Chapter

Molecular Breeding of Sweetpotato Carotenoids

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Abstract

Sweetpotato [sweet potato; *Ipomoea batatas* (L.) Lam.] is the seventh most valued food crop of the world. It has an inherent ability to grow under diverse agro-ecological and microclimatic zones ranging from tropical and subtropical zones to temperate areas with its tuberous roots enriched with the secondary metabolites of immense nutritional value. Among these, carotenoids are the most conspicuous one for having their use in nutritional, pharmaceutical, food, feed, aquaculture, and cosmetic industries. In food industries, carotenoids are used as food additives being antioxidants with attractive colors. Despite the immense economic importance, sweetpotato has received lesser attention in terms of its breeding with improved varieties. The conventional method of breeding by crossing has not been much successful due to the complexity of genome sterility and cross-incompatibility. Hence, the modern molecular breeding approaches, e.g. genetic, genomic, and metabolic (pathway) engineering, have been applied to this crop by some of researchers in Japan, Korea, and China to generate various cultivars with improved quantities and qualities of carotenoids. This has also opened a new gate for molecular breeders to engineer new sweetpotato cultivars enriched with carotenoids under current global scenario of dramatically rising climatic changes where novel food resources are bitterly needed, especially under alarmingly growing world population, the majority of which suffers from malnutrition.

Keywords: sweetpotato, carotenoids, molecular breeding, metabolic engineering, pathway engineering

1. Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.], also described as "sweet potato," belongs to the family Convolvulaceae and occupies the seventh position among the food crops of the world after wheat, rice, maize, potato, barley, and cassava [1, 2]. The largest genus in the family Convolvulaceae is *Ipomoea,* consisting of 600–700 species, among which, only *I. batatas* is cultivated widely as a food crop around the world [3, 4]. In comparison with other tuber crops, sweetpotato comprises higher contents of carbohydrates, many minerals, and more protein estimates than other vegetables [5, 6]. It also contains much higher levels of provitamin A, vitamin C, and minerals than those of rice or wheat [7]. Hundred grams of raw sweetpotato contain 1.57 g of protein, 20.12 g of carbohydrates, 3 g of total dietary fiber, 41.8 g

of total sugars, 30 mg of calcium, 0.61 mg of iron, 25 mg of magnesium, 47 mg of phosphorous, 337 mg of potassium, 55 mg of sodium, 0.3 mg of zinc, 2.4 mg of vitamin C, 0.5 mg of niacin, 0.2 mg of vitamin B6, 14,187 IU of vitamin A (VA), 0.2 mg of vitamin E, 11 μg of vitamin B-9, and 8509 μg of β-carotene (β,β-carotene) [8]. The starch in sweetpotato is easy to digest. Therefore, it is a valuable constituent in the preparation of excellent weaning meals [9]. It is a source of food supply to combat malnutrition in the developing nations, since the tuberous roots (tubers) are enriched with starch and dietary fiber, along with carotenoids, anthocyanin, ascorbic acid, potassium, calcium, iron, and other bioactive ingredients [10–13]. For people of South East Asia and Africa, this crop is the main source of β-carotene [10, 14]. The tubers of the Japanese cultivars are diverse concerning carotenoids accumulation [15]. Sweetpotato may exert diverse health positive effects, since it contains high amounts of numerous phytochemicals in roots or leaves [6, 16]. *I. batatas* cultivars with color-fleshed tubers have been reported for their excellent bioactivities, such as antimutagenic [17], free radical scavenging [18], hepatoprotactive, reduction of liver injury [19, 20], anticancer [21–23], antioxidative activities [23–25], antimicrobial activity, antihypertension, anti-inflammatory, antidiabetic, anticaries effect, ultraviolet protection [23], and chemopreventive activities [26]. Previous reports also suggest that its tubers may be useful for treating peptic ulcers [27]. The genome of *I. batatas* is structurally complex and has a size of 4.8–5.3 pg/2C nucleus [28]. Due to the existence of polyploidy, sweetpotato is a hexaploid species (2n = 6x = 90) that has a basic chromosome number of 15, [3] with a huge genome size of 2200–3000 Mbp [29]. The genetic studies on this species are exhausting, since it is difficult to generate seeds and to evaluate the effects of polyploidy on the genome [30, 31]. Complex structure of its genome also manifests self and cross-incompatibility, causing barrier for genetic studies on important agronomical characters [3, 32]. Its tubers exhibit various colors, such as white, yellow, orange and purple orange, and yellow and orange-fleshed lines, were shown to contain β-carotene as the predominant carotenoid [11–13, 33, 34]. Annual yield of sweetpotato is currently exceeding the value of 105 million metric tons, 95% of which is shared by the developing countries. China is the world's leader among all in sweetpotato consumption that counts about 66% of the total global consumption. China is followed by Nigeria and Tanzania, though, each of these last two countries shares only 4% of the total global consumption [35]. By applying the conventional breeding, biofortification of sweetpotato involved the selection of orange-fleshed varieties, to combat vitamin A deficiency among the developing nations [36]. In Japan, high β-carotene-accumulating varieties, such as "Benihayato," "J-Red," and "Sunny-Red," were initially developed by Japanese breeders at the Kyushu-Okinawa Agricultural Research Center (formerly the Kyushu National Agricultural Experiment Station), Miyakonojo, Miyazaki, Japan [37–39]. The enhancement of sweetpotato with provitamin A carotenoids (PVACs) has also been the area of research focus for the HarvestPlus (a company headquartered in Washington, DC, USA, involved in the development of nutritious food crops through biofortification and promotion of such crops) since the launch of its projects on biofortification [40]. Genetic modification of sweetpotato by using the transgenic tools and in order to improve the nutritional quality offers huge scope, and numerous research reports have already been published on genetic modification of sweetpotato using molecular gene engineering technologies [41]; however, it is the immense need to overcome hidden hunger, specially the one related with the insufficiency of provitamin A carotenoids among the poorly fed but rapidly growing populations in the developing countries by molecular breeding of sweetpotato varieties on sustainable basis.

2. Carotenoids and their distribution

Carotenoids, the visible colors of life, are the 40-carbon isoprenoids synthesized naturally by fungi, bacteria, algae, and cyanobacteria [42–44] and conspicuously by green plants including bryophytes [45] and higher plants [44, 46, 47]. Being intracellular, carotenoids are commonly located in the membranes of chloroplasts, mitochondria, or endoplasmic reticulum [48]. Approximately 750 carotenoids have been reported so far [49, 50].

Maoka et al. (unpublished) analyzed carotenoids that were extracted from the orange tubers of the cultivar W71. It was consequently found that there were β-carotene-5,8,5′,8′-diepoxide (13.8% of the total carotenoids), β-carotene-5,6,5′,8′-diepoxide (9.2%), β-carotene-5,8-epoxide (4.6%), β-cryptoxanthin (3.2%), β-cryptoxanthin-5′,6′-epoxide (2.2%), lutein (2%), and zeaxanthin (trace amounts), in addition to β-carotene (59.3%). The biosynthetic pathway of these carotenoids is proposed in **Figure 1**. The carotenoids with 5,6-epoxy-β-ring or with 5,8-epoxy-β-ring are unique to sweetpotato. The tubers of the Japanese cultivar "Benimasari" were also found to accumulate not only the unique carotenoids, such as β-carotene-5,8,5′,8′-diepoxide (40.5% of the total carotenoids), β-carotene-5,8-epoxide (6.5%), β-cryptoxanthin-5′,8′-epoxide (10.5%), and β-carotene (10.5%), but also typical carotenoids that included 5,6-dihydroxy-β-ring (named ipomoeaxanthins) [15].

2.1 Role of carotenoids in animals

Animals, with very few exceptions [51, 52] are unable to synthesize them [53, 54]; however, carotenoids are accumulated by crustaceans, crabs, fish, crayfish, prawns, mammals, and in insects such as butterflies. The animal and human diet must include carotenoids as essential nutrients [44].

