Journal of Genetic Engineering and Biotechnology 16 (2018) 553–562

HOSTED BY

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Elevated carotenoids in staple crops: The biosynthesis, challenges and measures for target delivery

Adebanjo Ayobamidele Badejo *

Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat 785013, Assam, India

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 20 February 2018
Accepted 20 February 2018
Available online 6 March 2018

Keywords:
Biofortification
Provitamin A
Genetic engineering
Conventional breeding
Hidden hunger

ABSTRACT

Poverty eradication and global food security are among the targets of world leaders, most especially combating the scourge of hidden hunger. Provitamin A carotenoids cannot be synthesized *de novo* by human and so it must be taken as part of the diet. The deficiency of which is causing almost 6000 sights to be lost daily in most developing countries because of the monotonous starchy diets lacking substantial amount of carotenoid. Conventional breeding as well as genetic engineering have been used to increase the level of carotenoid in many staples including rice, potato, maize and cassava. While products from genetic engineering are still been subjected to strict regulatory measures preventing the delivery of the products to target consumers, some of the products from conventional breeding are already on the table of consumers. Interestingly, both technologies are crucial to tackling micronutrient deficiencies. This review discusses the role of carotenoid in human, the biosynthesis in plant and some of the staple crops that have been modified for increased carotenoid. Some measures expected of the leaders of the countries in need of these products for safe delivery to the target population after two decades is also highlighted.

Contents

Peer review under responsibility of National Research Center, Egypt.

* Permanent Address: Department of Food Science and Technology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.
E-mail address: aabadejo@futa.edu.ng

https://doi.org/10.1016/j.jgeb.2018.02.010
1687-157X/

1. Introduction

One in nine, of all humanity, is suffering from hunger with the majority resident in developing countries [1]. Interestingly, the world is producing enough food to feed all its inhabitants with per capita food availability of 2640 kcal/person/day [2]. The kind of nutrients contained in these foods are as important as the quantity produced. These nutrients are generally classified as either Type I or Type II depending on the effect of the deficiency on the body. Type I nutrients are required for specific metabolic (cellular and molecular) functions in the body rather than the general metabolism. As a result, a deficiency of Type I nutrient normally results in specific physical signs on the individual; they are not obvious but 'hidden' hence 'hidden hunger'. Type II or 'growth' nutrient is required for normal tissue and body development, and deficiency will lead to a reduced overall growth commonly referred to as protein-energy malnutrition in children [3]. Micronutrient malnutrition or hidden hunger of public health concern globally are vitamin A deficiency, Iron deficiency anaemia and iodine deficiency disorder. The underlying cause of this hidden hunger is poverty with sub-segments in poor dietary intake, impaired absorption of the nutrients by the body due to diseases and unhealthy feeding practices. Globally, more than two billion individuals, or one in three people, are being afflicted by micronutrient deficiencies [1].

The Millennium Development Goals ended in 2015. Goal number 1 of the eight goals targeted eradicating poverty and hunger by the year 2015. This includes the provision of food adequate in calories, protein and micronutrients (vitamins and minerals). A total of 72 developing countries out of 129 (less than 60%) reached the Millennium Development Goal's overall hunger target [1]. Indeed, progress was made, yet a lot remains to be achieved. The UN has again come up with a lofty seventeen sustainable development goals of which the first two is to banish poverty and ensure zero hunger by the year 2030 [4].

Over 250 million children are vitamin A deficient with the highest prevalence in Central and West Africa and South Central Asia [5]. Vitamin A must be ingested in the diet as it cannot be synthesized *de novo* by mammals. Vitamin A deficiency is prevalent because more than one billion people living in the developing countries depend on starchy foods such as maize, sweet potato, cassava and rice as staples. Unfortunately, consumption of such staples over a long period has negative effect on mostly pregnant women and children under the age of five. Supplementation, fortification and diversification of diets have been applied to alleviate the problem, albeit with little success. The pressure on household income will not permit to utilize commercially available supplements or fortified food products with costs beyond the reach of myriads of low-income earners in the developing countries. Also, there are lots of constraints to the supplementation and fortification programme of various governments that rendered them less effective with huge financial implications [6]. This leaves biofortification, the development of micronutrient-dense staple crops, as the viable alternative to achieve this goal.

In this review, the biosynthesis of carotenoid is discussed along with some of the economically important crops that have been biofortified by conventional breeding and genetic engineering for increased carotenoid content. The perception and challenges of these technologies in solving the problem of hidden hunger and ensuring food security in the developing world are highlighted along with some measures required by the policy makers to make food security a reality.

2. Carotenoids and food sources

Carotenoids are tetraterpenoid pigmented compounds synthesized by plants. The inability of human to synthesize the compound is the main reason why consumption of fruits and vegetables is essential in their diet [7,8]. Carotenoids are classified into two groups: carotenes (non-oxygenated molecules) and xanthophylls (oxygenated molecules) (Fig. 1). Although over 600 forms of carotenoids have been identified, the bulk of the carotenoids in the diet are represented by β-carotene, α-carotene, lycopene, lutein and β-cryptoxanthin [9]. The provitamin A carotenoids comprising of β-carotene, α-carotene and β-cryptoxanthin can be converted to retinol (vitamin A) within the body. They are found mostly in fruits with yellow and orange colours such as mangoes, papaya, carrots, sweet potatoes and corn in varying

