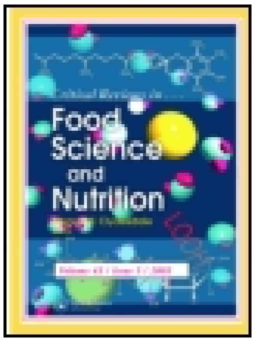

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The development and release of maize fortified with provitamin A carotenoids in developing countries

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ABSTRACT
Micronutrient deficiencies have been identified as major public health problems affecting a large part of the world's population. Biofortification of staple crops like maize has been proposed as one of the most cost effective and feasible approaches to combat micronutrient deficiencies. Studies have shown that provitamin A from biofortified crops is highly bioavailable and has the capacity to improve vitamin A status of vulnerable groups. Most people in sub-Saharan Africa subsist on maize and many people may benefit from consumption of provitamin A carotenoid biofortified maize, especially women and children. With the exception of transgenic golden rice, biofortified crops have received considerable acceptance by most communities. Negative perceptions associated with yellow maize do not affect orange maize, which is, for example, well-liked in rural Zambia. With proper policy frameworks and full commercialization, provitamin A maize can address the problem of vitamin A deficiencies among poor nations with maize-based diets.

KEYWORDS
Provitamin A; orange maize; bioavailability; consumer acceptance; micronutrient deficiency

Introduction

Several global demographic health surveys estimate that one third of the world's population does not meet their physical and intellectual potential because of vitamin and mineral deficiencies. World-wide an estimated 190 million children and 19 million pregnant women have low serum retinol concentrations (WHO, 2009). It has been identified as a major health problem among low and middle income countries (Zimmermann and Qaim, 2004; Naqvi et al., 2009; Meenakshi et al., 2012). Vitamin A is an essential nutrient, which is generally provided by retinyl esters in meat and dairy products and provitamin A carotenoids in plants (Chao et al., 2011). Vitamin A is a group of unsaturated organic compounds which is essential, as it cannot be produced by humans, and must be consumed as part of the diet. It includes retinol, retinal and retinoic acid (Tanumihardjo et al., 2016). Globally, vitamin A deficiency places 140 to 250 million people at risk for a number of health problems (Harjes et al., 2008). Vitamin A malnutrition leads to night blindness and increases the risk of child and maternal mortality (WHO, 2010) and also weakens the immune system of children, thus causing an increased risk of death from infectious disease (Tanumihardjo et al., 2016). The World Health Organisation (WHO) estimates that deficiencies in vitamin A rank among the top 10 leading causes of death in developing countries through several diseases (WHO, 2002).

Vitamin A malnutrition is estimated to affect approximately one third of children under the age of five around the world (WHO, 2009) and is estimated to claim the lives of 670 000 children under five annually (Black et al., 2008). Approximately 44 – 50% preschool children in the South Asian region suffer from severe vitamin A malnutrition (WHO, 2009). More than half of preschool children in Zambia are at risk of vitamin A deficiency (Micronutrient Initiative, 2009) and it accounts for 6% of all deaths and 5% of the total disease burden among preschool children (Black et al., 2008). Sixty four percent of 1 to 9 year old children in South Africa suffer from vitamin A deficiency (Labadarios et al., 2007). Thirty-four percent of women of child-bearing age, 35% of children under 5 years of age and 18% of school going children (between 6 and 14 years) in Zimbabwe are vitamin A deficient with serum retinol levels below 0.70 μmol l^{-1} (Muzhingi et al., 2008a; WHO, 2011). Unfortunately, most people affected by vitamin A malnutrition do not show clinical symptoms, nor are they themselves aware of the deficiency, a phenomenon called "hidden hunger" (World Food Programme, 2006). Vitamin A malnutrition is more serious in populations subsisting on cereals and tubers as staple food crops, because these food sources are deficient in provitamin A carotenoids.

Micronutrient deficiencies contribute to the degenerative cycle of poverty by limiting disposable income in households where people are too weak to work effectively because of hunger (WHO, 1999). Lack of adequate disposable income limits the capacity of parents to provide nutritious food to their families, leading to malnutrition, which negatively affects the health and normal development of children. Micronutrient deficiency prevalence is increasing due to a reduction in food diversity as

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a result of the effect of population increase on land pressure. As pressure for land increases, people tend to concentrate on energy giving crops, mostly cereals, because they are most productive, reliable and profitable (Welch and Graham, 1999). Unfortunately, cereals are poor in micronutrients. Various non-governmental organisations (NGOs) proposed the use of nutrition gardens to address the problem of micronutrient malnutrition. However, the initiative is limited by lack of land, space and water for irrigation due to frequent droughts. In urban areas, low income earners have no access to land for vegetable gardens and they normally buy vegetables. This results in insufficient vegetables being consumed to meaningfully address the problem of vitamin A deficiency. This leaves biofortification of staple food crops as one of the few sustainable ways of alleviating vitamin A deficiency, particularly on a large scale (Hotz and McClafferty, 2007).