In marine animals, astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) has been reported as the most commonly stored carotenoid pigment [55]. It is

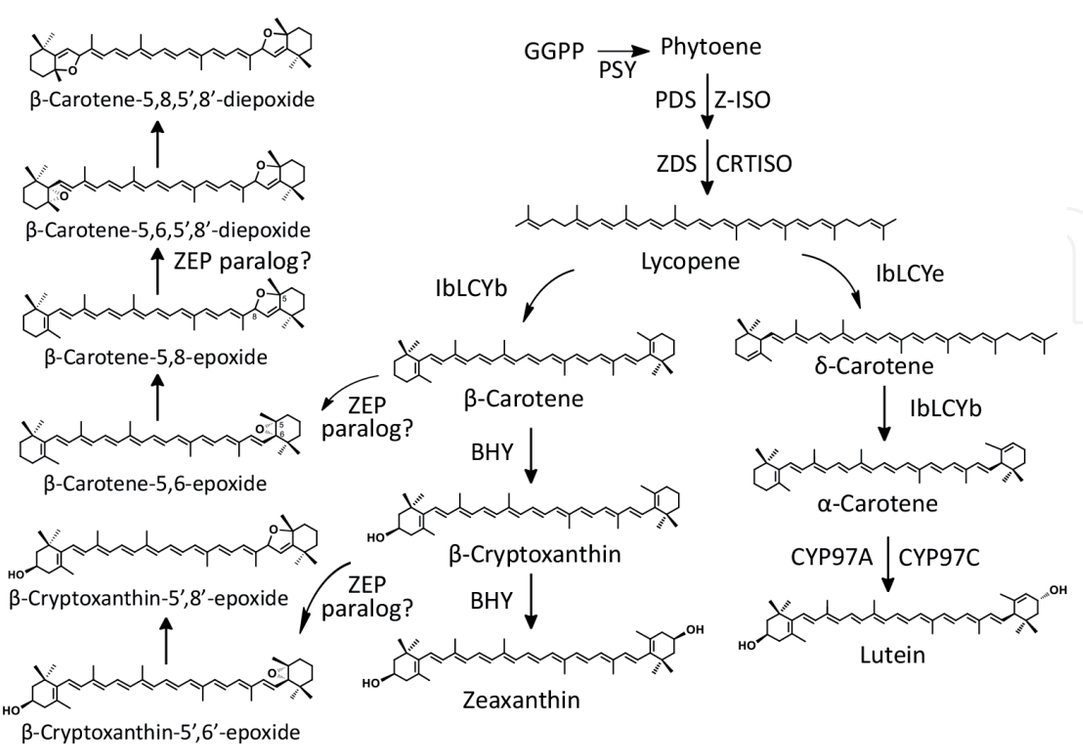

Figure 1.
Proposed carotenoid biosynthetic pathway in sweetpotato orange tubers [10].

responsible for the red/pink coloration of crustaceans [56, 57] and the flesh of salmonoids [58]. Astaxanthin has received much attraction for its likely role in preventing cardiovascular diseases and aging caused by UV light in human body [59]. Chemical structures of some major dietary carotenoids are shown in **Figure 2.**

Both á -carotene and β-carotene have provitamin A activity and are converted to retinol in the human body [60–64]. Carotenes such as the lycopene and β-carotene play a potential role in human nutrition and act as protectants against diseases, such as lycopene protects against cardiovascular [65], aging-related diseases, macular degradation of eye [66, 67], and certain types of cancers including gastrointestinal, cervix, breast, and prostate cancer [47, 61, 68–73].

Beneficial effects of dietary carotenes, α-carotene, and β-carotene on human health related to enhancement of immune system and minimizing the risk of cancer are due to their antioxidant potential [69, 74]. β-Carotene, α-carotene, and β-cryptoxanthin are provitamin A carotenoids (PVACs) and hence they are the main precursors of vitamin A (VA) in the human body [75].

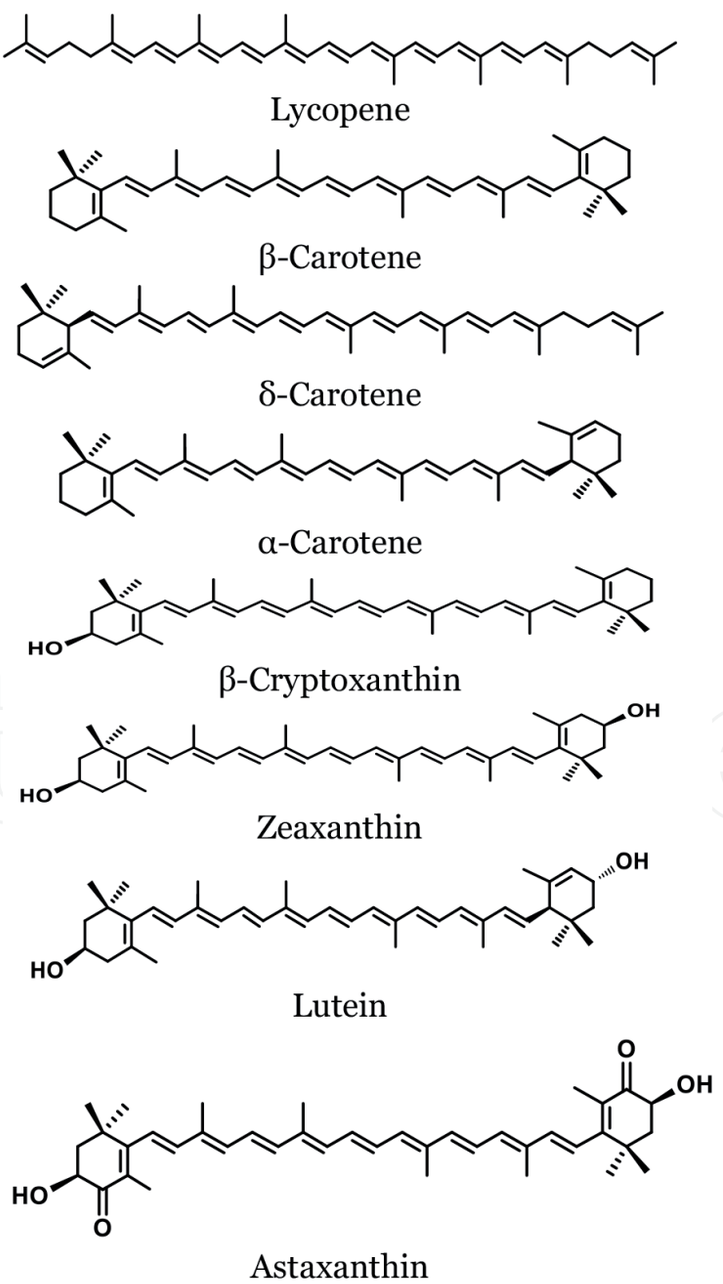

Figure 2.
Structures of some major dietary carotenoids.

2.2 Carotenoids use in disease prevention

Carotenoids, especially astaxanthin, have been reported to enhance both the non-specific and specific immune system and protect cell membranes and cellular DNA from mutation [44, 76]. Intake of fruits and vegetables rich in carotenoids mainly lycopene, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, and lutein lowers the risk of morbidity and mortality by cardiovascular diseases and atherosclerosis [69]. Epidemiological studies have reported that lycopene can lower the risk of prostate cancer [77] and in its ability to quench singlet oxygen; it is 2- to 10-fold stronger than β-carotene and α-tocopherol, respectively [78]. Clinical studies have reported that the lycopene-enriched foods are protective against oxidative DNA damage in leukocytes in vitro [79] and prostate tissue in vivo [80]. β-Carotene is useful in reducing the risk of ischemic heart disease and myocardial infarction [81]. In the macular region of human eye including eye lens, two xanthophylls, lutein and zeaxanthin, exist in high concentrations and are regarded very important carotenoids for eye health. Reports suggest that these two carotenoids protect eye from high energy UV light and are excellent reactive oxygen species scavengers [82]. The role of lutein and zeaxanthin as macular pigments and their function in eye health has been reported in previous studies [83]. It has been anticipated that phytoene and phytofluene which are colorless precursors of other carotenoids possess light absorption in UV-A and UV-B range and protect skin by their photo-protective characteristics [84, 85]. Astaxanthin is also known as the super antioxidant. Since, it contains particular molecular configuration, making it extremely powerful antioxidant consequently, protecting cells against oxidation by quenching singlet oxygen and dissipating the energy as heat. It has the strong potential for scavenging free radicals and effectively breaks peroxide chain reactions [86, 87]. Studies have showed that the low-density lipoprotein (LDL) high cholesterol levels in mice decreased when supplemented with astaxanthin. Neither β-carotene nor canthaxanthin produced the same effect. Astaxanthin or other carotenoids can decrease the oxidation of the lipid carriers and thereby reduce the risk of atherosclerosis [88]. It also has positive effects in case of antitumor activity [89].