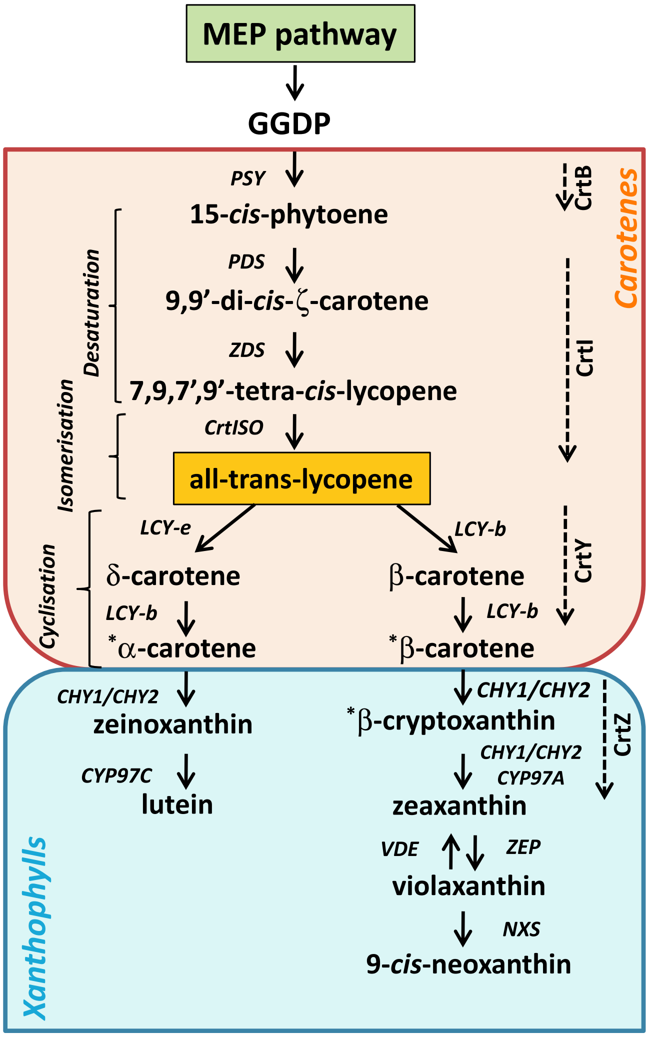

Fig. 1. The carotenoid biosynthesis pathway found in almost all plants. GGDP, Geranylgeranyl diphosphate is derived from the plastidial MEP (methylerythritol 4-phosphate) pathway; PSY, Phytoene synthase; PDS, Phytoene desaturase; ZDS, z-carotene desaturase; CRITSO, Carotenoid isomerase; LCY-b, Lycopene β-cyclase; LCY-e, Lycopene ε-cyclase; CHY1 and CHY2, non-heme β-carotene hydroxylases; CYP97A and CYP97C, heme-containing cytochrome P450 carotene hydroxylases; ZEP, Zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, Neoxanthin synthase; CrtB, CrtI, CrtY and CrtZ are multifunctional enzymes in bacteria catalyzing the steps covered by the broken arrows respectively; All the enzymes in upper cases and italics are functional in plant; *carotenoids with provitamin A activity.

concentrations [8]. Among the provitamin A carotenoid, β-carotene produces twice the vitamin A that is usually got from α-carotene and β-cryptoxanthin. On the other hand, no vitamin A activity is present in lutein, zeaxanthin and lycopene but they are of great importance nutritionally. Dark green vegetables such as spinach, broccoli, green collard and pumpkin are the major sources of lutein. Lycopene (lacking the β-ring end in the provitamin A carotenoids) is responsible for the bright red colour pigment found in tomatoes, watermelon, guava and red bell pepper. Several factors affect the bioavailability of carotenoids in the body including genetics, disease conditions, food matrix types and amounts. Carotenoids in oil droplet are more bioavailable in the body compared to the microcrystalline forms as in the case of lycopene in tomato and β-carotene in carrot [10].

3. Functions of carotenoids

Carotenoids in fruits and vegetables have been reported to be responsible for a number of health benefits conferred on human [7]. Fruits and vegetables rich in carotenoids and other phytochemicals have also been found to play a major role in preventing chronic diseases [7,11]. Particularly, increased lycopene intake has been reported to reduce prostate cancer progression [7] while lower lycopene level has been associated with increased cardiovascular diseases which were corrected when subjects were fed tomato juice [9].

The antioxidant properties of carotenoids have been used to tackle "the silent killer"-hypertension. The symptom of the disease is difficult to identify at the onset, it only become identifiable at an advanced stage when it is fatal. Lycopene has been reported to have a protective role against hypertension [9,12]. A diet approach, containing lycopene and other carotenoids alongside polyphenols and flavanols, has been recommended for the management of hypertension [13].

Xerophthalmia, Bitot's spots, and corneal ulcerations and lesions are disease conditions of the eyes that arise from the deficiency of vitamin A. The presence of provitamin A carotenoid in the diet prevents the manifestation of xerophthalmia. Zeaxanthin and lutein are the main xanthophylls in higher plants and they are the two major carotenoids found in the perifoveal and foveal regions of the retina macular where they scavenge free radicals and protect the macular from light-induced damage [7,14]. The macula of the eye absorbs dietary lutein and protects the retina from blue light thus maintaining optimal vision function [15]. Age-related macular degeneration (AMD) is one of the major causes of vision loss among the aged. Even though meta-analysis data showed that dietary lutein and zeaxanthin is not significantly associated with a reduced risk of early AMD, an increase in the intake of the duo may be protective against late AMD [16].

4. Biosynthesis of carotenoids

Carotenoids can be synthesized *de novo* in plants. The biosynthesis in the plastid begins with isopentenyl pyrophosphate (IPP) synthesized through the cytosolic Mevalonic (MVA) pathway or plastidic non-MVA dependent methylerythritol 4-phosphate (MEP) pathway [17,18]. Four molecules of IPP undergo several condensation reactions to form geranylgeranyl diphosphate (GGDP) through the action of IPP isomerase. Two molecules of GGDP acted upon by phytoene synthase (PSY or CrtB in bacteria) condense to form a 15-*cis* isomer of phytoene (15-*cis*-phytoene) which is the first dedicated compound in the carotenoid biosynthesis pathway (Fig. 1). The biosynthesis entails three main steps viz: desaturation, isomerisation and cyclisation. Phytoene desaturase (PDS) and zeta carotene desaturase (ZDS) are the two plant desaturases performing dehydrogenation reaction on the colourless 15-*cis*-phytoene. PDS is responsible for the desaturation of phytoene to zeta-carotene while ZDS catalyses the desaturation of zeta carotene to the red-coloured lycopene. Phytoene in higher plants exists in the 15-*cis* isomer form; however, the predominant form of lycopene is all-*trans* [19]. Carotenoid (pro-lycopene) isomerases (CrtISO), capable of isomerizing cis bonds at 7, 9 and 7′, 9′ positions, are responsible for the conversion of the poly *cis*-compounds to their all-*trans* forms. This is the place of isomerisation in the pathway. In bacteria, the desaturation and isomerisation reactions are performed by only one enzyme – bacterial *CrtI*. Cyclisation is very important in the pathway as it generates carotenoids diversity distinguished by the different cyclic end groups. Lycopene ε-cyclase (LCY-e) and lycopene β-cyclase (LCY-b) competitively act on the substrate lycopene to form either α-carotene or β-carotene [20]. LCY-e is capable of catalyzing the reaction by introducing two β-rings into lycopene to form β-carotene via δ-carotene. However, when LCY-e acts alone it cyclises only one end of lycopene to forms only the monocyclic δ-carotene, but in conjunction with LCY-b, α-carotene is formed. Downstream of the carotenes pathway is the oxygenated carotenoids (xanthophylls). The β-carotene and α-carotene are hydroxylated by non-heme carotene hydroxylases (CHY1 and CHY2) and heme-containing cytochrome P450 carotene hydroxylases (CYP97A and CYP97C). Hydroxylation of β-carotene forms zeaxanthin via the provitamin A β-cryptoxanthin. However, with α-carotene, zeinoxanthin is formed which when acted upon by cytochrome P450 enzyme forms the yellowish xanthophyll lutein. Violaxanthin and 9-*cis*-neoxanthin are other xanthophylls in the pathway. In the xanthophylls cycle, the activity of zeaxanthin epoxidase (ZDE) on zeaxanthin leads to the formation of violaxanthin which can be converted back to zeaxanthin by violaxanthin deepoxidase (VDE). Neoxanthin is produced from violaxanthin by neoxanthin synthase (NXS) which has been cloned from tomato [21].

