Maize Carotenoid Composition and Biofortification for Provitamin A Activity

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Abstract

Carotenoids are fat-soluble antioxidant vitamin compounds derived from the isoprenoid biosynthetic pathway. These natural pigments are secondary metabolites and can be divided into two classes—carotenes and xanthophylls—which play diverse biological roles in plants and animals. Carotenoids with unsubstituted β-ring end groups become more important because of their provitamin A activity. Maize is the third most staple food worldwide and also contains appreciable amount of provitamin A carotenoids with wide range of genetic variability. This makes it a good candidate crop for biofortification of provitamin A carotenoids. The quantity of provitamin A carotenoids needed to alleviate vitamin A deficiency (VAD) through biofortification depends upon its bioavailability, which is influenced by a number of factors in an individual. The bioavailability of biofortified maize can be known through determining vitamin A equivalence. Recent advances have shown that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A. So, a quantity of 15 μg provitamin A g^{-1} dry weight of kernel was targeted for biofortification. This chapter also includes the carotenoid biosynthetic pathway, biofortification strategies, recent advancements made toward biofortification of provitamin A, and future perspectives.

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7.1 Introduction

Carotenoids are natural pigments derived from the isoprenoid biosynthetic pathway and produced by most photosynthetic organisms that have been shown to be beneficial to both plants and animals. Carotenoids represent a diverse group of more than 750 structures found in

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bacteria, fungi, algae, and plants (Britton et al. 2004). Of the carotenoids found in nature, 20–50 are common in the human diet and about 20 are found in human blood and tissues (Johnson 2004). Biochemically, these secondary metabolites are terpenoids and constitute a class of fat-soluble antioxidant vitamin compounds containing polyisoprenic structure. There are two distinct classes of carotenoids—carotenes, which contain only carbon and hydrogen, and xanthophylls, which contain oxygen groups (Van den Berg et al. 2000). Carotenoids are lipophilic and are found in hydrophobic areas of cells in close proximity to proteins and lipids (Britton 1995; Van den Berg et al. 2000). Carotenoids play various biological roles in plants and animals. Some recent studies suggesting that greater intakes of carotenoid-containing foods result in reduced risks for several chronic diseases have stimulated greater interest in carotenoids (Canene-Adams et al. 2005). The exact chemical structure of individual carotenoids is decisive for their biological properties because it determines how they interact with other molecules and integrates into membranes (Rouseff et al. 1996).

Carotenoids can be divided into two groups, provitamin A and non-provitamin A carotenoids, depending upon their ability to release vitamin A. Vitamin A deficiency has emerged as a serious global health concern. A sustainable solution to eliminate VAD is increasing the provitamin A carotenoids in the major staple food crops, i.e., through biofortification. As maize is a staple food worldwide and also rich in natural genetic diversity for provitamin A carotenoids, qualify as a suitable candidate crop for biofortification (Wurtzel 2004). Recent advancements in areas of genetics, biotechnology, biochemistry, and analytical tools have led to the identification of QTLs, genes/alleles, or allozymes controlling the regulatory steps of carotenoid biosynthetic and utilizing pathways. Utilizing the available natural genetic variability for provitamin A carotenoids, maize breeders have succeeded in developing biofortified maize lines containing $\leq$15 μg β-carotene/g dry kernel weight (Yan et al. 2010). So, this chapter elaborates the importance of carotenoids, various findings related to metabolic pathway, developments, and future strategies toward biofortification of maize.

7.2 Biosynthetic Pathway

The biosynthesis of carotenoids in plants occurs on membranes of plastids with the regulating enzymes encoded in the nuclear genes and targeted to these plastids (Gallagher et al. 2004). Carotenoids are derived from the isoprenoid biosynthetic pathway and are precursors of the plant hormone abscisic acid and of other apocarotenoids. The first committed step of this pathway involves the condensation of two geranylgeranyl pyrophosphate (GGPP) to form 15-cis-phytoene, a colorless C40 compound. This constitutes the key regulatory step of the pathway and is catalyzed by phytoene synthase (PSY). Phytoene is converted to all-trans lycopene (a red pigment) through a series of reactions mediated by phytoene desaturase (PDS), ζ-carotene isomerase (Z-ISO), ζ-carotene desaturase (ZDS), and carotene isomerase (CRTISO). The first branch point of this pathway occurs at cyclization of lycopene cyclized by lycopene ε-cyclase (LCYE) and/or lycopene β-cyclase (LCYB) to generate α- and β-carotenes, respectively. Relative activities of LCYB and LCYE are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway. α- and β-carotenes are subsequently hydroxylated and modified to form the various xanthophylls. The carotenoid biosynthetic pathway in plants is shown in Fig. 7.1.