Industrial fortification of maize flour only benefits the urban populations who buy processed maize meal and is of little benefit to rural people who generally take their maize to the mill, without any additions. Furthermore, during times of economic challenges, companies do not normally fortify maize meal, since it increases the production costs. Currently, maize meal fortification is not a policy in many countries in Africa. Companies that fortify maize meal in countries like Zimbabwe do it as a marketing strategy, and they normally receive very little support, if any, from government. There is very little nutritional education from the Ministry of Health in several developing countries and other public health practitioners to educate the people to buy the fortified maize meal, which at times is slightly more expensive than unfortified meal. African governments put more emphasis on supplementation, which unfortunately is not very sustainable because of poor funding, governance and infrastructure (Graham et al., 2001). In most cases the supplementation is donor driven, which is again unsustainable and unreliable.

The most cost effective and feasible approach to combat the detrimental effects of dietary deficiencies in sub-Saharan Africa is to biofortify the staple food crops. Biofortification is a process by which crops are purposefully bred for higher nutritional density (Graham et al., 2001; Fraser and Bramley, 2004). The aim of this review is to uncover the value of maize as a candidate for biofortification.

Importance of maize

Maize is a very important crop in the world because of its various uses as a food crop, animal feed and as an important raw material in industry (Vasal, 2000; Prasanna et al., 2009). It is a staple food for more than 1.2 billion people in sub-Saharan Africa and Latin America and is regarded as a vital crop in the perspective of global nutrition (IITA, 2010; Nuss and Tanumihardjo, 2010). Maize is very important for food security in Southern Africa accounting for an average of 36% of all caloric intake in the region (Grant et al., 2012). It is important both as a human staple and as animal feed and is often used as an infant weaning food by resource poor households, with no additional animal products. In many countries, the crop can be an ideal source of a dietary supplement, as it provides carbohydrates, proteins, iron, vitamin A (yellow and orange maize only) and B (except vitamin B12) and some minerals to the human diet.

How can vitamin A deficiency be addressed?

Because of the great importance of maize as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value, including micronutrient deficiency, many efforts have been made to improve its nutritional value (FAO, 1992). In the developed world micronutrient deficiency is addressed by diet diversification, food fortification and supplementation. All people must have access to a varied diet, rich in fruits and vegetables but this is, however, limited by seasonality of crops, affordability and low bioavailability of green leafy plant carotenoids (West et al., 2002). Diversification, fortification and supplementation are unfortunately less effective in developing countries because of insufficient funding, poor governance, poor distribution networks (FAO/WHO, 2001), and political, socio-economic and technical constraints that are prevalent in these counties (Darnton-Hill and Nalubola, 2002). There have been many efforts to fortify maize, with outstanding results, but unfortunately fortification has not been implemented widely (FAO, 1992). This approach, however, may become important in the future as more people consume industrially processed foods, which can be more easily and efficiently fortified. Many people in the drier parts of Zimbabwe do not have access to fresh vegetables and fruits throughout the year (Gadaga et al., 2009). Vitamin A supplementation coverage rate (% of children ages 6–59 months) in Zimbabwe was 34% in 2013, its highest value over the past 11 years was 83% in 2007, while its lowest value was 20% in 2004 (World Bank, 2013). Staple crop biofortification is a promising and potentially feasible intervention to alleviate micronutrient deficiency in developing countries (Combs et al., 1996; Welch et al., 1997; Graham et al., 2001).

Maize as a source of provitamin A carotenoids

Human studies by Tang et al. (2009) and Li et al. (2010) have shown that β-carotene biofortified maize is a good source of provitamin A. From these observations, improvement in provitamin A content of maize has received increased interest in recent years. This is in an effort to overcome vitamin A malnutrition resulting from the consumption of white maize in poor communities who cannot afford animal products, and sufficient fruits and vegetables. Maize grain carotenoid concentrations are among the highest produced in cereals (Howitt and Pogson, 2006) and display considerable natural variation for carotenoid composition. In yellow maize, provitamin A carotenoids in the endosperm include α-carotene, β-carotene and β-cryptoxanthin, but concentrations are low, ranging from 0 to 1.3, 0.13 to 2.7 and 0.13 to 1.9 nmol g^{-1} respectively (Kurilich and Juvik, 1999). Recently developed maize varieties have β-carotene levels of about 15 $\mu g\ g^{-1}$ (HarvestPlus, 2007) and even as high as 25 $\mu g\ g^{-1}$ (USDA, 2007). This can support about 57% of daily needs of vitamin A required by human beings (Pixley, 2010).

Plant-based carotenoids are widely recognized for their antioxidant and nutritional value, which include provitamin A activity (Johnson, 2002). Upon symmetrical breakdown, provitamin A carotenoids produce one or two retinal molecules. There is no limit to the amount of plant based carotenoids that can be safely consumed in contrast to the toxic levels caused by excessive intake of preformed vitamin A (Tanumihardjo, 2008).