5. Increasing the micro-nutrient content of plants

The bulk of staples consumed worldwide are deficient in carotenoids and many essential minerals [22,23]. The occurrence of several diet-related diseases in human led to efforts in developing foods that are laden with micronutrients and vitamins essential for healthy living. Biofortification, which is the development of micronutrient-dense staple crops, can either be carried out through modern biotechnology (genetic engineering) or conventional plant breeding technique (Fig. 2) [24,25,8]. Conventional techniques require the selection of sexually compatible plants that carry hypermorphic alleles (i.e. encoding the required genes for active form of carotenogenic enzymes or the promoter that can enhance the quantity of the enzymes) to be crossed and backcrossed over a long period of time to achieve the desired trait [26]. Marker assisted selection and mutagenesis are often deployed for effective conventional breeding (Fig. 2). In most of the crops, conventional breeding is able to provide significant provitamin A activity; but with crops like rice, potato and wheat, the conventional method is still insufficient [8]. Genetic engineering has the advantage of introducing novel genes under the control of a strong promoter into the desired plant to alter the metabolism through overexpression of enzyme that catalyses the committed steps in the pathway, enhanced activity of enzymes in the rate limiting steps or enhancement of accumulation of target metabolites [27–29]. Antisense and RNAi techniques are also frequently used to decrease metabolic flux downstream of a target compound or on a competing metabolic branch as in the case of potato [30]. In other cases, attempts have been made to increase the metabolic sink where carotenoid is accumulated and also post-harvest stability

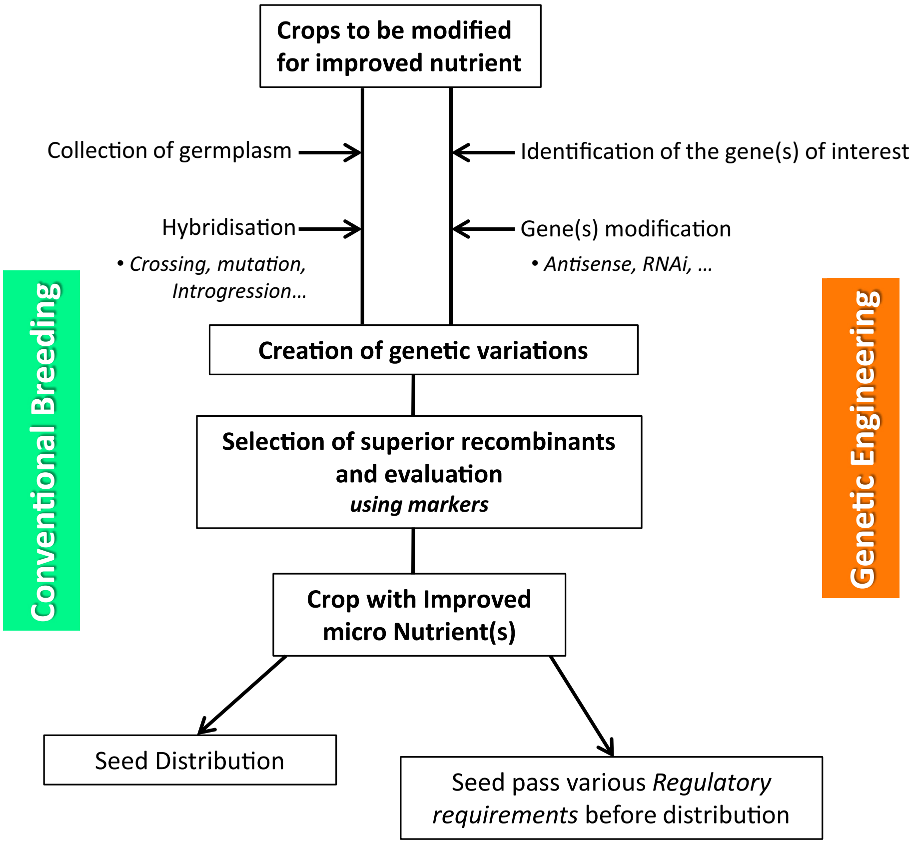

Fig. 2. The interconnectivity of conventional breeding and genetic engineering in the development of crops with enhanced micronutrients.

of carotenoids [8]. Biofortification is currently being used to increase the content of micronutrients in many staple crops. Table 1 shows some staple crops that have been engineered for increased carotenoids.

6. Engineering carotenoids in staple crops

6.1. Rice

The α- and β-carotenes which are provitamin A have been engineered into many food crops because the composition is very low (if present) in many developing countries' staples [26,8]. More than half of the world population eat rice as a staple [31] thus making rice prominently among the various crops that have been genetically engineered for increased carotenoids. Provitamin A and other carotenoids are grossly lacking in rice. Phytoene synthase (PSY) is a rate-limiting enzyme in carotenoid biosynthesis, and varying its expression or activity alters flux through the pathway [32]. The PSY coding sequence obtained by RT-PCR has been cloned by transferring it into pUC-based vector containing maize polyubiquitin promoter and discussed in detail [27]. When PSY from daffodil (*Narcissus pseudonarcissus*) was engineered into rice, it led to an increase in the phytoene content only. However, in combination with phytoene desaturase/isomerase from *Erwinia uredovora* (*crtI*), there were increases in β-carotene, α-carotene, lutein and zeaxanthin, raising carotenoid contents to 1.6 µg/g in the rice endosperm [33] with slight golden colour hence the name 'Golden rice'. This amount of carotenoid is far low compared to the daily requirement. The opponents of biotechnology in plants called it "fool's gold" as three kilograms must be consumed by a two-year old to get the amount required to prevent night blindness [34]. PSY from maize in combination with carotene desaturase (*crtI*) from *E. uredovora* were engineered to produce 'Golden rice 2' (Fig. 3) having 37 mg/g of carotenoid (of which 84% was β-carotene). This is about 23-fold increase in β-carotene content over the initial golden rice [27]. This amount is able to meet the recommended dietary allowance for children under the age of three (300 µg vitamin A) when 60–80 g of the rice is consumed. The Golden rice is currently deposited with the Golden Rice Humanitarian Board (http://www.goldenrice.org/). Conventional breeding technique has not been successful in increasing the β-carotene content in the endosperm of rice because there is no germplasm capable of synthesizing carotenoid in the endosperm.