2.3 Industrial uses of carotenoids

All carotenoids show antioxidants activities appearing in a variety of colors in red, yellow, and orange; therefore, carotenoids are used as natural pigments in food, food supplements, nutraceuticals, pharmaceuticals, and cosmetic industry and various biotechnological purposes [90, 91]. Global carotenoids market touched $1.5 billion ($1500 million) in 2017 with a projection of $2.0 billion by 2022 [92]. In a previous report, the global market for carotenoids was $766 million in 2007. The expected projection for the year 2015 was $919 million with a compound annual growth rate (CAGR) of 2.3%. In 2007, β-carotene alone shared the market value at $247 million; this segment was predicted to be worth $285 million by 2015 with CAGR of 1.8% [91, 93]. In horticultural crops, they appear as a trait of attractiveness, adding value to the marketing potential of fruits and vegetables [94, 95]. Green algae Haematococcus pluvialis, which is the natural source of astaxanthin, has been reported for huge amounts ranging from 10,000 to 40,000 ppm (mg/kg) of astaxanthin in addition to other important carotenoids such as β -carotene, lutein, and canthaxanthin [58, 96]. Industrially, astaxanthin has been utilized as a feed supplement for cultured fish and shellfish [97, 98]. Other diverse biological functions of astaxanthin include an involvement in cancer prevention [99], enhancer of immune responses [100], and a free radical quencher [58, 101]. It is evident, therefore, that astaxanthin is a biomolecule with huge biofunction potential both to the pharmaceutical and food industries [58].

3. Sweetpotato carotenoids

The carotenoids present in the sweetpotato leaves can scavenge free radical agents as singlet-oxygen quenchers [102–105]. In a recent analytical report [105], the total phenol, carotenoid, anthocyanin, and flavonoids contents of the sweetpotato leaves ranged from 2.0 to 22.5 (g/100 g DW), 0.9 to 23.4 (β-carotene equivalents/100 g; BET/100 g), 2.2 to 24.5 (color value/g DW), and 62.8–272.2 (catechin equivalents; μg/g), respectively [105]. Consumption of sweetpotato in Asia ranges from its use as additional food of minute status to a very vital supplementary food to rice and/or other root and tuber crops [106]. It is cooked or used to make cakes, chapatis, mandazia, bread, buns, and cookies [107]. In the United States and some other developed countries, sweetpotato is strictly used as a luxury food. In Japan, it is used in novel plant products and/or nutraceuticals [108]. By using absorption spectroscopy, Ishiguro et al. [109] analyzed carotenoids from eight cultivars of yellow-fleshed sweetpotato and compared them in terms of their carotenoids. By HPLC analyses, they revealed some 17 different carotenoids from yellow- and orange-fleshed sweetpotato. In yellow-fleshed sweetpotato, the major carotenoids included β-carotene-5,8, 5′,8′-diepoxide (32–51%) and β-cryptoxanthin 5,8-epxide (11–30%), whereas β-carotene with amounts ranging from 80 to 92% were dominant in the orange-fleshed cultivars. For other orange cultivars, e.g. W71 and "Benimasari," carotenoid composition in the tubers has already been described along with a comprehensive metabolic pathway [10, 15]. Kammona et al. [110] analyzed and compared the carotenoid composition in some Malaysian orange, yellow, purple, and white sweetpotato tubers. They reported the highest total carotenoid contents from orange sweetpotato followed by yellow, purple, and white sweetpotato. Among the individual carotenoids analyzed, β-carotene existed in all types ranging from 91.95 ± 2.05 μg/g DW in white sweetpotato to 376.03 ± 11.05 μg/g DW in orange sweetpotato tubers. Traces of zeaxanthin were reported with values 5.44 ± 3.23 μg/g DW and 20.47 ± 2.03 μg/g DW in yellow and white sweetpotato, respectively. Lutein was available only in orange sweetpotato at trace amount of 0.91 ± 1.03 μg/g DW. Purple sweetpotato contained only β-carotene (113.86 ± 14.17 μg/g DW) with absence of other carotenoids [110].

Islam et al. [111] performed HPLC analyzes of *trans*- and *cis*-β-carotene from raw and boiled sweetpotato which included three orange-fleshed, three yellowish-cream-fleshed, and one white-fleshed varieties of sweetpotato. The deep-orange-fleshed variety Kamalasundari (BARI SP-2) showed the highest amounts of β-carotene among all the varieties followed by yellow varieties. On the other hand, from one of the two white-fleshed varieties, only trace amounts of β-carotene were obtained with no amounts at all from the other one. Their results proposed that the orange-fleshed varieties of sweetpotato contain the highest amounts of β-carotene in raw as compared to those which were boiled.

Despite huge economic value, sweetpotato has not received due importance as compared with common staple crops such as wheat, maize, and rice. World increasing hidden hunger, especially in developing countries, needs new foods and nutrition sources on sustainable bases. In this regard, sweetpotato not only offers immense nutritional, medicinal, industrial, and potential benefits but is also a new horizon in modern industrial biotechnological uses for biofunction development through the latest molecular tools and technologies of molecular plant breeding.

3.1 Isolation and functional identification of carotenoids biosynthesis genes

The heterologous complementation expression system in *Escherichia coli* offers unique tool for functional analysis of isolated new carotenoids biosynthesis genes

from different organisms [112]. Carotenoid biosynthetic pathway in microorganisms, such as *Erwinia uredovora* and *Erwinia herbicola* (reclassified as *Pantoea ananatis* and *Pantoea agglomerans*, respectively), is specified by a gene cluster, encoding biosynthetic enzymes that function in a pathway starting with the synthesis of geranylgeranyl pyrophosphate (*GGPP*) and ending in the synthesis of zeaxanthin glucosides [113, 114]. Complete carotenoid gene clusters or part of it from *E. uredovora* or *E. herbicola* have been introduced into *E. coli,* which is otherwise a nonpigmented bacterium, and such transformed *E. coli* engineered in a way that they accumulate a range of colorful carotenoids [114, 115]. Since carotenoids are derived from isoprenoid precursors, *E. coli* can accumulate carotenoids by coupling an endogenous isoprenoid biosynthetic pathway with enzymes encoded by transformed genes of carotenogenic organisms such as *E. uredovora*. Hence, the biosynthetic pathway can be reconstructed in vivo even if the enzymes are of such diverse origin as those encoded by bacteria and plants [116–118]. The expression of carotenoid genes in *E. coli* has been useful for identifying function of gene products [118–120], the manipulation of the pathway [121, 122], investigating transcriptional regulators of carotenoids biosynthesis genes [123], and the isolation of new genes encoding enzymes of the carotenoid biosynthetic pathway [124] or enzymes catalyzing the synthesis of carotenoid precursors [125].

Misawa et al. [55] isolated and functionally identified the carotenoids biosynthesis genes cluster that included *crtB* (phytoene synthase), *crtI* (phytoene desaturase), *crtW* (β-carotene ketolase), and *crtZ* (β-carotene hydroxylase) from *Agrobacterium aurantiacum* (reclassified as *Paracoccus* sp. strain N81106). The functional identification of the isolated gene cluster led them to propose astaxanthin biosynthetic pathway for the first time.

Misawa et al. [114] isolated and functionally identified the carotenoid biosynthesis genes, such as *crtE* (GGPP synthase), *crtX* (Zeaxanthin glucosyltransferase), *crtY* (lycopene β-cyclase), *crtI* (phytoene desaturase), *crtB* (phytoene synthase), and *crtZ* (β-carotene hydroxylase), form *E. uredovora* by analyzing carotenoids accumulated in *E. coli* transformants in which these genes were expressed. By analysis of accumulated carotenoids in the transformed *E. coli* by these individual genes, they found that carotenoids in this pathway appeared to be close to those in higher plants rather than to those in bacteria. Although HPLC is a routine analytical tool to analyze various metabolic products from plants, highly developed and comprehensive metabolome analytical techniques with respect to particular tissues now offer precise analytical approaches such as nuclear magnetic resonance (NMR; COSY and NOESY) and accurate mass spectrometry (MS) techniques [47, 126]. A foreign *crtW* gene was expressed in the W71 cultivar of sweetpotato, and carotenoids generated there have been successfully analyzed by UV-vis, ESI-MS, ^{1}H-NMR, and CD spectral data [127]. As a result, novel carotenoids, shown in **Figure 3**, i.e. echinenone 5′,8′-epoxide, echinenone 5′,6′-epoxide, and 3′-hydroxyechinenone 5′,6′-epoxide, were identified besides ketocarotenoids including astaxanthin.