Chao et al. (2011) proposed that the potential impact of carotenoid enhancement should be judged against benchmarks, which include the importance of particular crops in terms of global food security and the amount of food that must be consumed to achieve the reference daily intake of vitamin A. Seventy-seven percent of maize produced in sub-Saharan Africa (except South Africa) is used for human food and only 12% serves as animal feed (Grant et al., 2012). Maize is consumed in large quantities, about three times a day in many settings in Africa, Latin American and Asia (Rooney et al., 1987; Rooney and Serna-Saldivar, 2003). The dietary habits of many Africans where maize is consumed for all three meals a day, makes maize a good target for biofortification (Li et al., 2007). Shifting consumption from white maize to provitamin A orange maize would reduce vitamin A deficiency among vulnerable children and expecting mothers, without problems of overdose because the body would regulate how much provitamin A to convert into vitamin A (Lindqvist and Verba, 2009). This will take advantage of the consistent daily consumption of large amounts of staple food crops by the poor, especially women and children, who are most vulnerable. This provides a low cost option for preventing or controlling vitamin A deficiency among the groups (Chowdhury et al., 2009).

Status and acceptance of provitamin A maize in Southern and Eastern Africa

The sub-Saharan region of Africa is a leader in the cultivation and consumption of white maize (IITA, 2010) which lacks provitamin A carotenoids (FAO, 1992; Johnson, 2000). Consumer acceptability plays a crucial role for provitamin A maize to be meaningful in alleviating vitamin A deficiency in maize-based diets. Although biofortified staple foods are inexpensive, locally adaptable and offer a long-term solution to diet deficiencies, cultural preferences may limit their acceptance (Harjes et al., 2008). It is unfortunate that yellow maize is unpopular among consumers in southern Africa and is presumed to have little or no human consumption demand (Muzhingi et al., 2008a; b). Rich in oils, carotenoids and fructose, yellow maize easily goes rancid and produces undesirable odours and flavour. It is also commonly perceived as a "poor man's grain" because it is associated with food aid (Tschirley and Santos, 1994; Muzhingi et al., 2008a). Although people prefer white maize over yellow maize, there is little evidence of differences in taste and processing qualities between yellow and white maize, except that coloured varieties are often flint, which is actually often associated with favourable cooking and processing characteristics (De Groote and Kimenju, 2008). Muzhingi et al. (2008a) found that nutritional education can potentially counter the negative perception of yellow maize consumption in countries like Zimbabwe, especially if targeted at low income household level. This will make people benefit from the nutritional value of orange maize and reduce vitamin A malnutrition.

Literature comparing acceptance of white maize with orange maize is limited. Most studies are comparing white maize with yellow maize, except one study by Stevens and Winter-Nelson (2008), which includes white, yellow and orange varieties of maize. The study by Muzhingi et al. (2008a) on consumer acceptability of yellow maize in urban and rural Zimbabwe found that more than 94% of households were willing to consume yellow maize if they knew it was more nutritious than white maize. Meenakshi et al. (2010) found that the negative perception associated with yellow maize does not affect orange maize which is well liked in rural Zambia. Nuss et al. (2012) also observed quick adoption of orange maize in Zambia in the form of thin and thick porridge. A successful intervention to introduce β-carotene rich, orange sweet potato in Mozambique, where only white sweet potato was previously cultivated, suggests that orange-coloured staple foods can be acceptable, and their regular consumption results in improved vitamin A status (Howe and Tanumihardjo, 2006). Orange-fleshed sweet potato production and use also spilled into neighbouring countries such as Zimbabwe, Malawi and South Africa. Results on acceptability of orange maize research in Mozambique suggest that orange maize meal is as preferred as white and that no price discounts are likely to be necessary to promote its consumption. In addition, families with young children and those that did not consume diets rich in animal products were more likely to accept orange maize. Because of the perceived acceptability and the potential to address vitamin A deficiency among poor households, Tumuhimbise et al. (2013) suggested that it is time to fully commercialise provitamin A crops by encouraging farmers to start large scale production and consumption.

For orange maize to be widely accepted, there is a need to develop strategies for taking the information of its benefits to the target people through nutritional education and advocacy. There is also a need to make it more available in shops and price it strategically lower than white maize, to make it affordable for the poor communities (Pillary et al., 2011), like what South Africa did with its nutritionally fortified brown bread. Unfortunately pricing it lower than white maize may result in stigma, as people might view it as food for people who cannot afford to pay a premium price for white maize. So this approach needs to be seriously scrutinized. It might be more effective to price it the same but intensify nutritional education on the benefits of eating orange maize.