6.2. Tomato

Tomato is one of the economically important crops in the world. Pathway engineering and improved sequestration machinery have been used in elevating the level of carotenoids (that are of health benefit to human) in tomato [35]. When the bacterial carotenoid gene (*crtI*) that encodes phytoene desaturase which is principally responsible for the conversion of phytoene to lycopene was engineered into tomato, carotenoid level did not change, lycopene content even decreased but β-carotene content increased by 3-fold [36]. Agrobacterium-mediated transformation of tomato with tomato lycopene β-cyclase cDNA isolated by RT-PCR from Red Setter RNA and cloned under the control of the CaMV 35S promoter resulted in 7-fold increase in β-carotene (Fig. 4) [37]. With PSY from *E. uredovora* (*crtB*) expressed in tomato in a fruit specific manner, the transgenic tomato fruits had total carotenoid level that was 3-fold of that present in the control while the lycopene and β-carotene were 1.8- and 2.2-fold higher, respectively [36,38]. In addition to the lycopene and β-carotene that have been the major targets in tomato, effort has also been geared toward increasing the

Table 1
Various crops engineered for increased carotenoid contents.

Crop	Genes Engineered (origin)	Total Carotenoid (FI#)	β-Carotene (FI#)	Other Carotenoids (FI#)	References
Rice	crtI (*Erwinia uredovora*), PSY and LYC-b (Daffodil)	1.6 µg/g	1.6 µg/g*		[33]
	crtI (*Erwinia uredovora*), PSY (*Zea mays*)	36.7 µg/g	31 µg/g	α-Carotene, β-cryptoxanthin	[27]
Potato	crtB, crtI and crtY (*Erwinia herbicola*) driven by tuber-specific patatain 1 and 35S constitutive promoters	114.4 µg/g (20)	47.4 µg/g (3643)	Lutein (23), Zeaxanthin (5.8), violaxanthin (7.9), Neoxanthin (9.5)	[28]
	Silencing of CHY1 and CHY2 (BCH of *Solanum tuberosum*)	14.26 µg/g (2.9)	0.085 µg/g (38)	Violaxanthin (2), Neoxanthin (3.2), Lutein (3.7)	[30]
Maize	LYCB (*Gentiana lutea*), crtI (*Pantoea ananatis*) and PSYI (*Zea mays*) driven by rice prolamin, pea small subunit of Rubisco and low molecular weight wheat glutenin promoters respectively	148.78 µg/g (135)	48.87 µg/g (349)	Lutein (24), Zeaxanthin (127), Lycopene	[62]
	crtB and crtI (*Pantoea ananatis*)	33.6 µg/g (34)	9.8 µg/g (10)	Lutein (22), Zeaxanthin (9)	[61]
Tomato	psy1 (tomato)	2276.7 µg/g (1.14)	819 µg/g (1.4)	Lutein (1.2)	[76]
	crt1 (*Erwinia uredovora*)	1372 µg/g**	520 µg/g (1.9)	Lutein (2.3), a-carotene	[36]
	crtB (*Erwinia uredovora*)	5918 µg/g (2)	825 µg/g (2.2)	Lycopene (1.8)	[38]
Cassava	Bacterial PSY; crtB (*Pantoea ananatis*) driven by Cassava CP1 promoter	21.84 µg/g (33.6)	6.67 µg/g (16)	Xanthophylls (8)	[22]

Fold increase relative to the wild type.
* Almost all the carotenoid are in β-carotene form.
** Total carotenoid did not increase only β-carotene and lutein. Fold increases that were not recorded in the original article were not included.

amount of xanthophylls. With the expression of *crtB* there were also increases in the levels of lutein, neoxanthin and zeaxanthin in tomato fruit [36]. Arabidopsis LCY-b and *Capsicum* β-carotene hydroxylase (CHY) under the control of tomato ripening induced promoter (Pds) engineered into tomato resulted in fruits with increased level of zeaxanthin and β-cryptoxanthin [39]. Upregulation of the isoprenoid precursors have also been reported to lead to increase in carotenoid contents of tomato [40].

6.3. Potato

Carotenoids levels are generally low in potatoes when compared to most fruits and vegetables. However, the South American *Solanum phureja* that lacks a dormant tuber had higher level of carotenoids of more than 3-fold compared with the common *S. tuberosum* variety [41]. The open reading frame of *crtB* gene has been cloned in the derivative vector pBIN19 and transformed into LBA4404 strain of *Agrobacterium tumefaciens* by electroporation [41]. Overexpression of this bacterial phytoene synthase gene in potato resulted in increases of about 4- and 7-fold in the carotenoid contents of *S. phureja* and *S. tuberosum* respectively with the major carotenoids being β-carotene, lutein and violaxanthin [41].

Silencing of the two β-carotene hydroxylases in sweet potato preventing the conversion of β-carotene to zeaxanthin resulted in increase of up to 38-fold in β-carotene level and up to 4.5-fold in total carotenoids content of the transgenic tubers [30]. Overexpression of LCY-b gene in potato however was only able to raise the β-carotene content level by 1.9-fold [42]. Multigene engineering used in transgenic potatoes expressing three genes, encoding *crtB*, *crtI* and *crtY* from *Erwinia* (Fig. 1) under the control of tuber-specific or constitutive promoters, resulted in 3600-fold increase compared to the wild type (Fig. 5). The β-carotene content of 47 µg/g in the transgenic potato made it the biofortified staple so far with the highest β-carotene content [28]. Other carotenoids downstream in the pathway were also elevated significantly; zeaxanthin (11 µg/g dry weight, a 5.8-fold increase) and lutein (23.1 µg/g, a 23-fold increase). The transgenic potato is able to provide 50% of the Recommended Daily Allowance of vitamin A with the consumption of 250 g fresh weight.

When different varieties of potato were transformed by the sense and antisense constructs encoding zeaxanthin epoxidase (ZEP) under the control of potato granule-bound starch promoter the resulting transgenic tubers had zeaxanthin level of over 100-fold and total carotenoid of more than 5-fold compared to the control [43]. This is because the conversion of zeaxanthin to violaxanthin has been suppressed, thus the resultant accumulation of zeaxanthin.

The orange coloured potatoes developed through conventional breeding method have all-*trans*- β-carotene (major provitamin A carotenoid) contents ranging from 108 to 315 µg/g of dry matter with more than 70% retention after processing [44,45]. These potatoes have been widely accepted in many countries [46]. The South Korean 'Golden Valley' cultivar showed high expression of the first three genes in the carotenoid biosynthesis pathway and this correlates with the total carotenoids within the tubers [47].