7.3 Biological Roles

In plants and animals, these pigments play diverse biological roles as mentioned below:

As natural pigments, carotenoids are responsible for different colors present in plant parts, which is determined by the number and location of the double bonds present within the structure (Watson 1962). As accessory pigments in the photosynthetic apparatus, they play various roles

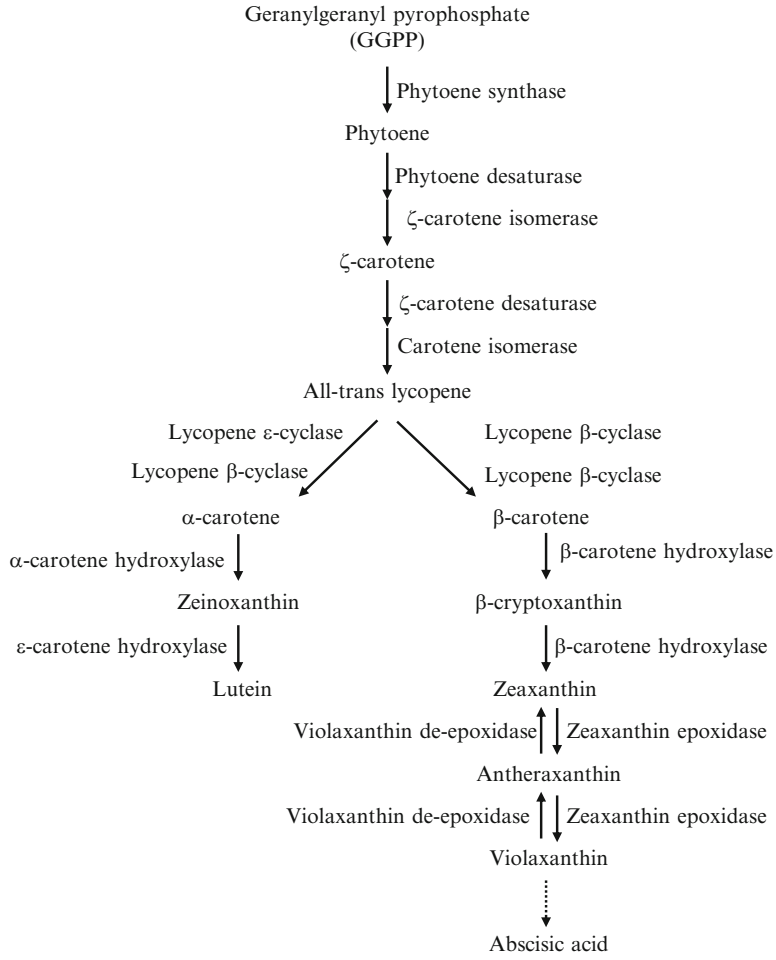

Fig. 7.1 Carotenoid biosynthetic pathway in plants

such as in light harvesting and photoprotection, as attractants for seed dispersal and pollination, and as precursors of some scents as antioxidants (Cunningham 2002; Fraser and Bramley 2004; Howitt and Pogson 2006). In spite of various biological functions, their role as antioxidants appears to be ubiquitous, which is best understood in chloroplasts, where desaturated carotenoids quench triplet chlorophyll and singlet oxygen, preventing the formation of reactive oxygen species and photo-oxidation of the contents of the organelle (Niyogi 1999). Carotenoids also act as precursors to various cleavage products, such as the apocarotenoid abscisic acid (ABA), which regulates plant growth, embryo development, dormancy, and stress responses (Nambara and Marion-Poll 2005), and additional apocarotenoid products, such as strigolactone and others, whose functions are still not known but are considered essential elements that may affect plant yield (Booker et al. 2004; Akiyama and Hayashi 2006). Carotenoid antioxidants protect membranes from lipid peroxidation under heat and light stress conditions (Havaux et al. 2007; Johnson et al. 2007).