Several authors suggested that nutritional information can influence consumer acceptance of orange maize. Muzhingi et al. (2008a; b) found that nutritional information is the single most important factor in determining a household's decision to purchase nutritionally enhanced maize; a nutrition campaign can significantly alter consumers' perceptions and lead to a much higher probability that non-white maize would be consumed. A study done in Nairobi gave evidence of a substantial reduction in the discount for commercially fortified yellow maize flour as a consequence of nutrition education (De Groote and Kimenju, 2008). They found that while there is an interest in commercially fortified maize, the average premium for fortification is less than half the discount on yellow maize. However, what is worrying is the observation that poorer people tend to place lower premiums on nutritionally enhanced foods (Morawetz et al., 2006; De Groote and Kimenju, 2008) as they seem to be more worried about addressing their caloric needs. This may mean pricing orange maize cheaper than white maize could lead to the adoption of orange maize by poor households wanting to address their energy need and subsequently also address vitamin A deficiency.

Effective nutrition campaigns can be conducted using various methods which include nutritional messages and food labelling that encourage provitamin A maize as a healthy choice, through mass media, theatre and community leaders and involvement of the private sector. In developed countries, health information is typically conveyed through the use of written labels and the literature suggests that premiums for health labelling can be significant (Kinnucan et al. 1997; Tanumihardjo et al., 2017). The use of labelling is not practical in poor communities, given low levels of literacy, costs of labelling, and the fact that maize is sold from farmer to farmer. Using community leaders, extension workers, NGOs and radio messages for conveying health information is more realistic (Zimicki, 1997).

There is also need to address the problem of unacceptable organoleptic properties of yellow maize (Muzhingi et al., 2008a) to enhance it acceptability. This can be achieved through breeding by strategically selecting against high oil content, which causes the crop to quickly go rancid and produce a bad smell if not properly stored. There is also a need to carry out research on the best on-farm storage conditions for the crop; so that it will not lose its provitamin A carotenoids due to degradation and also that it will not produce undesirable characteristics in storage.

To enhance acceptability by farmers there is need to research the agronomic performance and stability of provitamin A maize across varying environments, so that the provitamin A maize varieties will not yield less than varieties currently grown by farmers. Provitamin A maize hybrids developed in Zambia are agronomically sound. Li et al. (2007) stated that the positive nutritional and acceptance results will need to be coordinated with comprehensive breeding and seed distribution efforts to realize the potential of provitamin A biofortified maize. One possibility is to counter negative perceptions of increased β-carotene content with other new traits that farmers find useful. Governments may also subsidise the production of provitamin A maize to encourage cultivation and consumption by resource-poor consumers. Economists typically assume that adoption by farmers is *prima facie* evidence that it provides them with benefits (Dawe and Unnevehr, 2007).

In order to have maximum impact on biofortification, high yielding varieties are needed to convince the poor farmers to grow it, even though the target consumer is in no position to pay a higher price for quality. Researchers found that it is possible to combine the high-density trait with high yield, unlike protein content and yield that are negatively correlated. The micronutrient traits are stable across environments and the genetic control is relatively simple. Given equal or superior agronomic performance of the orange maize varieties, they may attract a premium in the market. Consumer education, extension, properly designed policies encouraging adoption, mitigating the higher seed cost, and lessons learned from sweet potato are all part of the desired policy mix to enhance adoption (Tumuhimbise et al., 2013).

Effect of maize storage and processing on retention of carotenoids

Maize can be stored from one harvest to the next, which is equivalent to 6–12 months, depending on the number of growing seasons per year in any given agro-ecological zone. The highly unsaturated structure of carotenoids makes them susceptible to post harvest degradation by heat, oxygen and light (De Moura et al., 2015). The mechanisms of carotenoid degradation may involve the reaction of carotenoids with heat (thermal degradation), atmospheric oxygen (auto-oxidation) and light (photo degradation), as well as degradation by the interactions of carotenoids with singlet oxygen, acid, metals and free radicals. In food systems, the degradation mechanisms are more complex (Boon et al., 2010). After 6 months of storage, Weber (1987) reported the average total carotenoid retention among four inbred lines of maize as 58%. The genotype with the lowest initial total carotenoids content (27.4 μg g^{-1}dry weight) showed the highest retention (67%). Burt et al. (2010), while studying two genotypes over a period of 18 months, observed that total carotenoids remained constant for the first three months and declined significantly by six months and then remained stable, giving a total loss of 35–40%. The carotenoids are stored in the endosperm, therefore they are less subjected to milling losses. Milling provitamin A biofortified maize into mealie meal resulted in a higher retention of carotenoids compared to milling into samp (Pillary et al., 2011). However, the study demonstrated that provitamin A retention in maize is affected by the cooking method and therefore cooking methods that result in good retention of provitamin A need to be identified and recommended.

De Moura et al. (2015) reported that provitamin A retention in maize grain during storage was found to be highly linked to genotype and retention ranged between 45–93% after four months of storage. It was suggested that provitamin A degradation be determined before a new variety is released for production. Soaking and milling of maize caused a 7.3% loss, and cooking porridge an additional 18% loss in β-carotene content. Burt et al. (2010) stated that the understanding of genotype effect has the potential to guide the development of high carotenoid maize inbred lines with good stability during drying and storage.