3.2 Sweetpotato carotenoids biosynthesis genes, cloning, and genetic engineering

Although, sweetpotato is highly important as a valuable source of carotenoids especially β-carotene, very little research has been done on molecular biological aspects of its carotenoid biosynthesis [10, 14, 31, 128]. The development of an efficient and reproducible transformation system is needed for genetic manipulation of sweet potato to either improve the crop or establish it as a novel "transgenic plant bioreactor" [129]. Otani et al. [130] developed and reported the first successful transformation protocol for the production of transformed (transgenic) sweetpotato

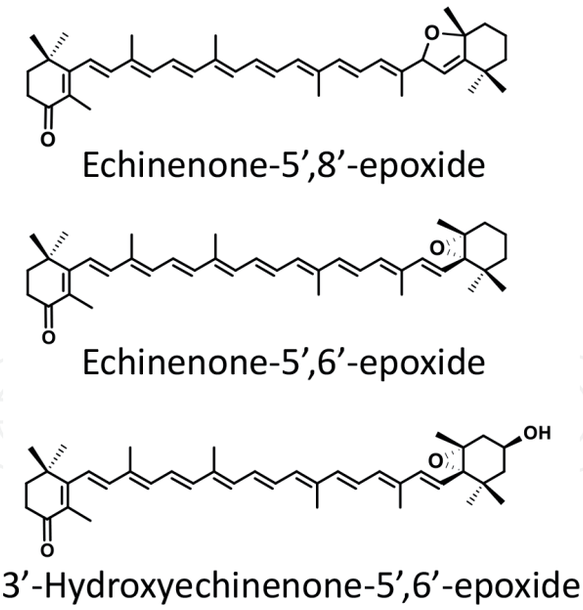

Figure 3.
Novel carotenoids produced in the tuber of the transgenic sweetpotato engineered with the crtW gene of bacterial origin.

plants that was based on the formation of hairy roots using leaf disks as explants for *Agrobacterium rhizogenes*. However, the regenerated transgenic plants showed some morphological abnormalities such as short storage root and internodes. Later on, to overcome such anomalies, a modified and successful *Agrobacterium tumefaciens*-mediated transformation protocol was developed via somatic cell embryogenesis [131–133]. Liao et al. [14] isolated and functionally characterized an isopentenyl diphosphate isomerase (*idi*) gene from sweetpotato cultivar YUSU 303 from Southeast China. They isolated a full-length cDNA of *idi* gene by SMART™ RACE cDNA Amplification Kit (Clontech, USA). Isolated *idi* was 1155 bp with an open reading frame of 892 bp encoding a polypeptide of 296 amino acids (GenBank accession No. DQ150100). Isolated *idi* gene was cloned in pTrc expression vector and was fed to *E. coli* which contained pAC-BETA plasmid for β-carotene accumulation. *E. coli* were cultured and carotenoids were analyzed by color complementation. Cultures of *E. coli* which were transformed with *idi* gene turned orange indicative for β-carotene and suggested its potential activity in promoting β-carotene biosynthesis. Kim et al. [134] isolated a partial sequence of phytoene synthase (*PSY*) which contained 354 bp from a cultivar Shinhwangmi (accession No. HQ828092). It showed 94% sequence identity with a *PSY* isolated from Ipomoea species Kenyan (GenBank accession No. AB499050.1). However, no gene function of isolated *PSY* from sweetpotato could be reported. Ling et al. [135] isolated a lycopene ε-cyclase (*LCYe*) gene from sweetpotato cultivar Nongdafu 14 from China. However, they did not functionally characterize it. They isolated a full-length cDNA of *idi* gene by GeneRacer TM Kit (Invitrogen Carlsbad, CA, USA). Isolated *LCYe* was 1805 bp with an open reading frame of 1236 bp encoding a polypeptide of 411 amino acids. Quantitative real-time PCR analysis showed that *IbLCYe* expression levels were desirably higher in roots as compared to those in leaves. Isolated *LCYe* gene was expressed in tobacco cultivar Winconsin 38. Carotenoids from transgenic tobacco plants were extracted and analyzed by HPLC which revealed transgenes accumulating more β-carotene as compared to control plants. Kim et al. [128] isolated a partial lycopene β-cyclase (*IbLCYb*) from a cultivar Yulmi of sweetpotato. They synthesized primers by using a partial sequence of *IbLCYb* from database with accession number JX393306 and amplified a partial cDNA of *IbLCYb* by

RT-PCR. By using isolated *IbLCYb*, an IbLCYb-RNAi vector was constructed and then used to transform white-fleshed sweetpotato. Transformed sweetpotatoes were cultured and analyzed for accumulated carotenoids. Their results showed a total increase in the carotenoids contents along with increase in resistance against salt stress in transgenic sweetpotato as compared to the control. Significant levels of carotenoids genes expression were observed in all plant parts with highest expression in leaves to lowest in the fibrous roots. But in case of transgenic calli, expressions of *IbLCYb* were dramatically reduced and found high in non-transgenic calli. Lycopene was not produced both by transgenic and non-transgenic sweetpotato. In an another experiment, Kim et al. [134] cloned a partial cDNA encoding β-carotene hydroxylase (*BHY*) from storage roots of sweetpotato cultivar Shinhwangmi and constructed an RNA-i-IbCHY-β vector for transformation of white-fleshed cultivar Yulmi and evaluation of inhibition effects of β-carotene hydroxylase (*BHY*) in transgenic lines. Downregulation of *IbBHY* gene expression altered the content and degree of carotenoids between transgenic and non-transgenic cells with an increase in the β-carotene and total carotenoids in transgenic sweetpotato cells along with an increase in their antioxidation potential.

3.3 Metabolic engineering of the carotenoid biosynthetic pathway to enhance carotenoid contents in higher plants

The pathway engineering approach using a variety of carotenoid biosynthesis genes is becoming a potential approach as one of the most effective methods to generate large quantities of structurally diverse carotenoids [59, 136, 137]. Astaxanthin (3,30-dihydroxy-4,40-diketo-β-carotene) is a high-value ketocarotenoid that is biosynthesized only by a few organisms typically at low levels. This red pigment (produced through chemical synthesis) has been used in large amounts in aquaculture. Currently, natural astaxanthin is employed as a health boosting food and is investigated for the treatment of a number of human diseases including cancers [138]. The limited renewable sources and growing demand for natural astaxanthin have attracted tremendous interest in its engineering into heterologous hosts, especially plants with the ability of sequestering 10- to 50-fold higher carotenoids than microorganisms, to produce the high-value pigment, during the past decade [139, 140]. The most promising approach reaching high astaxanthin yields was by chloroplast transformation using a bacterial ketolase gene [141]. Plastid genome transformation of lettuce (*Lactuca sativa*) has similarly been site-specifically modified with the addition of three transgenes, which encoded β,β-carotenoid 3,3′-hydroxylase (*crtZ*) and β,β-carotenoid 4,4′-ketolase (4,4′-oxygenase; *crtW*) from a marine bacterium *Brevundimonas* sp. strain SD212, and isopentenyl-diphosphate-isomerase (*idi*) from a marine bacterium *Paracoccus* sp. strain N81106. The resultant transplastomic lettuce leaves generated 49.2% astaxanthin fatty acid diester, 18.2% astaxanthin monoester, and 10.0% astaxanthin in its free forms along with the 17.5% of other ketocarotenoids. The ketocarotenoids produced in transplastomic lettuce were 94.9% of total carotenoids. The wild-type native carotenoids analyzed were 3.8% lactucaxanthin and 1.3% lutein in the transplastomic lettuce [142]. Likewise, by the introduction and heterologous expression of *crtW* gene, astaxanthin and other intermediates have been produced and reported in carrot (*Daucus carota*) roots [143], canola (*Brassica napus*) seeds [144], and maize (*Zea mays*) endosperms [145]. A comprehensive carotenoid biosynthetic pathway in these higher plants is shown in **Figure 4** with a summarized illustration for the metabolic pathway engineering with heterologous *crtW* and *crtZ* genes expression.

