enzyme lycopene epsilon cyclase which catalyses the conversion of lycopene into α-carotene or β-carotene (Harjes et al., 2008). CrtRB1 encodes for β-carotene hydroxylase enzyme that converts β-carotene into β-cryptoxanthin (Yan et al., 2010). It is through the manipulation of these genes using different breeding strategies that breeders enhance the proVA content of maize.

Breeding objectives and pre-breeding activities

The primary objective of proVA biofortification is to develop cultivars with high proVA content of approximately 50% of the estimated average requirements for Vitamin A. The initial maize proVA target is set at 15 $\mu g g^{-1}$ (Bouis et al., 2011; Andersson et al., 2017). However, the cultivars should also be robust in other traits to increase adoption by farmers (Pillay et al., 2011). Suwarno et al. (2014) reported no significant correlation between grain yield and provA concentration, an indication that both traits can be improved simultaneously without affecting each other. It should be noted that, like any other breeding programme, the success of a biofortification programme relies on the availability of enough genetic variation in proVA concentration among the available germplasm (Pixley et al., 2013; Suwarno et al., 2014). Thus, genetic diversity and population structure analysis for proVA concentration among the available germplasm should be undertaken as part of pre-breeding activities. This can be achieved using molecular markers in combination with different proVA screening methods (Kimura et al., 2007; Azmach et al., 2013; Frascaroli et al., 2013).

Yellow maize has wide genetic variation and allelic diversity for carotenoid content, a characteristic that allows the application of both conventional and molecular breeding strategies. The availability of enough genetic variation allows breeders to exploit additive gene effects, transgressive segregation, and heterosis to improve proVA density in maize kernels. Conversely, when there is insufficient genetic variation among the available germplasm, transgenic approaches can be employed (Andersson et al., 2014). Given the above described genetics of proVA, biofortification objectives and pre-breeding activities the following breeding strategies under both conventional and molecular breeding can be deployed in maize biofortification.

Conventional Breeding Strategies

Backcross breeding has been a key strategy in developing proVA biofortified maize varieties during the early stages of biofortification in tropical and sub-tropical countries including SSA (Menkir et al., 2008; Azmach et al., 2013; Pixley et al., 2013). Temperate based germplasm has been found to be superior over the tropical and sub-tropical germplasm in proVA content especially in β-carotene content (Babu et al., 2013). Therefore, the base germplasm of proVA maize breeding in SSA was developed from backcrossing tropically adapted elite white maize with temperate yellow proVA donor lines (Pixley et al., 2013).

Recurrent selection is another breeding strategy that has been employed in maize biofortification. Under this approach, the breeding pipeline can be started by intermating landraces, popular or introduced varieties with superior proVA concentrations, followed by selecting the best progenies and repeating the process until high average stable proVA concentrations are achieved. Dhliwayo et al. (2014) improved the proVA content of open pollinated varieties (OPVs) from 25 to 67% through recurrent selection.

Hybridization has been an important strategy in breeding cross pollinated crops like maize, mainly to exploit the associated heterosis and because of increasing adoption of hybrids in maize producing countries including SSA (Derera et al., 2007). In maize biofortification, hybridization involves the development of inbred lines with stable, robust, high-yielding and high proVA concentration, followed by crossing the selected inbred lines into single, three-way and double cross improved hybrids. The value of an inbred line in a hybrid combination depends on its ability to combine with other lines to produce high performing hybrids. Therefore, the chosen inbred parents should first undergo a rigorous screening and combining ability analysis for proVA concentration and other key agronomic traits (Menkir et al., 2015). To date many proVA hybrids have been released for SSA production.

Molecular Breeding

The identification of key genes that govern the key steps of the carotenoid pathway and their allelic polymorphism enabled the incorporation of marker assisted selection (MAS) technology into biofortification (Andersson et al., 2014). Fu et al. (2013) identified two polymorphisms in the gene PSY1, explaining 7 to 8% of the variation in total carotenoids. Favourable alleles of PSY1 increase proVA content by increasing the amount of substrate flowing into the carotenoid biosynthesis pathway (Sagare et al., 2015). Major breakthrough in the history of molecular biofortification came when three polymorphic sites in CRTRB1 gene that accounts for 40% of variation in β-carotene concentration in maize endosperm were identified (Yan et al., 2010). On the other branch of the carotenoid biosynthetic pathway (see Figure 3), Harjes et al. (2008) reported allelic polymorphism in the LCYE gene with the favourable allele associated with increase in total proVA content at the expense of lutein content. Based on the functional polymorphisms of these key genes of the carotenoid biosynthesis pathway, several maize molecular markers have been developed and validated for use in maize biofortification (Harjes et al., 2008; Yan et al., 2010; Babu et al., 2012; Fu et al., 2013). This resulted in accelerated genetic gain in breeding for increased provitamin A content in maize. Table 1 gives a summary of maize genes encoding key enzymes in the carotenoid biosynthesis pathway and their respective favourable alleles.

Table 1. Three maize genes encoding key enzymes in the carotenoid biosynthesis pathway, and their allelic polymorphism.