Provitamin A maize breeding

To draw maximum benefit from agricultural research as a vehicle for addressing public health issues, in July of 2003 the Consultative Group on International Agricultural Research (CGIAR) established HarvestPlus: the Biofortification Challenge Program (BCP), adding food quality to its agricultural production research programme. Biofortification research is a comprehensive programme that spans from genetic crop improvement to research on the impact of biofortified crops on human health (Haas et al., 2005; Van Jaarsveld et al., 2005; Tanumihardjo et al., 2017) and is conducted mainly under the auspices of HarvestPlus. The focus of HarvestPlus is on three micronutrients, Fe, Zn and vitamin A which have been identified by WHO as limiting for most poor households. The programme on Agriculture for Nutrition and Health (A4NH) under HarvestPlus focuses on conventional plant breeding techniques for biofortification. Their target concentration of provitamin A carotenoids (pVAC) is half of the estimated average requirement for the population of interest, where an average amount of the biofortified food is consumed (De Moura et al., 2015). Scientists generally agree that exploiting the genetic variation in crop plants for micronutrient density is one of the most powerful tools available to change the nutrient balance of a given diet on a large scale. With this realisation the BCP was set up to carry out research on possible ways to address Fe, Zn and vitamin A deficiency among poor people.

Maize displays considerable natural variation for carotenoid composition, with some lines accumulating as much as 66 μg g^{-1} (Harjes et al., 2008), which means selection for provitamin A in maize is possible. Researchers at the Agricultural Research Service of the USA identified genetic sequences in maize associated with higher levels of β-carotene. It was discovered that breeders can cross breed certain variations of maize with the aim of producing a crop with an 18-fold increase in β-carotene (USDA, 2010).

Historically, work on maize carotenoids has been limited to available material rather than germplasm developed for high carotenoid levels (Kurilich and Juvik, 1999). Of interest now is research on improving carotenoid content, which has been conducted by several groups. For example, Egesel et al. (2003) were able to determine combining ability for several Corn Belt dent inbred lines and discovered that selection for improved carotenoid content can yield improved varieties. Suwarno et al. (2014) found that provitamin A concentration in maize is controlled primarily by additive gene action. However, the significant environmental effects for total provitamin A concentration they observed represents a challenge to developing cultivars with widespread impact on vitamin A malnutrition. Menkir et al. (2015) reported that crosses of elite yellow or orange lines with exotic donors, followed by backcrossing to elite lines, followed by repeated selection for bright yellow to orange kernel colour with acceptable kernel characteristics, was successful in increasing β-carotene content. Likewise visual selection in inbred lines for bright yellow and orange colour with good kernel characteristics was effective for developing inbred lines with good agronomic and adaptive traits.

Harjes et al. (2008) demonstrated the power of targeting specific steps of the metabolic pathway in order to achieve the desired carotenoid profile, high β-carotene maize, and they showed the potential for marker assisted selection to improve carotenoid content within the breeding pools. Early research indicated that maize carotenoid content in the grain varies considerably and breeding maize for high provitamin A is possible (Brunson and Quackenbush, 1962). Genetic variation for specific carotenoid content has been reported in maize lines adapted to the tropics (Harjes et al., 2008; Menkir et al., 2008).

Experimental evidence from association and linkage populations in maize demonstrates that the gene encoding β-carotene hydroxylase 1 (*crtRB1*) underlies a principal quantitative trait locus (QTL) associated with β-carotene concentration and conversion in maize kernels. The *crtRB1* alleles associated with reduced transcript expression correlate with higher β-carotene concentrations (Yan et al., 2010). These alleles are rare in frequency and unique to temperate germplasm, but maize breeders have successfully introduced the alleles into some tropical germplasm. The implementation of this programme requires backcross selection to convert African adapted white germplasm to orange (Chandler et al., 2013). Under the best scenario, the *crtRB1* gene variations can increase concentration of β-carotene from a little above zero, to about 57% of the micronutrient target (15 μg g^{-1} β-carotene). HarvestPlus has determined that this would improve people's nutrition and health (Pixley et al., 2010).

Suwarno et al. (2014) also conducted a study to assess the heritability of visual scores for relative intensity of orange kernel colour and they identified genetic markers associated with orange colour. They identified visually scored kernel colour to have a moderately high heritability and identified five common QTLs and six rare QTLs for intensity of orange colour. Notably, half of them coincided with carotenoid biosynthetic genes. Their results indicate that breeders can have flexibility to select for orange kernel colour visually and/or with gene-specific markers. The moderately high heritability of visual scores for relative intensity of orange kernel colour indicates that this trait should respond favourably to phenotypic selection. The identification of strong positive effect QTLs in the vicinity of carotenoid biosynthetic pathway genes *y1, zds1*, and *lcyE* implies that phenotypic selection for dark orange colour will likely result in higher amounts of total carotenoids in the maize kernel. However, the discovery of only weak positive effect QTLs in close proximity to *zep1* and *ccd1* underscores the need to further explore and characterize genetic diversity at these two loci and search for more favourable alleles (Suwarno et al., 2014).