Through pathway engineering that utilizes the marine bacterial carotenoid 4,4′-ketolase (4,4′-oxygenase) gene named *crtW,* unique keto-carotenoids such as

Figure 4.
Carotenoid biosynthetic pathway in higher plants. A summarized illustration for the introduction and function of heterologous crtW and crtZ genes expressed in tobacco [141] and lettuce [142] leaves. The carotenoids shown in black represent native carotenoids accumulated by both tobacco and lettuce, and black and underlined are those reported from both of the transgenic tobacco and lettuce. Carotenoids underlined red are reported from lettuce only, where GGPS, is geranylgeranyl pyrophosphate synthase, PSY is phytoene synthase, PDS is phytoene-desaturase, ZDS is ζ-carotene desaturase, CRTISO is carotenoid isomerase, LCYb is lycopene β-cyclase, LCYe is lycopene ε-cyclase, LsLCYe is lettuce LCYe, LsCYP97C is lettuce heme-containing cytochrome P450-type carotene ε-ring hydroxylase, BHY is non-heme di-iron-type carotene β-ring hydroxylase, EHY is carotene ε-ring hydroxylase, ZEP is zeaxanthin-epoxidase, VDE is violaxanthin de-epoxidase, and NSY is neoxanthin synthase.

astaxanthindiglucoside, 2,2′-dihydroxyastaxanthin, and 2,2′-dihydroxycanthaxanthin have been produced in *Escherichia coli* [146, 147] and 4-ketoantheraxanthin in tobacco (*Nicotiana tabacum*) plants [148]. Breitenbach et al. [149] also synthesized α-echinenone (4-keto-α-carotene) in rice callus using *crtW*. Recently, 4-ketozeinoxanthin was produced in *E. coli* cells by introducing the bacterial *crtW* gene and carotenogenic genes from liverwort [150].

3.4 Metabolic engineering of the carotenoids biosynthetic pathway in sweetpotato

Metabolic engineering of carotenoid biosynthetic pathway using a combinatorial approach has led to the efficient production of interesting carotenoids of high commercial value and pharmaceutical potential [44, 59, 151].

Starting with transgenic approach, prerequisite is to have a sound knowledge on the metabolic pathways regulating the carotenoid biosynthesis and their accumulation. Due to efforts of many scientists, the carotenoid metabolic pathway and the function of the biosynthetic enzymes involved in carotenoids biosynthesis have been elaborated well [152]. It was reported that sweetpotato contained not only β-carotene but also several epoxy carotenoids unique to the sweetpotato tubers, e.g. β-carotene-5, 8-epoxside and β-carotene-5, 8, 5′8′-diepoxside [15]. Therefore, it

was assumed that the new structural carotenoids with epoxy and keto groups can be produced by expressing the ketolase *crtW* gene in sweetpotato tubers. Recently, marine bacterial genes that include the *crtW* gene encoding carotenoid 4,4′-ketolase [148] was introduced into sweetpotato cultivar W71 under the control of the CaMV promoter. Consequently, novel carotenoids with epoxy and keto groups 1, 2, and 3 were obtained along with a series of ketocarotenoids. The structural elucidation of these novel epoxy-keto carotenoids along with biosynthetic pathway in sweetpotato was also proposed [127]. A tabulated summary of recent developments in molecular breeding of sweet potato by genetic, metabolic, and pathway engineering approaches is presented in **Table 1**.

"White Star" (WS) and W71, which produce white- and orange-fleshed tubers, respectively, are important sweetpotato cultivars, since they are amenable to *Agrobacterium*-mediated transformation [10, 127, 158, 159]. Chemical analysis of the carotenoids and isolation and functional characterization of the carotenoids biosynthesis genes of these two cultivars was reported in more details by Khan et al. [10] for the first time. One of the initial works that led to the sweetpotato genetic improvement for enhancing provitamin A amounts was done by Kim et al. [160] which involved isolation and functional analysis of the orange (*Or*) gene, from orange-fleshed sweetpotato controlling the carotenoids accumulation in the transgenic calli of sweetpotato. White-fleshed sweetpotatoes were transformed by the orange (*Or*) gene, which resulted in the 10-fold increased accumulation of β-carotene and total carotenoids. Later, identical results were presented by Park et al. [161] who observed that the overexpression of *IbOr* gene boosted the carotenoid composition in purple-flesh sweetpotato cultivar. In higher plants, the biosynthesis of carotenoids from lycopene involves the enzymatic activity

Gene engineered	Promoter used	Carotenoids enhancement	References
ZDS	Cauliflower mosaic virus (CaMV) 35S	3.96–2.37 increase in β-carotene and lutein and, 2.23-fold increase in total carotenoids accompanied with enhanced salt tolerance	[153]
PSY	Tuber-specific primer	6.3-fold increase in carotenoid, 19-fold increase in β-carotene	[154]
LCYb	Cauliflower mosaic virus (CaMV) 35S	1.4–1.8 times higher in β-carotene, increased tolerance to drought stress	[128]
LCYe		5.44-fold to 6.59-fold Increase in β-carotene and 1.77–2.75 times increase in total carotenoids	[155]
BHY	Cauliflower mosaic virus (CaMV) 35S	Twofold increase in total carotenoids and 16-fold increase in β-carotene	[152]
CrtW	Cauliflower mosaic virus (CaMV) 35S	Novel carotenoids with epoxy and keto groups were produced including a series of ketocarotenoids	[127]
CrtO	Cauliflower mosaic virus (CaMV) 35S	10–12% increase in total carotenoid	[156]
BHY	Silencing of BHY	117 μg/g (dry weight) increase in total carotenoids and 34.43 lg/g (dryweight) in β- carotene	[134]
IbOr-R96H	cauliflower mosaic virus (CaMV) 35S promoter	19.6- and 186.2-fold higher total carotenoid and β-carotene contents, respectively	[157]

Table 1.
Role of metabolic engineering in carotenoids enhancement in sweetpotato˚.

of lycopene ϵ-cyclase (*LCYe*) gene, via β-branch-specific biosynthetic pathway yielding β-carotene. By downregulating the expression of *IbLCYe* through RNA interference (RNAi) technology, higher amounts of β-carotene were recorded [162]. To increase the β-carotene contents in sweetpotato, researchers have also made use of the molecular markers along with the evaluation and screening of available germplasm.

This combined approach is thought very useful in selecting the desirable parents for breeding new sweetpotato varieties with the higher levels of β-carotenes [163]. To analyze the gene diversity and evolutionary relationships among various cultivars of sweetpotato, Hwang et al. [164] have applied the use of Simple Sequence Repeats (SSRs*)*. Their results showed that polycross-derived cultivars have higher levels of genetic diversity suggesting the application of polycross breeding that overcomes the challenges of cross-incompatibility. For breeding high β-carotene sweetpotato varieties, Quantitative Trait Loci (QTLs) for β-carotene content in a cross sweetpotato were reported for the first time by Cervantes-Flores et al. [165] which led to the understanding of the inheritance pattern and is considered the foundation of the development of marker-assisted breeding techniques for breeding high β-carotene (provitamin A) accumulating sweetpotato cultivars.

Orange-fleshed sweetpotato, which is a genetically modified crop, is now well accepted by consumers [166, 167] and has appeared as a sound supply of provitamin A. To achieve the daily provitamin A needs, mere 125 g of fresh orange-fleshed sweetpotato roots from most varieties are enough [35].

4. Conclusion

The rapidly increasing world population demands sustainable supply of ample quantities of quality food, especially under the changing climatic conditions. Food insecurity accompanied with already existing malnutrition among the developing countries is a grand challenge of the day. Foods rich in phytonutrients not only contribute toward enhancing the health but also reduce the risk of many diseases including early aging. In this regard, genetic improvement of the major staple crops such as sweetpotato needs additional strategies of the molecular plant breeding to overcome the genetic complexity. The application of metabolic engineering supplemented with the omics and the recently developed gene editing tools and technologies are the potential strategies to be adopted which promise scope for improving the quantity of phytonutrients, especially the carotenoids in sweet potato. It will contribute to prevent the malnutrition and the diseases linked with foods deficit in quality nutrients. There is a dire need to apply multiple gene engineering approaches for multi-phytonutrients improvement to meet the need.