Gene	Polymorphic site	Allelic diversity	Favourable allele	Reference
PSY1	PSY1-SNP7	A, C	A	(Babu et al., 2013; Fu et al., 2013)
	PSY1-InDel1	0,378	378	
LCYE	LCYE-5'TE	1,2,3,4	1,4	(Harjes et al., 2008)
	LCYE-SNP 216	G, T	G	
	LCYE-3'InDel	8,0	8	
CRTRB1	CRTRB1-5'TE	1,2,3	2	(Yan et al., 2010)
	CRTRB1-InDe14	12,0	12	
	CRTRB1-3'TE	1,2,3	1	

Adopted from Sagare et al. (2015) with modifications.

Molecular markers based on functional polymorphisms within PSY1, LcyE and CRTRB1 provide a quick means of developing provitamin A enriched lines and cultivars. Marker assisted backcrossing can be handy in speeding up the introgression of favourable alleles of LCYE and CRTRB1 into tropical materials from temper-

ate donors. Applying MAS, CIMMYT and IITA breeders have developed several tropical maize lines and populations with proVA content that surpasses the current set target of 15 µg g-1 (Andersson et al., 2017; Menkir et al., 2017).

Transgenic technology is another approach that is applicable in proVA biofortification since proVA content is controlled by few genes. However, it has been deemed less necessary in maize proVA biofortification because maize has enough natural genetic variation.

Genotype by Environment Interaction

There is a general consensus in literature that there is no significant genotype by environment interaction (GXE) effect in proVA expression in maize (Egesel et al., 2003; Menkir & Maziya-Dixon, 2004; Pfeiffer & McClafferty, 2007). Thus, the expression of proVA in maize is relatively stable across different growing environments. Menkir and Maziya-Dixon (2004) found that β-carotene, which is the most efficient proVA carotenoid is strongly influenced by the genotype and less so by the environment. This fits together with the fact that proVA is controlled by relatively few genes and more simply inherited (Pfeiffer & McClafferty, 2007). However, this should not rule out the need to perform multi-environment trials (MET) in maize biofortification since other key traits like yield, and biotic and abiotic resistance have significant GXE effects.

Provitamin A quantification

ProVA quantification is one of the daunting and crucial steps in maize biofortification. It is a challenging task because (1) maize has a complex mix of carotenoids (proVA and non proVA carotenoids) which takes a thorough laboratory analysis to extract and quantify each molecule; (2) carotenoids can be found in complex interaction with other molecules such as starch and proteins and (3) given their organic nature carotenoids are prone to degradation (Guild et al., 2017). These challenges can be reduced by carefully choosing the analysis method. Several methods have been considered and evaluated based on their accuracy, cost and speed to screen carotenoid content in maize kernels. These methods include visual colour scoring, near infrared reflectance and spectroscopy (NIRS) and liquid chromatography.

Despite its low cost, visual colour scoring has been found less efficient in quantifying carotenoids in maize because of poor correlation between the key proVA carotenoids (β-carotene and β-cryptoxanthin) and the visual colour score (Harjes et al., 2008). Spectroscopic techniques such as NIRS are excellent when determining total carotenoid (proVA and non-ProVA) content but not good at partitioning the carotenoids as the absorption maxima is a similar wavelength region for all carotenoids. Therefore, this method is not suitable for crops like maize that have a complex mixture of carotenoids (Guild et al., 2017).

Liquid chromatography analysis which is either High Performance Liquid Chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) can partition and quantify the different carotenoids present. This is useful in crops like maize which contain a mixture of carotenoids. High Performance Liquid Chromatography (HPLC) has been the method of choice for precision analysis; but the high cost, low throughput and consequently longer time required for analysis are acting as deterrents for most resource constraint biofortification programmes in SSA. Due to its high throughput capacity, low cost for reagents, ultra-performance liquid chromatography (UPLC) is becoming a better choice for most breeders (Pixley et al., 2013).

Cultivars released

Since the inception of maize biofortification in Africa, over 50 proVA maize cultivars in the form of open pollinated varieties, synthetics, single-cross hybrids, and three-way hybrids have been released for production in many maize consuming SSA countries. These countries fall within the HarvestPlus's maize top Biofortification Priority Index (BPI) (http://www.harvestplus.org/knowledge-market/BPI). Figure 4 shows the general performance in terms of grain yield and proVA content of some of the cultivars that were released in two phases between 2012 and 2017. These cultivars were released in Zimbabwe, Ghana, Malawi, Mali, Nigeria, Tanzania, Zambia and DR Congo. ProVA content ranges from 5 to 15 with percentage target increment varying from 33% to 100% (HarvestPlus, 2014; Andersson et al., 2017).