In another study to identify genomic regions controlling variation for carotenoid concentration in grain with genome-wide association analysis, several genes not in the carotenoid pathway but which may affect the amount of carotenoids, were suggested. The study validated the significance of *CRTRBI* and genes such as *HYD5* and *HYD1*, which are involved in carotenoid synthesis. Other candidate genes were also identified (*GGPS2, SXS1, ZEP1* and *CCD1*) which can be used for selection for increased carotenoids (Suwarno et al., 2015). The absence of provitamin A in cereals means the corresponding metabolic pathway is absent, truncated or inhibited in the endosperm, hence genes encoding enzymes free from feedback need to be introduced (Christou and Twyman, 2004; Zhu et al., 2007). Maneesha et al. (2008) found that over-expression of the bacterial genes *crtB* (for phytoene synthase) and *crtl* (for the four desaturation steps of the carotenoid pathway catalysed by phytoene desaturase and β-carotene desaturase in plants), under the control of 'super γ-zein promotor' for endosperm expression resulted in an increase of total carotenoids of up to 34-fold with preferential accumulation of β-carotene in the maize endosperm. The β-carotene trait was found to be reproducible over at least four generations.

Phenotypic selection for orange colour should be effective, simple and low cost for converting white or yellow grain maize germplasm to orange. To further enhance β-carotene levels, this phenotypic selection could be combined with marker assisted selection for favourable *crtRB1* alleles. Genotyping single kernels and selecting for favourable alleles at the six loci would further expedite breeding efforts by assuring that the most desirable genotypes are selected before planting in winter or summer nurseries (Suwarno et al., 2014). These authors proposed that this approach could be combined with a strategy that uses a set of genome-wide markers to rapidly select against the undesirable genetic background of less adapted or lower yielding orange donor lines.

Zambia has been successful in the release and dissemination of provitamin A maize. The first three provitamin A biofortified maize varieties were released in Zambia in 2012 after extensive evaluation. They were found to be competitive with normal maize varieties. By 2015 one of the provitamin hybrids had gained 1% of the seed market. Momentum for the orange maize has been accelerating, and the acceptance is such that it has become sustainable. The selling capacity of the orange maize is still much lower than that of white maize, as there is a preference for white maize, and the Food Reserve Agency only allows for the purchase of white maize, so farmers are growing it for their own use. Yet there has been significant market development for orange maize and its products (Simpungwe, 2017; Tanumihardjo et al., 2017).

Although current genetic results and strategy are encouraging, they need to be placed in context as part of an overall biofortification effort encompassing breeding infrastructure, seed distribution, societal acceptance, dietary habits and nutritional impact. Available information on some of these issues is encouraging (De Groote and Kimenju, 2008). Plant breeding strategies hold promise in reducing recurrent expenditure associated with other nutrition programs, such as supplementation, fortification and dietary diversification.

The importance of provitamin A maize varieties as sources of vitamin A in a breeding program also depends on the stability of expression of these compounds across different growing conditions. Limited information is available on the effect of different growing conditions and its interaction with the genotype on provitamin A carotenoids content in sub-Saharan Africa.

Promising provitamin A enriched synthetics and hybrids are included in regional trials in Africa and in 2016 as many as 64 provitamin A enriched synthetics and 74 hybrids were dispatched to partners in 14 African countries for extensive evaluation. Of the best five synthetics selected, some had significantly higher levels of provitamin A carotenoids and equal or higher yields than the benchmark orange OPV. The number of provitamin A enriched varieties and hybrids released from regional trials between 2012 and 2016 were three synthetics each in the Democratic Republic of the Congo, and Mali, and two each in Ghana and Nigeria. In terms of hybrids, two each were released in Ghana and Mali and five in Nigeria. These genotypes had provitamin A concentrations of between 6–11 $\mu g\ g^{-1}$ and had yield advantages of between 0 and 80% over farmer preferred varieties. It was suggested that the concentration of provitamin A should be increased to much higher levels to offset losses of provitamin A during storage, milling and preparation of food (Menkir et al., 2017).

Conventional versus transgenic breeding on maize biofortification: Challenges and opportunities

One of the significant disadvantages of conventional breeding compared to transgenic strategies is its reliance on alleles already in the species genepool (Zhu et al., 2007). But the major advantage of conventional breeding is that it uses intrinsic properties of the crop. As a result there are few regulatory requirements. The dependence on the existing gene pool will mean that a long time is needed to develop a new variety. Traits might need to be introgressed from wild relatives, which might again take a longer period of time.

Gene expression analysis suggests that increased accumulation of β-carotene is due to an up-regulation of the endogenous lycopene β-cylase. Modification of *crtl* as well as phytoene synthase (*PSY)* boosts kernel provitamin A content in maize (Maneesha et al., 2008; Babu et al., 2012). Polymorphism at the *lcye* locus in maize was shown to alter the flux between the α-carotene and β-carotene branches of the carotenoid pathway, potentially allowing breeding for enhanced β-carotene levels (Harjes et al., 2008).