5. Recent development and future scope

Recent developments in carotenoids gene manipulations have helped to make insight that engineering sweetpotato with IbOr gene manipulations would be a potential strategy to improve the total carotenoids and specially the β-carotene through enhancing sink strength in storage roots of sweet potato. Moreover, site-directed mutagenesis supplanted with the genome editing tools such as CRISPR-Cas9 such as CRISPR-Cas9 and its different modifications will further lead to a fruitful biofortification of sweet potato for nutritional enhancement through carotenoids improvement.

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Conflict of interest

The authors declare that they have no conflict of interest.

Notes/thanks/other declarations

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References

[1] Ahn YO, Kim SH, Kim CY, Lee JS, Kwak SS, Lee HS. Exogenous sucrose utilization and starch biosynthesis among sweetpotato cultivars. Carbohydrate Research. 2010;**345**(1): 55-60

[2] Rodriguez-Bonilla L, Cuevas HE, Montero-Rojas M, Bird-Pico F, Luciano-Rosario D, Siritunga D. Assessment of genetic diversity of sweet potato in Puerto Rico. PLoS One. 2014;**9**(12):e116184

[3] Hirakawa H, Okada Y, Tabuchi H, Shirasawa K, Watanabe A, Tsuruoka H, et al. Survey of genome sequences in a wild sweet potato, Ipomoea trifida (HBK) G. Don. DNA Research. 2015;**22**(2):171-179

[4] Austin DF, Huáman Z. A synopsis of Ipomoea (Convolvulaceae) in the Americas. Taxon. Feb 1996;**45**(1):3-38

[5] Shih PH, Yeh CT, Yen GC. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. Journal of Agricultural and Food Chemistry. 2007;**55**(23):9427-9435

[6] Ji H, Zhang H, Li H, Li Y. Analysis on the nutrition composition and antioxidant activity of different types of sweet potato cultivars. Food and Nutrition Sciences. 2015;**6**(01):161

[7] Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural and Food Chemistry. 1997;**45**(2): 304-309

[8] USDA. (U.S. Department of Agriculture), National Nutrient Database for Standard Reference, Release 28. Basic Report: Raw, 11507, Sweetpotato, Unprepared. USA: Online, 2016. Available from: https://ndb.nal.usda.gov/ndb/foods/show/ [Accessed: December 16, 2016]

[9] Antonio GC, Takeiti CY, de Oliveira RA, Park KJ. Sweet potato: Production, morphological and physicochemical characteristics, and technological process. Embrapa Agroindústria de Alimentos-Artigo em periódico indexado (ALICE). 2011;**5**(2):1-18

[10] Khan MZ, Takemura M, Maoka T, Otani M, Misawa N. Carotenoid analysis of sweetpotato Ipomoea batatas and functional identification of its lycopene β-and ε-cyclase genes. Zeitschrift für Naturforschung C. 2016;**71**(9-10): 313-322

[11] Purcell AE, Walter WM. Carotenoids of centennial variety sweet potato, Ipomea batatas. Journal of Agricultural and Food Chemistry. 1968;**16**(5):769-770

[12] Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV, Yencho GC. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry. 2007;**103**(3):829-838

[13] Grace MH, Yousef GG, Gustafson SJ, Truong VD, Yencho GC, Lila MA. Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweetpotato storage and impacts on bioactive properties. Food Chemistry. 2014;**145**: 717-724

[14] Liao Z, Chen M, Yang Y, Yang C, Fu Y, Zhang Q, et al. A new isopentenyl diphosphate isomerase gene from sweet potato: Cloning, characterization and color complementation. Biologia. 2008;**63**(2):221-226

[15] Maoka T, Akimoto N, Ishiguro K, Yoshinaga M, Yoshimoto M. Carotenoids with a 5, 6-dihydro-5, 6-dihydroxy-β-end group, from yellow sweet potato

"Benimasari", *Ipomoea batatas* Lam. Phytochemistry. 2007;**68**(13):1740-1745

[16] Tsuda T, Horio F, Osawa T. Dietary cyanidin 3-O-β-d-glucoside increases ex vivo oxidation resistance of serum in rats. Lipids. 1998;**33**(6):583

[17] Mazza G, Kay CD, Cottrell T, Holub BJ. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. Journal of Agricultural and Food Chemistry. 2002;**50**:7731-7737

[18] Tsoyi K, Park HB, Kim YM, Chung JI, Shin SC, Lee WS, et al. Anthocyanins from black soybean seed coats inhibit UVB-induced inflammatory cylooxygenase-2 gene expression and PGE2 production through regulation of the nuclear factor-κB and phosphatidylinositol 3-kinase/Akt pathway. Journal of Agricultural and Food Chemistry. 2008;**56**(19):8969-8974

[19] Wang J, Mazza G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-γ-activated RAW 264.7 macrophages. Journal of Agricultural and Food Chemistry. 2002;**50**(4): 850-857

[20] Pisha E, Pezzuto JM. Fruits and vegetables containing compounds that demonstrate pharmacological activity in humans. Economic and Medicinal Plant Research. 1994;**6**:189-233

[21] Hagiwara A, Yoshino H, Ichihara T, Kawabe M, Tamano S, Aoki H, et al. Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color, of 2-amino-1-methyl-6-phenylimidazo [4, 5-B] pyridine (phip)-associated colorectal carcinogenesis in rats. The Journal of Toxicological Sciences. 2002;**27**(1): 57-68

[22] Kurata R, Adachi M, Yamakawa O, Yoshimoto M. Growth suppression of human cancer cells by polyphenolics from sweetpotato (*Ipomoea batatas* L.) leaves. Journal of Agricultural and Food Chemistry. 2007;**55**(1):185-190

[23] Islam S. Sweetpotato (*Ipomoea batatas* L.) leaf: Its potential effect on human health and nutrition. Journal of Food Science. 2006;**71**(2):R13-R21

[24] Kano M, Takayanagi T, Harada K, Makino K, Ishikawa F. Antioxidative activity of anthocyanins from purple sweet potato, Ipomoera batatas cultivar Ayamurasaki. Bioscience, Biotechnology, and Biochemistry. 2005;**69**(5):979-988

[25] Cho J, Kang JS, Long PH, Jing J, Back Y, Chung KS. Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and cordyceps mushroom extract. Archives of Pharmacal Research. 2003;**26**(10): 821-825

[26] Kamei H, Kojima T, Hasegawa M, Koide T, Umeda T, Yukawa T, et al. Suppression of tumor cell growth by anthocyanins in vitro. Cancer Investigation. 1995;**13**(6):590-594

[27] Panda V, Sonkamble M. Anti-ulcer activity of Ipomoea batatas tubers (sweet potato). Functional Foods in Health and Disease. 2012;**2**(3):48-61

[28] Ozias-Akins P, Jarret RL. Nuclear DNA content and ploidy levels in the genus Ipomoea. Journal of the American Society for Horticultural Science. 1994;**119**(1):110-115

[29] Yan L, Gu YH, Tao X, Lai XJ, Zhang YZ, Tan XM, et al. Scanning of transposable elements and analyzing expression of transposase genes of sweet potato [Ipomoea batatas]. PLoS One. 2014;**9**(3):e90895

[30] Roullier C, Duputié A, Wennekes P, Benoit L, Fernández Bringas VM, Rossel G, et al. Disentangling the origins of cultivated sweet potato (Ipomoea

batatas (L.) Lam.). PLoS One. 2013; **8**(5):e62707

[31] Arizio CM, Tártara SC, Manifesto MM. Carotenoids gene markers for sweetpotato (*Ipomoea batatas* l. lam): Applications in genetic mapping, diversity evaluation and cross-species transference. Molecular Genetics and Genomics. 2014;**289**(2): 237-251

[32] Martin FW. Incompatibility in the sweet potato. A review. Economic Botany. 1965;**19**(4):406-415

[33] Holden JM, Eldrige AL, Beecher GR, Buzzard MI, Bhagwat S, Davis CS, et al. Carotenoid content of U. S. Foods: An update of the database. Journal of Food Composition and Analysis. 1999;**12**:169-196

[34] Burns J, Fraser PD, Bramley PM. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. Phytochemistry. 2003;**62**(6):939-947

[35] CIP, International Potato Center. 2019. Available from: https://cipotato.org/