6.4. Cassava

Cultivation of cassava requires less human input and it produces more energy per unit area than most other crops [48]. It is the staple for more than 500 million people in Africa where the root is the most consumed part of the plant. Recently, its use in poultry diet has also increased [49]. Over 70% of the total world cassava is produced in five countries – Nigeria, Brazil, Indonesia, DR Congo and Thailand [50]. Cassava root is laden with cyanogenic glucoside (linamarin) which has to be removed by several food

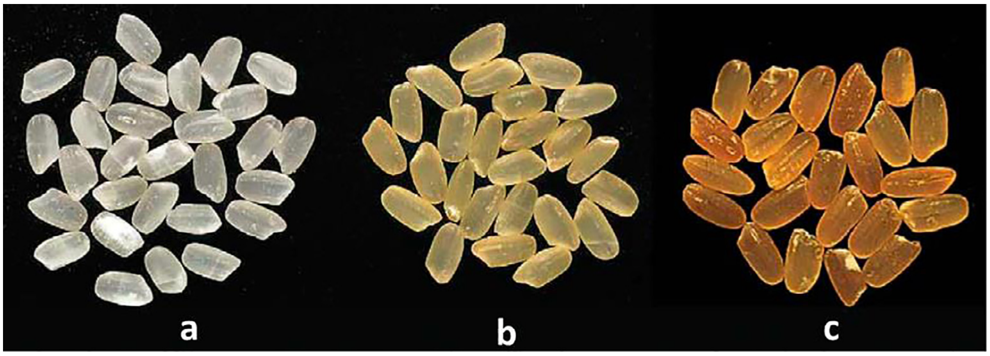

Fig. 3. Enhancement of carotenoid content of rice endosperm. Wild-type rice (a); Golden Rice 1, expressing the phytoene synthase from daffodil (*Narcissus pseudonarcissus*) along with *Erwinia uredovora crtI* gene (b); Golden Rice 2 expressing the phytoene synthase from maize (*Zea mays*) along with *crtI* (c). *Source:* Paine et al. [27].

Fig. 4. Transversal (a) and longitudinal (b) sections of ripe tomatoes from transgenic (top) expressing tomato lycopene b-cyclase showed all parts having intense orange colour, and control plants (bottom). *Source:* D'Ambrosio et al. [37].

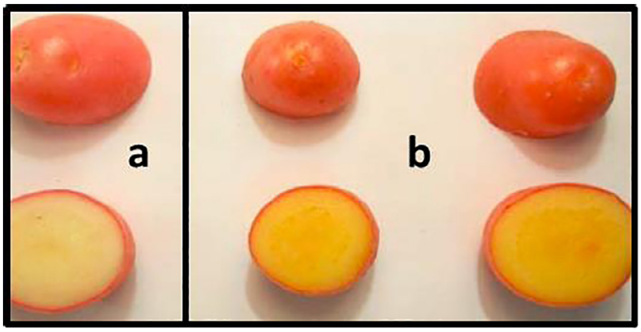

Fig. 5. Enhancement of carotenoid content of potato. Wild type variety (a) and transgenic varieties (b) expressing *crtY*, *crtB* and *crtI* under the control of *Pat1* and *Pat2* promoters. *Source:* Diretto et al. [28].

processing operations for it to be safe for human consumption. Cassava meal of about 500 g will meet the energy requirement of an adult but only 10% of the daily provitamin A (β-carotene) requirement with very low and poor quality protein [51,49]. The BioCassava Plus (BC+) programme under Donald Danforth Plant Science Center, St. Louis, MO, USA has targeted development of transgenic cassava with provitamin A among other nutrients [48]. It has been established that PSY is being overexpressed in the yellow coloured cassava cultivars (Fig. 6) and this has been linked to the high concentration of carotenoid in the cultivars [22]. The number of PSY genes varies depending on the type of plant. However, two PSYs (PSY1 and PSY2) have been reported to be present in cassava and responsible for the elevated carotenoid contents with PSY2 playing the major role in the root with about ten-fold in expression compared to PSY1 [52]. Between 2005–2010, the BC + programme was able to develop cassava cultivars with improved provitamin A through modern biotechnology [48] and these cassava varieties have been used for field trial in Nigeria. Unfortunately, till date no genetically modified cassava has been released in Africa.

Fig. 6. Peeled cassava roots showing the white wild type (a) and the transgenic carotenoid-laden variety (b) expressing bacterial PSY (*crtB*) gene. *Source:* Welsch et al. [22].

HarvestPlus, one of the program of Consultative Group for International Agricultural Research (CGIAR) funded by Bill and Melinda Gates, the UK Government, European Commission and the US Government Feed the Future Initaitive, also developed many yellow-fleshed cassava cultivars with elevated provitamin A content through plant breeding (non-genetically modified) and are currently being explored for use by farmers and cassava processors [53,54,22,24,48,55,23]. Welsch et al. [22] showed that single nucleotide polymorphism present in PSY2 was able to enhance flux of carbon through the pathway leading to elevated provitamin A carotenoid in the cassava root after breeding pedigree. Several other crops that have been biofortified by HarvestPlus for high content of provitamin A include maize, banana/plantain, pumpkin, sweet potato and squash, most of which are either already released for use by farmers or under testing in various countries across the globe [53,56,23].

6.5. Maize

Some tropical yellow-maize varieties analyzed for carotenoids content showed that the non-provitamin A xanthophylls (lutein and zeaxanthin) are higher in concentration compared to the provitamin A carotenoid (α-carotene, β-carotene and β-cryptoxanthin) and they vary across the varieties [57]. An allelic variation at the Y1 locus that regulates the synthesis of phytoene synthase has been reported along with the polymorphic sites at the Y1 locus that are associated with endosperm colour phenotype [58]. The variation in the provitamin A and non-provitamin A carotenoid of these tropical varieties is a potential for selection of parent lines for breeding and genetic improvement [57,26]. Four natural lycopene ε-cyclase polymorphisms have been reported in maize and when bred for low lycopene ε-cyclase, there was an increase in the level of β-carotene, lutein and zeaxanthin [59]. Also, Yan et al. [60] showed that three polymorphisms in the gene encoding β-carotene hydroxylase (*crtRBI*) is associated with β-carotene concentration in maize kernels. The *crtRBI* alleles unique to temperate germplasm was introgressed via PCR-assisted marker selection to tropical maize germplasm for use in developing countries [60]. Overexpression of the bacterial *crtB* and *crtI* under the control of endosperm specific promoter 'super γ-zein' resulted in up to 33-fold increase in the total carotenoid content and up to 25-fold increase in provitamin A carotenoid of the maize endosperm (Fig. 7) and this was reproducible over four generations [61]. The increase in the provitamin A carotenoid was shown to be a result of up-regulation of the endogenous lycopene β-cyclase. The flux through the overexpression is distributed across the entire pathway, making the possibility of not just increasing the β-carotene content but also most of the other downstream carotenoids very high [26]. Similarly, with multigene metabolic engineering, the overexpression of phytoene synthase 1, phytoene desaturase, β-carotene hydroxylase, lycopene β-cyclase and β-carotene ketolase in maize under the control of different endosperm-specific promoters resulted in transgenic maize with enhanced carotenoid content of more than 130-fold compared to the white maize with most of the transgenic lines having elevated lycopene, β-carotene and zeaxanthin as the predominant carotenoids [62]. Detailed multi-gene metabolic engineering of other crops had been reviewed [63].