Transgenic approach advantages are rapid; unconstrained by a genepool; they target expression in plant organs which are not used for human consumption and are applicable directly to elite lines. However, for transgenic biofortification strategies to be successful there are also regulatory and public perception issues to overcome, such as the current negative perception of genetically modified (GM) foods in most developing countries. These should be addressed purely through science-based analysis and separated from socio-political and regional economic interests, for example, through the oversight of independent, NGO sponsored panels (Zhu et al., 2007).

NGOs have a lot of influence on adoption of technologies by farmers and consumers. These NGOs are unlikely to support GM maize because they mostly advocate traditional farming. The NGO's objections are due to ethical or ideological considerations, not scientific scepticism (Dawe and Unnevehr, 2007). So there is need for educational campaigns targeting farmers and the general public. Most importantly, nutritionally enhanced crops should be available to those most in need without intellectual property constraints and licensing restrictions, which are often in place for commercial use in the western communities.

Transgenic approaches offer the most rapid way to develop high-nutrient commercial lines. Transgenic strategies differ from other approaches in that novel genetic information is introduced directly into the plant's genome (Zhu et al., 2007). The best biofortification strategies should include genetic engineering in conjunction with conventional breeding, particularly when the direct enhancement of local lines is required (Naqvi et al., 2009). However, adoption will depend on legalisation of GM organisms. From a political standpoint, in Africa, like in Asia, there are still very few advocates for GM technology. Government agents mostly promote organic farming. Any biofortification effort needs the support of National Research Centres and NGOs in order to be designed and targeted appropriately. If NGOs who work with the poor households embrace it, it will be easily accepted. Any biofortification of a staple crop using GM technology will encounter greater political resistance, as well as more challenges in safety assessments and delivery, than non-GM approaches.

While conventional breeding will most likely never allow us to reach the high levels possible with transgenics, it can achieve the substantial and important increments necessary to improve human nutrition (Pixley, 2010). In the case of maize, CIMMYT and other HarvestPlus partners are developing high provitamin A maize lines and have identified lines with 15 ug g^{-1} of provitamin A. This is far lower than the 60 ug g^{-1} achieved transgenically, but it is much higher than the 0 ug g^{-1} found in white maize, or 1.5 ug g^{-1} common in yellow maize (Pixley, 2010). Naqvi et al. (2009) developed elite inbreds in which three vitamins were increased, specifically in the endosperm through simultaneous modification of three separate pathways. The kernels produced contained 169-fold the normal amount of β-carotene, six-fold the normal amount of ascorbate and double the amount of folate. The vitamins produced remained stable upto T_3 homozygous generation. This means the development of nutritionally complete cereals to address deficiency of important nutrients in resource-poor households is possible.

HarvestPlus has estimated that up to 50% of the estimated average requirement for vitamin A in Zambia could be met by eating 15 ug g^{-1} HarvestPlus orange-maize instead of non-biofortified lines. Transgenic maize, which can have up to 169 fold of provitamin A carotenoids, can largely address the daily human needs of vitamin A. This suggests that increasing the amount by using transgenic approaches could meet most of the recommended dietary allowance. In addition to enhancing provitamin A carotenoids, transgenic approaches could also increase other micronutrients not present in maize. However, once a transgenic line is obtained, at least three expensive and time-consuming actions are required. First, several years of conventional breeding are needed to assure that the transgenes are stably inherited and that it does not result in inadvertent, undesirable associated effects. Secondly, concurrent conventional breeding is needed to incorporate the transgenic line into varieties which also perform well agronomically so that farmers will want to grow it. Thirdly, there is also an expensive and lengthy process of research to document and defend the human and environmental safety of transgenic crops, and to obtain the legal approvals to release and commercialize these lines (Pixley, 2010). Furthermore, in quite a number of countries there currently is no legal framework that allows commercial release of transgenic maize varieties. Thus, while transgenic approaches are clearly the fastest way to “prove the concept” that biofortified crops, and in this case, multi-micronutrient-biofortified crops are possible, they may not be the quickest to obtain usable products and desired impact. As carotenoid biosynthesis is very complex and involves quite a number of genes, a strategy of stacking favourable genes was proposed, which should lead to improvement of a number of carotenoids and health benefits from maize (Menkir et al., 2017).

Analysis of provitamin A carotenoids to support breeding

Accurate assessment of provitamin A carotenoids in maize must be performed to direct breeding efforts. Carotenoid analysis of foods is inherently difficult due to a large number of naturally occurring carotenoids, highly variable composition of foods, wide ranges of carotenoid concentrations, and isomerization and degradation of carotenoids prior to and during analysis. Breeding for carotenoids requires high throughput screening methods, which are inexpensive. If the colour intensity has a link with the amount of provitamin A carotenoids, rapid screening will be possible, since genetic gain in carotene content will be visually estimated with accuracy (Simon, 1992). There is need to do profiling research to see the relationship of colour intensity and carotenoid concentration of grains, so that breeders can exploit this for selection for high carotenoid genotypes. Selection indices are very often used in selection for high-yielding genotypes under drought stressed and irrigated conditions (Cattivelli et al., 2008). However colour intensity might give an indication of total carotenoid content only, without indicating the amount of provitamin A carotenoids and other carotenoids, including non provitamin A.