Provitamin A activity is the classical biological function of carotenoids in mammalian systems (USDA 2008). Carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin have provitamin A activity as these contains unsubstituted β-ring end groups. Among these, β-carotene contains two provitamin A structures, i.e., two non-hydroxylated β-ionone rings, in comparison to β-cryptoxanthin and α-carotene,

which contains single non-hydroxylated β-ionone ring, so it has twice the activity of the others. In case of mammals, provitamin A carotenoids are cleaved in the intestinal lumen to produce retinal (vitamin A). In addition, xanthophylls such as lutein and zeaxanthin are the essential components of the macular pigment of the eye (Beatty et al. 1999). Macular pigment protects retinal rods and cones by filtering harmful UV/blue light wavelengths (Mares-Perlman and Klein 1999; Kopsell and Kopsell 2006). Apart from these activities, several other essential biological functions of carotenoids, particularly in human health, include reducing the risk of degenerative diseases such as cardiovascular, cancers, cataract, and muscular degeneration via their role as antioxidants and/or as regulators of the immune system (Fraser and Bramley 2004; Johnson 2004; Tang et al. 2005).

Economic benefits to animal production systems are also associated with carotenoids. Diets of chickens with high levels of carotenoid pigments have also been associated with a desirable color of the egg yolks and of the skin of broilers and fryers (Blessin et al. 1963; Perez-Vendrell et al. 2001). β-carotene levels may work synergistically with other fat-soluble vitamins in the protection of broiler leg meat from oxidation (Ruiz et al. 1999). This coloration of yolks and skin is perceived as associated with good health and quality by some consumers (Hadden et al. 1999). The industrial use of carotenoids involves their application for nutrient supplementation, pharmaceutical purposes and as food colorants and animal feeds (Weber 1987).

7.4 Implications of Vitamin A Deficiency

Provitamin A activity is the classical biological function of carotenoids in mammalian systems as only its deficiency in the body may result into causal factor for numerous other diseases. It is well known that adequate vitamin A intake is important for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system (Sommer 1982). Globally, approximately one-third of preschool-age children and 15 % of pregnant women are estimated to be vitamin A deficient (WHO 2009). The problem becomes more severe particularly in the developing countries whose poor populations rely on a single staple crop for their sustenance, e.g., Africa and Southeast Asia have the highest burden of vitamin A deficiency (WHO 2009). The consequences of VAD include blindness, reduced growth in children, and increased morbidity and mortality (Sommer and West 1996; Shankar et al. 1999; Rice et al. 2004; Maida et al. 2008).

7.5 Maize Carotenoid Composition

Maize exhibits considerable natural variation for kernel carotenoids, with some genotypes accumulating as high as 66.0 μg/g (Harjes et al. 2008). Yellow maize kernel carotenoids are present in different isoforms, including two carotenes, α- and β-carotene, and three xanthophylls, β-cryptoxanthin, zeaxanthin, and lutein (Watson 1962; Weber 1987). The predominant carotenoids in maize kernels, in decreasing order of concentration, are lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene. Generally, provitamin A carotenoids constitute only 10–20 % of total carotenoids in maize, whereas zeaxanthin and lutein each commonly represent 30–50 %. The amounts of provitamin A in traditional yellow maize varieties range from 0.25 μg to 2.5 μg g^{-1} dry weight. In typical maize, concentrations of provitamin A carotenoids, i.e., α-carotene, β-carotene, and β-cryptoxanthin, range from 0 to 1.3, 0.13 to 2.7, and 0.13 to 1.9 nmol/g, respectively (Kurilich and Juvik 1999). Although β-carotene has the highest provitamin A activity, it is present in a relatively low concentration (0.5–1.5 μg/g) in most yellow maize grown and consumed throughout the world (Harjes et al. 2008).

7.6 Biofortification

Biofortification is the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology. In developing countries, staple food crops predominate in the diets of the poor, so, biofortification inherently targets the most vulnerable populations (Bouis 2003). Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. The biofortified seeds can be easily reproduced by poor farmers, and thus the seeds are a sustainable means to target remote rural communities not served by conventional seed markets (Qaim et al. 2007).

To alleviate the vitamin A deficiency from rural areas of developing countries, biofortification of major staple food crops of respective areas or countries with provitamin A carotenoids is the only feasible way, since this will better ensure the targeting and compliance. Furthermore possible economic benefits to animal production systems are also associated with carotenoids, which can be increased through biofortification for carotenoids.

As the third most important cereal staple food crop worldwide (FAPRI 2009), a major cereal staple for African consumers (FAOSTAT 2010), maize has also the advantage of being the only crop that contains appreciable amount of carotenoids (Wurtzel 2004) with wide range of genetic variability. The first solid food given to African infants includes white maize porridges devoid of provitamin A (Faber et al. 2005; Poggensee et al. 2004) and are also widely consumed by older children and adults. The nutritional improvement of maize with provitamin A carotenoids would have a significant impact on the target populations. Utilizing the available natural genetic variability for provitamin A carotenoids, maize breeders have succeeded in developing biofortified maize lines containing $\leq$15 μg β-carotene/g dry kernel weight (Yan et al. 2010).