7. Perception and challenges facing genetically biofortified crops

More than 250 million children under the age of six are suffering from vitamin A deficiency and unfortunately about half a million of them become blind each year with more than 200,000 losing their lives after the blindness. Since 1999 till date no scientific fault has been documented relating to the safety of the genetically biofortified golden rice, yet it has not been made available to those who needed it the most. Also, none of the genetically biofortified crops have been released for cultivation or consumption [64]. The 'bureaucratic and unjustified' delay is killing and has deprived more than eight million children of sight since 1999 [65]. Fortification and supplementation of micronutrients in food, that could have delivered similar result as the golden rice, have been used for decades with little success stories. They have been largely hampered by expensive distribution logistics in developing nations and total reliance on interventions from donors [66].

The unfortunate announcement of genetically engineered crops at a time when consumption of meat with bovine spongiform encephalopathy was causing incurable Creutzfeld-Jakob disesase in human across Europe in 1996 led to the pre-emptive regulations before any facts were gathered relating to human health and environment [67]. The controversies notwithstanding, there has been a steady increase in the global land area covered by genetically engineered crop in the past two decades and no reported adverse effects upon those land areas or the health of the inhabitants [68]. Part of the measures to safeguard the human, plant and the

Fig. 7. Enhancement of carotenoid content of maize. (a) Ears from *crtB/crtI* cotransformants regenerated from callus and crossed with Hi-II (white maize). (b) Wild type (top) and transgenic (bottom) obtained from overexpression of *Zmpsy1*, corn phytoene synthase 1 and *PacrtI*, *Pantoea ananatis* phytoene desaturase. *Sources:* Aluru et al. [61] and Naqvi et al. [77].

environment in the pursuit of genetic engineering is the Precautionary Principle, which can be triggered with full understanding only when threat is identified [69].

Obviously, no technology is pursued without accruing benefits. When tangible benefits are not available however, technologies can be rejected even in the absence of any documented risk [67]. Multifaceted researches (including genetic engineering) to develop drugs are greatly encouraged as the benefit to all is immeasurable. If it is allowed in drugs, the big question then will be, why not in foods? Perhaps because of the absence of compelling benefit. This double standard on 'only genetically modified foods' is against the Precautionary Principle as recommended by the European Commission [69]. Europe is highly food secured compared to most African and Asian countries suffering greatly from food insecurity, thus the urgent need for critical decision on the part of policy makers regarding what will benefit the people [70].

Intellectual Property right relating to agricultural biotechnology has contributed to the growth of the sector. Research-intensive private sector uses Intellectual Property rights to protect their agricultural biotechnology-based products. This is to enable them profit from the huge resources expended on the research. The rise in the number of patents made 'freedom-to-operate' (FTO) opinion indispensable to prevent infringement. FTO is a legal assessment detailing whether a research project or the development of a new product can proceed with a low, or tolerable, likelihood that associated activities will not infringe existing patents or other types of Intellectual Property rights [71]. FTO opinion is vital at the inception of humanitarian projects intended to develop genetically modified crops to forestall wastage of resources. Intellectual property has always been a major hurdle to genetically modified crops, along with public perception and regulatory processes. A study reported that Golden rice is a product of about 70 patented technologies from 30 different institutions [72]. This can be described as the 'tragedy of the anti-common' where, proliferation and fragmentation of Intellectual Properties ownership across multiple owners, prevents any single institution or company from assembling all of the necessary rights to produce a product, thus resulting in the underuse (or non-use) of resources [71]. While Patents and Intellectual properties are intended to encourage investment in research and development, the development of an 'anti-commons' has the opposite effect of blocking innovation and in this case, the at-risk population suffers.

The media and activists greatly influence public opinion on acceptance and willingness-to-pay (the amount the consumers are willing to pay for biofortified crops over the price of the non-biofortified). This ultimately dictates the kind of policies rolled out by the government. In this regard, transparent communication measures revealing both sides of the coin scientifically rather than presumption will play invaluable roles. With positive information, consumers' willingness-to-pay can be as high as 20% [73].

8. Role of government and policy makers in the developing countries

Connecting the perception and challenges together, it is high time policy makers in the developing countries decide appropriately while weighing critically scientific evidences on genetic modification and genetically assisted conventional breeding in providing adequate staple crops with micronutrients for the teaming population. This can be achieved among other measures by:

1. Consciously funding agricultural biotechnology by the governments of the various developing countries with short, medium and long term goals will doubtless bring about great return and a leap out of poverty and hunger. The high willingness-to-pay for biofortified staples indicated by the consumers will in no small measure contribute to the economic growth of the countries. The government should therefore set up a unifying mechanism for all the stakeholders across the value chain through various national agricultural policies from crop breeding, cultivation and food processing, to extension services to bring an end to 'hidden' hunger.
2. Making clear demarcation between profit-oriented genetically modified crops that benefit the farmers more, and public health-oriented biofortified crops which benefits the consumers. Since biofortified crops will be meeting 'hidden' health needs rather than the obvious, there is the need for purposeful policy/governmental interventions that aim to augment the cost of buying multi-biofortified crops by the target group as these crops may be priced more than the non-biofortified varieties. Subsidized fertilizers for the growers of biofortified crops will facilitate incorporation of the biofortified staples into the various countries nutrition plan to feed vulnerable age groups.
3. Providing incentives to attract international private organizations to developing countries can make research and development domiciliary, which may result in the use of the technologies with minimal constraints. Obviously, the private sector cannot single-handedly solve the problem. Some Agencies and Foundations are currently focusing on conventional breeding, genomics applications and genetic-marker-based selection as well as genetic modification. There is the need for strategic communication and strong advocacy on purposeful public–private partnership, where funding and expertise collaborate to find lasting solution to hidden hunger.
4. Lending support, at international forums, for relaxed regulatory measures on genetically modified crops targeted to and for developing nations with functional Biosafety regulations to guide the use of genetically biofortified crops. Also, subjecting media reports to scrutiny similar to those of scientific reports will reduce publishing of negative data without verification and deliberate suppression of positive information by omission.

9. Conclusion

While the gene pool of the host and genetic variation is key to conventional plant breeding, genetic modification operates beyond this level with great potential for success. After 20 years since genetically modified crops have been commercialized and subjected to safety tests, no identifiable health issues have been reported. However, the regulatory bottleneck has kept all the energies at the inertia state. Currently, more than 20 million people in developing countries are either cultivating or consuming the conventionally biofortified crops [23]. However, report has shown that transcriptome alteration was lesser in transgenic (genetically modified) than mutagenized (conventional breeding) plants [74]. Without doubt both technologies give ray of hope to those who desperately need the output as this can stop the 6,000 death per day that is currently being experienced due to vitamin A deficiency. Policy makers in the developing countries need to consider the peculiarities of each local community in order to formulate embracing strategies to tackle the menace of vitamin A deficiency. There is need also to explore the new emerging conventional breeding techniques such as cisgenesis and intragenesis [75] which promise faster delivery than what is currently obtained and possibly workable in most of the important staple crops.

Conflict of interest

There is no conflict of interest to be declared.

Acknowledgement

The author was supported by DBT-TWAS postdoctoral fellowship with FR number: 3240287112. Prof. M.K. Modi is greatly appreciated for giving access to use his laboratory while writing the manuscript.