Bioavailability of provitamin A carotenoids from maize

Human nutrient studies have shown that provitamin A from biofortified crops is highly bioavailable and has the capacity to improve vitamin A status (Nestel et al., 2006; Pfeiffer and McClafferty, 2007). A study by Palmer et al. (2016) showed, in a study in preschool aged Zambian children, that routine consumption of provitamin A biofortified maize markedly increased serum β-carotene, showing the bioavailability of the β-carotene. This was the case even using various traditional preparation methods. Due to other factors in this study (such as malaria prevalence) the β-carotene did not translate into higher circulating concentrations of vitamin A status in these children.

The total amount of a micronutrient in plant food does not represent the actual micronutrient content of the food which is utilizable by the consumer. In human nutrition terms, bioavailability is commonly defined as the amount of a nutrient in a meal that is absorbable and can be utilized by the person eating the meal (Van Campen and Glahn, 1999). Micronutrients can occur in various chemical forms of differing proportions in plant foods and their amounts vary depending on numerous factors including the growth environment, plant species, genotype, and cultural methods and management practices used to grow the plant. These forms have characteristically different solubility and reactivity with other plant constituents and other meal components. There are multiple interactions occurring between micronutrients in plant foods and other plant substances once the food is consumed, such as with other interacting nutrients and chemical substances which can either inhibit (such as anti-nutrients) or enhance (such as promoters) absorption and/or utilization of the micronutrients and consequently their bioavailability. Additionally, many other interacting factors, both genetic and environmental, affect micronutrient bioavailability to the consumer, such as food processing methods, meal preparation techniques, and an individual’s personal characteristics (such as gender, age, genetic predisposition, ethnic background, economic status, physiological state, nutritional and disease status). Reports (Garcia-Casal et al., 1998; Garcia-Casal and Layrisse, 1999; Layrisse et al., 1997; 1998) indicated that fortifying cereal-based diets with vitamin A or β-carotene and Fe(II)-fumarate enhanced the bioavailability of the Fe to humans dramatically (e.g., β-carotene increased Fe bioavailability more than three fold in rice-based meals and more than 1.8 fold in wheat and maize-based meals). Li et al. (2010) found that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A. Human subjects may have different abilities to convert provitamin A carotenoids to vitamin A. The conversion efficiency of dietary β-carotene to retinol was reported to be in the range of 3.6-28:1 by weight, but this varied according to, amongst other factors, body weight of the test subjects (Tang, 2010).

Heying et al. (2013) observed that consumption of daily provitamin A carotenoids by sows during gestation and lactation increased liver retinol status in weanling piglets, illustrating the potential for provitamin A carotenoid consumption from biofortified staple foods to improve vitamin A reserves. They concluded that frequent intake of provitamin A carotenoids from biofortified maize may sustain adequate vitamin A status in deficient populations if widely adopted as their staple food. A study in India found a 54% reduction in childhood mortality in children who were given small weekly doses of preformed vitamin A, which represented achievable daily consumption amounts from food (Rahmatthullah et al., 1990). The β-carotene in the biofortified maize adequately maintained vitamin A status in vitamin A depleted gerbils and was efficacious as β-carotene supplement (Howe and Tanumihardjo, 2006).

Conclusions

In developing countries there is little if any support for food fortification from governments. There is poor funding for vitamin A supplementation programmes and there is also poor infrastructure making it difficult to reach remote areas where poor people live. Furthermore, supplementation is mostly donor driven, making it unsustainable. Biofortification of staple food crops like maize is the panacea to address vitamin A deficiency in developing countries because of poor infrastructure, poor governance and poverty. Carotenoids are stored in the endosperm; therefore they are less subjected to milling losses. The highly unsaturated structure of carotenoids makes them susceptible to post harvest degradation by heat, oxygen and light so there is need for research on proper on-farm storage facilities. Although many people prefer white maize, it is devoid of provitamin A carotenoids. Shifting consumption from white maize to provitamin A orange maize would reduce vitamin A deficiency among vulnerable groups. Both animal and human studies have shown that provitamin A from biofortified crops is highly bioavailable and has the capacity to improve vitamin A status. There is need to make provitamin A maize varieties available to farmers and encourage farmers to start large-scale production. It is very interesting to note that provitamin A maize developed in Zambia is agronomically sound and is well accepted by farmers. For provitamin A maize to be widely accepted there is need to develop strategies for taking the information of its benefits to the target people through nutritional education and advocacy. With proper policies, provitamin A maize may potentially address vitamin A deficiency among poor households in sub-Saharan Africa.

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