Before devising a strategy to enhance the provitamin A carotenoids in maize, one question that must be addressed is how much quantity is needed to alleviate VAD. This is related to the bioavailability or the fraction of an ingested nutrient that becomes available to the body for utilization in physiological functions or for storage (Jackson 1997; Fraser and Bramley 2004). There are numerous factors that influence bioavailability, including nutrient status of the host, species of carotenoid, food matrix, and amount of food consumed in the meal. In biofortified maize, the bioefficacy of the β-carotene can be predicted by determining experimentally its vitamin A equivalence. The β-carotene quantity needed to provide vitamin A activity equivalent to 1 μg of retinol is known as vitamin A equivalence. The extent of conversion of β-carotene to vitamin A is highly variable among well-nourished people. A recent study evaluated the effect of genetic polymorphisms in the *BCMO1* gene that encodes β-carotene 15,15′-monooxygenase on the conversion of β-carotene to retinyl palmitate (Leung et al. 2009). Taking these factors into account, nutritionists have estimated that 15 μg provitamin A g^{-1} dry weight of kernel could greatly alleviate vitamin A deficiency (www.harvestplus.org). Li et al. (2010) carried out an experiment on women to quantify the vitamin A equivalence of the β-carotene in β-carotene-biofortified maize based on consumption of a single serving of maize porridge and found that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A.

So, improving the micronutrient balance of staple crops such as maize through biofortification is therefore an economically and socially sound way to address micronutrient malnutrition, including VAD, on a global scale (Tanumihardjo et al. 2008).

7.7 Strategies for Biofortification of Maize

To increase the provitamin A potential of maize, there is a need to maximize biosynthetic flux toward carotenoid synthesis, limit the

carotenoids degradation, inhibit branching points leading to non-provitamin A carotenoids and inhibit enzymes leading to the conversion of provitamin A to non-provitamin A carotenoids. This can be achieved by regulating the rate-limiting steps of the pathway or identifying the alleles contributing toward synthesis of provitamin A carotenoids as mentioned below.

The enzyme phytoene synthase catalyzes the first rate-limiting step of the carotenoid biosynthetic pathway and is the most important as its activity controls the carbon flux toward carotenoid biosynthesis. The first branch point of this pathway occurs at cyclization of lycopene where action of lycopene beta cyclase (LCYB) at both ends of linear lycopene produces a molecule with two β-rings. Alternatively, the coaction of LCYB and lycopene epsilon cyclase (LCYE) generates a β, ε-carotene that is a precursor to lutein. Relative activities of LCYB and LCYE are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway. If somehow flux can be shifted toward more synthesis of β-carotene, it will be a boost for provitamin A synthesis. But as the concentration of provitamin A carotenoids increases, a large amount is hydroxylated to β-cryptoxanthin (β-CX) and zeaxanthin (Z), which have 50 % and 0 % of the provitamin A activity of β-carotene, respectively. So, there is need to downregulate the activities of carotene hydroxylases. Furthermore the inhibition of the steps leading to degradation of the carotenoids or directing toward other metabolic pathways such as synthesis of ABA will also help in increasing the concentration of provitamin A.

7.8 Recent Advancements Related to Metabolic Pathways Toward Increasing Provitamin A Carotenoids

Use of composite interval mapping led to identification of two candidate genes, *yellow 1* and *viviparous 9*, which may be responsible for quantitative variation in carotenoids. The *yellow 1* gene is associated with phytoene synthase, the enzyme catalyzing the first dedicated step, and the *viviparous 9* gene is associated with zeta-carotene desaturase, an enzyme catalyzing an early step, in the carotenoid biosynthetic pathway (Wong et al. 2004). Similarly, Chander et al. (2008) constructed a genetic linkage map using a 233 recombinant inbred lines derived from a cross between By804 and B73 and identified 31 putative QTL in total including 23 for individual and 8 for total carotenoids. Two loci, i.e., *y1* and *y9*, that explained most of the phenotypic variation in carotenoids contents were identified along with a gene-targeted marker (Y1ssr) in the candidate gene phytoene synthase 1 (*psy1*) tightly linked to a major QTL explaining 6.6–27.2 % phenotypic variation for levels of carotenoids. They also emphasize the role of QTL cluster located at *y9* locus for pyramiding favorable alleles controlling contents of carotenoids from diverse maize germplasm. Later on, Chen et al. (2010) found that maize *y9* locus encodes ζ-carotene isomerase (Z-ISO), an enzyme necessary for endosperm carotenogenesis in plants. Another catabolic gene affecting seed carotenogenesis, i.e., *CCD1* from maize, was cloned and found to cleave carotenoids effectively (Sun et al. 2008; Vogel et al. 2008). The position of *ZmCCD1*, chromosome 9.07, was found linked to the dominant *white cap 1* (*wc1*) locus involved in the depletion of endosperm carotenoids through gene dosage effect (Vallabhaneni et al. 2010).