References

[1] FAO, IFAD, WFP. The state of food insecurity in the world 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO; 2015.

[2] FAO. Food and Agriculture Organization. FAO Statistical Yearbook 2012; 2012 <http://www.fao.org/docrep/015/i2490e/i2490e03a.pdf> [accessed December 2016].

[3] Golden MHN. Specific deficiencies versus growth failure: Type I and Type II nutrients. J Nutri Environ Med 1996;6:301–8.

[4] FAO. FAO and the 17 Sustainable Development Goals. <www.fao.org>; 2015.

[5] United Nations. System Standing Committee on Nutrition - 6th report on the world nutrition situation. Geneva; 2010.

[6] Farre G, Ramessar K, Twyman RM, Capell T, Chritou P. The humanitarian impact of plant biotechnology: recent breakthroughs vs bottlenecks for adoption. Curr Opin Plant Biol 2010;13:219–25.

[7] Woodside JV, McGrath AJ, Lyner N, McKinley MC. Carotenoids and health in older people. Maturitas 2015;80:63–8.

[8] Giuliano G. Provitamin A biofortification of crop plants: a gold rush with many miners. Curr Opin Biotechnol 2017;44:169–80.

[9] Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res 2007;55:207–16.

[10] Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 2014;6(2):466–88.

[11] Vieira AR, Aba L, Vingeliene S, Chan DSM, Aune D, Navarro-Rosenblatt D, Stevens C, Greenwood D, Norat T. Fruits, vegetables and lung cancer risk: a systematic review and meta-analysis. Ann Oncol 2016;27:81–96.

[12] Hozawa A, Jacobs Jr DR, Steffes MW, Gross MD, Steffen LM, Lee DH. Circulating carotenoid concentrations and incident hypertension: the coronary artery risk development in young adults (CARDIA) study. J Hypertension 2009;27:237–42.

[13] Most MM. Estimated phytochemical content of the dietary approaches to stop hypertension (DASH) diet is higher than in the control study diet. J Am Diet Assoc 2004;104:1725–7.

[14] Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 2001;385:28–40.

[15] Ahmed SS, Lott MN, Marcus DM. The macular xanthophylls. Surv Ophthalmol 2005;50(50):183–93.

[16] Ma L, Dou H-L, Wu Y-Q, Huang Y-M, Huang Y-B, Zou Z-Y, Lin X-M. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. Brit J Nutr 2012;107:350–9.

[17] Rodriguez-Concepcion M. Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. Phytochem Rev 2006;5:1–15.

[18] Moise AR, Al-Babili S, Wurtzel ET. Mechanistic aspects of carotenoid biosynthesis. Chem Rev 2014;114:164–93.

[19] Nisar N, Li L, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. Mol Plant 2015;8:68–82.

[20] Giuliano G, Tavazza R, Diretto G, Beyer P, Taylor MA. Metabolic engineering of carotenoid biosynthesis in plants. Trends Biotechnol 2008;26:139–45.

[21] Neuman H, Galpaz N, Cunningham Jr FX, Zamir D, Hirschberg J. The tomato mutation nxd1 reveals a gene necessary for neoxanthin biosynthesis and demonstrates that violaxanthin is a sufficient precursor for abscisic acid biosynthesis. Plant J 2014;78:80–93.

[22] Welsch R, Arango J, Bar C, Salazar B, Al-Babili S, Beltran J, Chavarriaga P, Ceballos H, Tohme J, Beyer P. Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. Plant Cell 2010;22:3348–56.

[23] Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global Food Security 2017;12:49–58.

[24] Njoku DN, Gracen VE, Egesi CN, Asante I, Offei SK, Okogbenin E, Kulakow P, Eke-okoro ON, Ceballos H. Breeding for enhanced β-carotene content in cassava: constraints and accomplishments. J Crop Improve 2011;25:560–71.

[25] Khush GS, Lee S, Cho JI, Jeon JS. Biofortification of crops for reducing malnutrition. Plant Biotechnol Rep 2012;6:195–202.

[26] Farre G, Bai C, Twyman RM, Capell T, Christou P, Zhu C. Nutritious crops producing multiple carotenoids – a metabolic balancing act. Trends Plant Sci 2011;16:532–40.

[27] Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 2005;23:482–7.

[28] Diretto G, Al-Babili S, Tavazza R, Papacchioli V, Beyer P, Giuliano G. Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial minipathway. PLoS ONE 2007;2:e350.

[29] Badejo AA, Eltelib HA, Fukunaga K, Fujikawa Y, Esaka M. Increase in ascorbate content of transgenic tobacco plants overexpressing the acerola (*Malpighia glabra*) phosphomannomutase gene. Plant Cell Physiol 2009;50:423–8.

[30] Diretto G, Welsch R, Tavazza R, Mourgues F, Pizzichini D, Beyer P, Giuliano G. Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. BMC Plant Biol 2007;7:11.

[31] Mohanty S. Trends in global rice consumption. Rice Today 2013;12:44–5.

[32] Rodriguez-Villalon A, Gas E, Rodriguez-Concepcion M. Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark grown Arabidopsis seedlings. Plant J 2009;60:424–35.

[33] Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, et al. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 2000;287:303–5.

[34] Enserink M. Tough lessons from golden rice. Science 2008;320. 486-471.

[35] Fraser PD, Enfissi EMA, Bramley PM. Genetic engineering of carotenoid formation in tomato fruit and the potential application of systems and synthetic biology approaches. Arch Biochem Biophys 2009;483:196–204.

[36] Romer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM. Elevation of the provitamin A content of transgenic tomato plants. Nat Biotechnol 2000;18:666–9.

[37] D'Ambrosio C, Giorio G, Marino I, Merendino A, Petrozza A, Salfi L, Stigliani AL, Cellini F. Virtually complete conversion of lycopene into β-carotene in fruits of tomato plants transformed with the tomato lycopene b-cyclase (tLCY-b) cDNA. Plant Sci 2004;166:207–14.

[38] Fraser PD, Romer S, Shipton CA, Mills PB, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc Natl Acad Sci USA 2002;99:1092–7.

[39] Dharmapuri S, Rosati C, Pallara P, Aquilani R, Bouvier F, Camara B, Giuliano G. Metabolic engineering of xanthophyll content in tomato fruits. FEBS Lett 2002;519:30–4.

[40] Botella-Pavia P, Rodriguez-Concepcion M. Carotenoid biotechnology in plants for nutritionally improved foods. Physiol Planta 2006;126:369–81.

[41] Ducreux LJM, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. J Exp Bot 2005;56:81–9.

[42] Song XY, Zhu WJ, Tang RM, Cai JH, Chen M, Yang Q. Overexpression of StLCYb increases β-carotene accumulation in potato tubers. Plant Biotechnol Rep 2016;10:95–104.