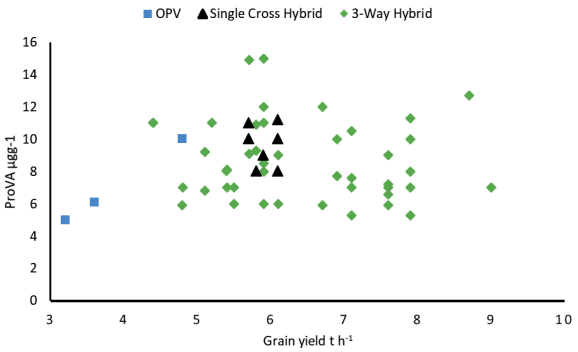

Figure 4 - ProVA content and grain yield performance of some of the released proVA maize cultivars in the form of OPVs, single cross and 3-way hybrids. Data sources: (HarvestPlus, 2014; Andersson et al., 2017) and Cultivar release proposals from some of the National Research Institutes in SSA .

The phase three products are still in inbred line form and are expected to be released within the next few years. The phase three inbred lines were developed using both conventional and molecular breeding methods with average proVA content as high as >15 µgg-1. They have the CRTB genes introgressed using marker assisted backcrossing (Andersson et al., 2017). Apart from having high proVA content, the released cultivars and identified elite lines have high grain yield and strong farmer preferences. The International Maize and Wheat Improvement (CIMMYT), International Institute of Tropical Agriculture (IITA), selected National Research Institutes and some of the private seed companies form the research and breeding component of the maize biofortification programme in Africa. Zambia and Nigeria are the primary countries where maize proVA biofortification is coordinated from while Zimbabwe, Mozambique, Malawi, Ghana, Benin, Ghana, Liberia, Sierra Leone, Mali among others constitute regional testing sites (HarvestPlus, 2014).

Challenges and Limitations

Early maize proVA biofortification efforts in SSA were constrained by high preference of white maize over yellow maize by consumers and other maize value chain actors (De Groote & Kimenju, 2008). This resulted in poor adoption of yellow coloured biofortified maize, a challenge that slowed down the uptake of maize biofortification technology in SSA. Pillay et al. (2011) found that this skewed preference is due to lack of knowledge on the nutritional benefits of biofortified yellow maize. In Southern Africa, notably in Zimbabwe, Zambia, Malawi and Mozambique yellow maize is shunned because it is perceived as a symbol of suffering and poverty. This is because yellow maize was imported into these countries during times of drought and famine (Muzhingi et al., 2008). To remedy the problem of skewed colour preferences, breeders changed the colour of biofortified maize to orange or deep yellow through conventional breeding, a measure which greatly improved the acceptability of biofortified maize in SSA. Furthermore, to inform farmers and other maize value chain actors about the nutritional benefits of biofortified maize, HarvestPlus and partners created parallel programmes to reach out to end users in the form of awareness campaigns. This resulted in improved acceptability of biofortified orange maize in SSA (HarvestPlus, 2014).

Quantification of carotenoids in the maize endosperm is another challenge facing maize biofortification for high provitamin A. High performance liquid chromatography (HPLC) which is the current method of choice is expensive, time consuming and low throughput, compromising its suitability for high volume breeding programmes in resource-constraint plant breeding programmes in sub-Saharan Africa and other developing countries. The cost of carotenoid analysis using HPLC is $50-$100 per sample which is beyond the reach of most breeding programmes. Ultra-performance liquid chromatography (UPLC) provides a good alternative to HPLC due to lower cost and slightly higher throughput. However, the UPLC throughput still falls far below the quantities required by most of the breeding programmes.

The aspects of maize carotenoids degradation and retention during postharvest storage still require more elucidation and documentation. Although several researchers have raised it, there is no consensus on the average rate of degradation and level of proVA carotenoid retention (Burt et al., 2010; Messias et al., 2014; Mugode et al., 2014; De Moura et al., 2015). This poses a challenge to the quantification of the gains of biofortification especially in rural areas where maize is stored in different storage facilities for a longer period by subsistence farmers before consumption. Genotype, kernel physical properties, storage temperature, light, oxygen and humidity are the main factors that affect postharvest storage rate of carotenoid degradation and level of retention (Taleon et al., 2017). Elevated temperatures and humidity during postharvest period accelerate carotenoid degradation (Ortiz et al., 2016). Disparities among genotypes in carotenoid stability are partially attributed to the differences in kernels physical properties. This means that kernel physical properties are other trait that breeders should consider when breeding for enhanced proVA content. Thus, kernels with small surface and low porosity can be selected to breed for increased carotenoid retention during postharvest storage (Ortiz et al., 2016). However, given the inadequate and diverging claims by several researchers concerning carotenoid retention during postharvest storage, there is need for further detailed research

Conclusions and prospects

Biofortification of maize for enhanced vitamin A has proved to be an important innovation for addressing both food and nutrition insecurity in SSA. Given the genetics and heritability of proVA both conventional and molecular breeding can be applied in maize biofortification. The application of molecular markers quickens the process of proVA biofortification. To increase the adoption of biofortified maize varieties in SSA, the released cultivars should be competitive in other traits such as grain yield, biotic and abiotic resistance. Enhancing drought tolerance in proVA maize cultivars developed for SSA could be handy in increasing acceptability of the biofortified maize

in Southern Africa given the precedency of drought in this region. Given the predicted potential growth of the biofortification industry there is need for the development of cheaper, efficient and high throughput proVA quantification technologies.

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