Several haplotypes of the gene encoding lycopene epsilon cyclase (*lcyE*) that substantially increase the ratio of β- to α-carotenoids in maize grain were identified by Harjes et al. (2008). To find out the correlation between carotenoid content and candidate gene transcript levels, a maize germplasm collection was used by Vallabhaneni and Wurtzel (2009), and it was observed that transcript levels of paralogs encoding isoprenoid isopentenyl diphosphate and geranylgeranyl diphosphate-producing enzymes, *DXS3*, *DXR*, *HDR*, and *GGPPS1*, were positively correlated with endosperm carotenoid content. *PSY1* and *CrtISO* transcripts were found to be positively and inversely correlated, respectively, for carotenoid pathway enzymes.

Furthermore, the *ZEP* (zeaxanthin epoxidase) transcript level, the enzyme involved in the depletion of carotenoid pool for its conversion to ABA, was also examined, and carotenoid accumulation was found inversely associated with *ZEP1* and *ZEP2* transcript levels.

Using metabolite sorting on maize diversity core collection, Vallabhaneni et al. (2009) identified the enzyme carotene hydroxylase encoded by the *Hydroxylase3* (*HYD3*) locus, whose transcript levels were negatively correlated with high β-carotene levels and positively correlated with zeaxanthin levels. Using PCR genotyping of 51 maize lines, they also showed that the *HYD3* locus could explain 36 % variation and fourfold difference in β-carotene levels. Yan et al. (2010) demonstrated through association and linkage population studies in maize that the gene encoding β-carotene hydroxylase 1 (*crtRB1*/*HYD3*) underlies a principal quantitative trait locus associated with β-carotene concentration and conversion in maize kernels. *crtRB1* alleles associated with reduced transcript expression were found correlated with higher β-carotene concentrations.

7.9 Progress Made Toward Maize Biofortification for Provitamin A Carotenoids

In the past few years, significant progress has been made toward maize biofortification. Conventional breeding has led to the development of a few high β-carotene maize lines having a maximum of 13.6 μg g^{-1} of provitamin A g^{-1} dry weight kernel that approach the target of 15 μg g^{-1} of provitamin A g^{-1} dry weight kernel (Kurilich and Juvik 1999; Islam 2004; Harjes et al. 2008). Biofortified maize lines containing ≤15 μg β-carotene/g dry kernel weight have been successfully developed by the breeders (Yan et al. 2010). Now these identified source lines are being routinely used as parents for new crosses to obtain new sources with greater provitamin A carotenoids. As breeding programme is cyclic in nature, there is need of continuous screening of germplasm and to exploit the natural variability available through molecular marker-assisted selection (MAS) for biofortification purpose.

Transgenic strategies provide important tools to transfer the desired trait from one species to another. Aluru et al. (2008) show that maize seeds can be metabolically engineered to produce high levels of provitamin A comparable to 50 % EAR values by overexpressing the bacterial *crtB* and *crtI* genes in an endosperm-specific manner, using a modified and highly active c-zein promoter. As maize exhibit considerable natural variation for provitamin A carotenoids, transgenic approaches have not been used for its biofortification.

7.10 Future Perspectives

Maize biofortification for provitamin A carotenoids benefits human health and also adds to commercial value of food as these are natural colorants. National germplasm collections hold untapped potential for maize improvement. Biofortification of maize requires large-scale germplasm screening and utilization of identified high provitamin A carotenoid material into the breeding programme. There is need to identify more QTLs through population development, whereas mutational studies can provide an insight about the regulatory points. Similarly, stage-specific metabolite profiling and its correlation to candidate gene expression will provide important information regarding regulation chemistry and expression patterns. So, an interdisciplinary approach including biochemistry, genetics, plant breeding, and nutrition is required for understanding and identifying more QTLs, the rate-limiting steps of the pathway, gene expression patterns with respect to time, allozymic diversity, etc., which, in turn, might provide important information for deciding futuristic strategies. Furthermore, the developed biofortified maize material should not only contain higher quantities of provitamin A carotenoids but also have all other crucial traits including higher yield, insect-pest and disease resistance, and better nutritional quality.

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