[36] Institute of Food Technologists. Chapter 4: Nutritionally Improved Sweetpotato. Comprehensive Reviews in Food Science and Food Safety. 2008;**7**:81-91.(1t)

[37] Kukimura H, Yoshida T, Komaki K. New sweet potato cultivars, Benihayato and Satsumahikari, making a new turn for processing. JARQ. 1988;**22**(1):7-13

[38] Yamakawa O, Yoshinaga M, Kumagai T, Hidaka M, Komaki K, Kukimura H, et al. "J-red": A new sweetpotato cultivar. Bulletin of the Kyushu Agricultural Experiment Station (Japan). 1997;**33**:49-72

[39] Yamakawa O, Kumagai T, Yoshinaga M, Ishiguro K, Hidaka M, Komaki K, et al. "Sunny-red": A new sweetpotato (*Ipomoea batatas*) cultivar for powder. Bulletin of the Kyushu Agricultural Experiment Station (Japan). 1999;**35**:19-40

[40] Tanumihardjo SA, Ball AM, Kaliwile C, Pixley KV. The research and implementation continuum of biofortified sweet potato and maize in Africa. Annals of the New York Academy of Sciences. 2017;**1390**(1): 88-103

[41] Liu Q. Improvement for agronomically important traits by gene engineering in sweetpotato. Breeding Science. 2017;**67**:15-26. DOI: 10.1270/jsbbs.16126

[42] Walter MH, Strack D. Carotenoids and their cleavage products: Biosynthesis and functions. Natural Product Reports. 2011;**28**(4):663-692

[43] Okada K, Kasahara H, Yamaguchi S, Kawaide H, Kamiya Y, Nojiri H, et al. Genetic evidence for the role of isopentenyl diphosphate isomerases in the mevalonate pathway and plant development in Arabidopsis. Plant and Cell Physiology. 2008;**49**(4):604-616

[44] Misawa N. Carotenoids. Comprehensive Natural Products. 2010;**II**:733-753. DOI: 10.1016/b978-008045382-8.00009-5

[45] Takemura M, Maoka T, Misawa N. Biosynthetic routes of hydroxylated carotenoids (xanthophylls) in Marchantia polymorpha, and production of novel and rare xanthophylls through pathway engineering in *Escherichia coli*. Planta. 2015;**241**(3):699-710

[46] Sugawara T, Yamashita K, Asai A, Nagao A, Shiraishi T, Imai I, et al. Esterification of xanthophylls by human intestinal Caco-2 cells. Archives of Biochemistry and Biophysics. 2009;**483**(2):205-212

[47] Fraser PD, Bramley PM. Metabolic profiling and quantification of carotenoids and related isoprenoids in crop plants. In: Plant Metabolomics. Berlin, Heidelberg: Springer; 2006. pp. 229-242

[48] Margalith PZ. Production of ketocarotenoids by microalgae. Applied Microbiology and Biotechnology. 1999;**51**(4):431-438

[49] Farré G, Maiam Rivera S, Alves R, Vilaprinyo E, Sorribas A, Canela R, et al. Targeted transcriptomic and metabolic profiling reveals temporal bottlenecks in the maize carotenoid pathway that may be addressed by multigene engineering. The Plant Journal. 2013;**75**(3):441-455

[50] Britton G, Liaaen-Jenson S, Pfander H. (Eds), Compiled by Mercadante AZ, and Egeland ES, Birkhauser Verlag, Basle, Switzerland. Free Radical Research. 2004;**38**(8):885. DOI: 10.1080/10715760410001727849

[51] Moran NA, Jarvik T. Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science. 2010;**328**(5978):624-627

[52] Tonhosolo R, Fabio LD, de Rosso VV, Gazarini ML, Matsumura MY, Peres VJ, et al. Carotenoid biosynthesis in intraerythrocytic stages of *Plasmodium falciparum*. Journal of Biological Chemistry. 2009;**284**(15):9974-9985

[53] Ruiz-Sola MA, Rodríguez-Concepción M. Carotenoid biosynthesis in Arabidopsis: A colorful pathway. Arabidopsis Book. 2012;**10**:e0158

[54] Cazzonelli CI. Carotenoids in nature: Insights from plants and beyond. Functional Plant Biology. 2011;**38**(11): 833-847

[55] Misawa N, Satomi Y, Kondo K, Yokoyama A, Kajiwara S, Saito T, et al. Structure and functional analysis of a marine bacterial carotenoid biosynthesis gene cluster and astaxanthin biosynthetic pathway proposed at the gene level. Journal of Bacteriology. 1995;**177**(22):6575-6584

[56] Matsuno T. In: Krinsky NI, Mathews-Roth MM, Taylor F, editors. "Carotenoids:" In Chemistry and Biology. New York: Plenum Publishing Corp; 1989. pp. 59-74

[57] Renstrøm B, Borch G, Skulberg OM, Liaaen-Jensen S. Optical purity of (3S,3'S)-astaxanthin from Haematococcus pluvialis. Phytochemistry. 1981;**20**(11):2561-2564. DOI: 10.1016/0031-9422(81)83094-4

[58] Fraser PD, Miura Y, Misawa N. In vitro characterization of astaxanthin biosynthetic enzymes. Journal of Biological Chemistry. 1997;**272**(10): 6128-6135

[59] Misawa N. Pathway engineering for functional isoprenoids. Current Opinion in Biotechnology. 2011;**22**(5):627-633

[60] Zeb A, Mehmood S. Carotenoids contents from various sources and their potential health applications. Pakistan Journal of Nutrition. 2004;**3**(3):199-204

[61] Jaswir I. Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. Journal of Medicinal Plants Research. 2011;**5**:33. DOI: 10.5897/jmprx11.011

[62] Thane C, Reddy S. Processing of fruit and vegetables: Effect on carotenoids. Nutrition & Food Science. 1997;**97**(2):58-65. ISSN: 0034-6659

[63] Park SY, Nomura AM, Murphy SP, Wilkens LR, Henderson BE, Kolonel LN. Carotenoid intake and colorectal cancer risk: The multiethnic cohort study. Journal of Epidemiology. 2009;**19**(2): 63-71. DOI: 10.2188/jea.je20080078

[64] Carrillo-Lopez A, Yahia EM, Ramirez-Padilla GK. Bioconversion of carotenoids in five fruits and vegetables to vitamin A measured by retinol accumulation in rat livers. American Journal of Agricultural and Biological Sciences. 2010;**5**(2):215-221

[65] Böhm V. Lycopene and heart health. Molecular Nutrition & Food Research. 2012;**56**(2):296-303

[66] Gupta SK, Trivedi D, Srivastava S, Joshi S, Halder N, Verma SD. Lycopene attenuates oxidative stress induced experimental cataract development: An in vitro and in vivo study. Nutrition. 2003;**19**(9):794-799

[67] Chichili GR, Nohr D, Frank J, Flaccus A, Fraser PD, Enfissi EM, et al. Protective effects of tomato extract with elevated β-carotene levels on oxidative stress in ARPE-19 cells. British Journal of Nutrition. 2006;**96**(4):643-649

[68] Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature. 1981;**290**(5803):201-208

[69] Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014;**6**(2):466-488

[70] Stahl W, Heinrich U, Jungmann H, von Laar J, Schietzel M, Sies H, et al. Increased dermal carotenoid levels assessed by noninvasive reflection spectrophotometry correlate with serum levels in women ingesting Betatene. The Journal of Nutrition. 1998;**128**(5): 903-907

[71] Woodall AA, Lee SW, Weesie RJ, Jackson MJ, Britton G. Oxidation of carotenoids by free radicals: Relationship between structure and reactivity. Biochimica et Biophysica Acta (BBA)-General Subjects. 1997;**1336**(1): 33-42

[72] Erdman JW Jr, Ford NA, Lindshield BL. Are the health attributes of lycopene related to its antioxidant function? Archives of Biochemistry and Biophysics. 2009;**483**(2):229-235

[73] Giovannucci E. Obesity, gender, and colon cancer. Gut. 2002;**51**(2):147-147

[74] Das A, Yoon SH, Lee SH, Kim JY, Oh DK, Kim SW. An update on microbial carotenoid production: Application of recent metabolic engineering tools. Applied Microbiology and Biotechnology. 2007;**77**(3):505-512

[75] Arscott SA, Tanumihardjo SA. Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Comprehensive Reviews in Food Science and Food Safety. 2010;**9**(2):223-239

[76] Bendich A. Carotenoids and the immune system. In: Carotenoids. Boston, MA: Springer; 1989. pp. 323-335

[77] Nakazawa Y, Sashima T, Hosokawa M, Miyashita K. Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. Journal of Functional Foods. 2009;**1**(1):88-97

[78] Rao LG, Guns E, Rao AV. Lycopene: Its role in human health and disease. Agro Food. 2003;**7**:25-30

[79] Pool-Zobel BL, Bub A, Müller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: First results of a human intervention trial with carotenoid-rich foods. Carcinogenesis. 1997;**18**(9):1847-1850

[80] Bowen P, Chen L, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, et al. Tomato sauce supplementation and prostate cancer: Lycopene accumulation and modulation of biomarkers of carcinogenesis.