[43] Romer S, Lubeck J, Kauder F, Steiger S, Adomat C, Sandmann G. Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. Metab Eng 2002;4:263–72.

[44] Bengtsson A, Namutebi A, Alminger ML, Svanberg U. Effects of various traditional processing methods on the all-trans- β-carotene content of orange-fleshed sweet potato. J Food Comp Anal 2008;21:134–43.

[45] HarvestPlus. Disseminating Orange-Fleshed Sweet Potato: Uganda Country Report. Washington, D.C.; 2012.

[46] Hotz C, Loechl C, Lubowa A, Tumwine JK, Ndeezi G, Masawi AN, Baingana R, Carriquiry A, de Brauw A, Meenakshi JV, Gilligan DO. Introduction of β-carotene–rich orange sweet potato in rural Uganda results in increased vitamin A intakes among children and women and improved vitamin A status among children. J Nutr 2012;142:1871–80.

[47] Goo Y-M, Kim T-W, Ha S-H, Back K-W, Bae J-M, Shin Y-W, Lee C-H, Ahn M-J, Lee S-W. Expression profiles of genes involved in the carotenoid biosynthetic pathway in yellow-fleshed potato cultivars (*Solanum tuberosum* L.) from South Korea. J Plant Biol 2009;52:49–55.

[48] Sayre R, Beeching JR, Cahoon EB, Egesi C, Fauquet C, Fellman J, Fregene M, Gruissem W, Mallowa S, Manary M, Maziya-Dixon B, Mbanaso A, Schachtman DP, Siritunga D, Taylor N, Vanderschuren H, Zhang P. The Bio-Cassava Plus Program: biofortification of Cassava for Sub-Saharan Africa. Ann Rev Plant Biol 2011;62:251–72.

[49] Morgan NK, Choct M. Cassava: nutrient composition and nutritive value in poultry diets. Animal Nutr 2016;2:253–61.

[50] FAO. Food and Agriculture Organization of the United Nations). Food Outlook Biannual report on global food markets; 2014.

[51] FAO/WHO. Vitamin A. In: FAO/WHO Expert consultation on human vitamin and mineral requirements. Rome: World Health Org./Food Agric. Org. United Nations. p. 87–107; 2002 [chapter 7].

[52] Arango J, Wust F, Beyer P, Welsch R. Characterization of phytoene synthases from cassava and their involvement in abiotic stress-mediated responses. Planta 2010;232:1251–62.

[53] Adenle AA, Aworh OC, Akromah R, Parayil G. Developing GM super cassava for improved health and food security: future challenges in Africa. Agric Food Security 2012;1:11.

[54] Thakkar SK, Maziya-Dixon B, Dixon AG, Failla ML. Β-carotene micellarization during in vitro digestion and uptake by Caco-2 cells is directly proportional to β-carotene content in different genotypes of cassava. J Nutr 2007;137:2229–33.

[55] Nduwumuremyi A, Melis R, Shanahan P, Asiimwe T. Introgression of antioxidant activity into cassava (*Manihot esculenta* C.): an effective technique for extending fresh storage roots shelf life. Plant Breeding 2016;135:1–8.

[56] HarvestPlus. Biofortified staple food crops: who is growing what; 2016 <www.harvest plus.org> [accessed December 12, 2016].

[57] Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T. Carotenoid diversity in tropical-adapted yellow maize inbred lines. Food Chem 2008;109:521–9.

[58] Palaisa KA, Morgante M, Williams M, Rafalski A. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. Plant Cell 2003;15:1795–806.

[59] Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, et al. Natural genetic variation in lycopene ε-cyclase tapped for maize biofortification. Science 2008;319:330–3.

[60] Yan J, Kandianis CB, Harjes CE, Bai L, Kim EH, Yang X, Skinner DJ, Fu Z, Mitchell S, Li Q, Fernandez MGS, Zaharieva M, Babu R, Fu Y, Palacios N, Li J, DellaPenna D, Brutnell T, Buckler ES, Warburton ML, Rocheford T. Rare genetic variation at *Zea mayscrtRB1* increases β-carotene in maize grain. Nat Genet 2010;42:322–7.

[61] Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Wang K, Rodermel S. Generation of transgenic maize with enhanced provitamin A content. J Exp Bot 2008;59 (13):3551–62.

[62] Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P, Capell T. Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. Proc Natl Acad Sci USA 2008;105:18232–7.

[63] Giuliano G. Plant carotenoids: genomics meets multi-gene engineering. Curr Opin Plant Biol 2014;19:111–7.

[64] De Steur H, Mehta S, Gellynck X, Finkelstein JL. GM biofortified crops: potential effects on targeting the micronutrient intake gap in human populations. Curr Opin Biotechnol 2017;44:181–8.

[65] Potrykus I. Regulation must be revolutionized. Nature 2010;466:561.

[66] Beyer P. Golden rice and ‘golden’ crops for human nutrition. New Biotechnol 2010;27(5):478–81.

[67] Paarlberg R. Starved for science: how biotechnology is being kept out of Africa. Harvard University Press; 2008.

[68] Farre G, Twyman RM, Zhu C, Capell T, Christou P. Nutritionally enhanced crops and food security: scientific achievements versus political expediency. Curr Opin Biotechnol 2011;22:245–51.

[69] Tagliabue G. The precautionary principle: its misunderstandings and misuses in relation to “GMOs”. New Biotechnol 2016;33(4):437–9.

[70] Whitty CJM, Jones M, Tollervey A, Wheeler T. Africa and Asia need a rational debate on GM crops. Nature 2013;497:31–3.

[71] Alandete-Saez M, Chi-Ham C, Graff G, Boettiger S, Bennett AB. Intellectual property in Agricultural biotechnology: strategies for open access. In: Stewart Jr CN, editor. Plant biotechnology and genetics: principles, techniques, and applications. Hoboken, New Jersey: John Wiley & Sons Inc; 2015. p. 347–63.

[72] Kryder RD, Kowalaski SP, Krattiger AF. The intellectual and technical property components of pro vitamin-A rice (Golden RiceTM): a preliminary freedom-to-operate review, ISAAA Brief No. 20, ISAAA, Ithaca, New York; 2000.

[73] De Steur H, Demont M, Gellynck X, Stein AJ. The social and economic impact of biofortification through genetic modification. Curr Opin Biotechnol 2017;44:161–8.

[74] Batista R, Saibo N, Lourenco T, Oliveira MM. Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. Proc Natl Acad Sci USA 2008;105:3640–5.

[75] Schaart JG, van de Wiel CCM, Lotz LAP, Smulders MJM. Opportunities for products of new plant breeding techniques. Trends Plant Sci 2016;21 (5):438–49.

[76] Fraser PD, Enfissi EMA, Halket JM, Truesdale MR, Yu D, Gerrish C, Bramley PM. Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids, and intermediary metabolism. Plant Cell 2007;19:3194–211.

[77] Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, Conesa DP, Ros G, Sandmann G, Capell T, Christou P. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci USA 2009;106:7762–7.