Experimental Biology and Medicine. 2002;**227**(10):886-893

[81] Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. New England Journal of Medicine. 1996;**334**(18):1145-1149

[82] Edge R, McGarvey DJ, Truscott TG. The carotenoids as anti-oxidants—A review. Journal of Photochemistry and Photobiology B: Biology. 1997;**41**(3): 189-200

[83] Abdel-Aal ES, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients. 2013;**5**(4): 1169-1185

[84] Stahl W, Sies H. β-Carotene and other carotenoids in protection from sunlight. The American Journal of Clinical Nutrition. 2012;**96**(5):1179S-1184S

[85] Engelmann NJ, Clinton SK, Erdman JW Jr. Nutritional aspects of phytoene and phytofluene, carotenoid precursors to lycopene. Advances in Nutrition. 2011;**2**(1):51-61

[86] Kurashige M, Okimasu E, Inoue M, Utsumi K. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiological Chemistry and Physics and Medical NMR. 1990;**22**(1):27-38

[87] Jørgensen K, Skibsted LH. Carotenoid scavenging of radicals. Zeitschrift für Lebensmittel-Untersuchung und Forschung. 1993;**196**(5):423-429

[88] Murillo E. Efecto hipercolesterolémico de la cantaxantina y la astaxantina en ratas. Archivos Latinoamericanos de Nutrición. 1992;**42**(4):409-413

[89] Jyonouchi H, Sun S, Iijima K, Gross MD. Antitumor activity of astaxanthin and its mode of action. Nutrition and Cancer. 2000;**36**(1):59-65

[90] Martín JF, Gudiña E, Barredo JL. Conversion of β-carotene into astaxanthin: Two separate enzymes or a bifunctional hydroxylase-ketolase protein? Microbial Cell Factories. 2008;**7**(1):1-10

[91] Kushwaha K, Saini A, Saraswat P, Agarwal MK, Saxena J. Colorful world of microbes: Carotenoids and their applications. Advanced Biology. 2014;**2014**:837891

[92] The Global Market for Carotenoids. FOD025F. 2018

[93] FOD025C. BBC Research. Available from: http://www.bccresearch.com/report/FOD025C.html

[94] Azadi P, Otang NV, Chin DP, Nakamura I, Fujisawa M, Harada H, et al. Metabolic engineering of Lilium× formolongi using multiple genes of the carotenoid biosynthesis pathway. Plant Biotechnology Reports. 2010;**4**(4): 269-280

[95] Clotault J, Peltier D, Berruyer R, Thomas M, Briard M, Geoffriau E. Expression of carotenoid biosynthesis genes during carrot root development. Journal of Experimental Botany. 2008;**59**(13):3563-3573

[96] Turujman SA, Wamer WG, Wei RR, Albert RH. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. Journal of AOAC International. 1997;**80**(3): 622-632

[97] Fujita T, Satake M, Watanabe T, Kitajima C, Miki W, Yamaguchi K, et al. Pigmentation of cultured red sea bream with astaxanthin diester purified from krill oil. Nippon Suisan Gakkaishi. 1983;**49**(12):1855-1861

[98] Matsuno T. Xanthophylls as precursors of retinoids. Pure and Applied Chemistry. 1991;**63**(1):81-88

[99] Tanaka T, Morishita Y, Suzui M, Kojima T, Okumura A, Mori H. Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. Carcinogenesis. 1994;**15**(1):15-19

[100] Jyonouchi H, Zhang L, Tomita Y. Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation. Nutrition and Cancer. 1993;**19**:269-280

[101] Miki W, Otaki N, Yokoyama A, Izumida H, Shimidzu N. Okadaxanthin, a novel C 50-carotenoid from a bacterium, Pseudomonas sp. KK10206C associated with marine sponge, Halichondria okadai. Experientia. 1994;**50**(7):684-686

[102] Foote CS. Photosensitized oxidation and singlet oxygen: Consequences in biological systems. In: Pryor WA, editor. Free Radicals in Biology Vol II. Vol. 35. New York: Academic Press; 1976. pp. 3-22

[103] Hue SM, Boyce AN, Somasundram C. Comparative study on the antioxidant activity of leaf extract and carotenoids extract from Ipomoea batatas var. Oren (sweetpotato) leaves. International Journal of Nutrition and Food Engineering. 2011;**5**(10):604-607

[104] Hue SM, Boyce AN, Somasundram C. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato ('*Ipomoea batatas*'). Australian Journal of Crop Science. 2012;**6**(3): 375-380

[105] Islam S. Some bioactive constituents, antioxidant, and antimutagenic activities in the leaves of *Ipomoea batatas* Lam. Genotypes. Journal of Food Science and Technology. 2016;**4**(3):70-80

[106] Sosinski B, He L, Cervantes-Flores J, Pokrzywa RM, Bruckner A, Yencho GC. Sweetpotato genomics at North Carolina State University. In: International Conference on Sweetpotato. Food and Health for the Future. Vol. 583. Lima, Peru: Acta Horticulture; 2001. pp. 51-60. DOI: 10.17660/ActaHortic.2002.583.4

[107] Aguoru CU. Varietal characterisation and taxonomic evaluation of sweet potato (*Ipomoea batatas*) using macro-and micromorphological evidence. Open Access Library Journal. 2015;**2**(08):1

[108] Wanda CW. Genetic improvement for meeting human nutrition needs. In: Quebedeaux B, Bliss F, editors. Proceedings of the First International Symposium on Horticulture and Human Nutrition, Contributor of Fruits and Vegetables. London: Prentice Hall; 1987. pp. 191-199

[109] Ishiguro K, Yoshinaga M, Kai Y, Maoka T, Yoshimoto M. Composition, content and antioxidative activity of the carotenoids in yellow-fleshed sweetpotato (*Ipomoea batatas* L.). Breeding Science. 2010;**60**(4):324-329

[110] Kammona S, Othman RA, Jaswir IR, Jamal P. Characterisation of carotenoid content in diverse local sweet potato (*Ipomoea batatas*) flesh tubers. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;**7**(2): 347-351

[111] Islam SN, Nusrat T, Begum P, Ahsan M. Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. Food Chemistry. 2016;**199**:628-631

[112] Wurtzel ET, Valdez G, Matthews PD, Wurtzel E. Variation in expression of carotenoid genes in transformed *E. coli* strains. Bioresearch Journal. 1997;**1**:1-1

[113] Sandmann G, Woods WS, Tuveson RW. Identification of carotenoids in Erwinia herbicola and in a transformed *Escherichia coli* strain. FEMS Microbiology Letters. 1990;**71**(1-2):77-82

[114] Misawa N, Nakagawa M, Kobayashi K, Yamano S, Izawa Y, Nakamura K, et al. Elucidation of the Erwinia uredovora carotenoid biosynthetic pathway by functional analysis of gene products expressed in *Escherichia coli*. Journal of Bacteriology. 1990;**172**(12):6704-6712

[115] Misawa N, Yamano S, Linden H, de Felipe MR, Lucas M, Ikenaga H, et al. Functional expression of the Erwinia uredovora carotenoid biosynthesis gene crtl in transgenic plants showing an increase of β-carotene biosynthesis activity and resistance to the bleaching herbicide norflurazon. The Plant Journal. 1993;**4**(5):833-840

[116] Misawa N, Truesdale MR, Sandmann G, Fraser PD, Bird C, Schuch W, et al. Expression of a tomato cDNA coding for phytoene synthase in *Escherichia coli*, phytoene formation in vivo and in vitro, and functional analysis of the various truncated gene products. The Journal of Biochemistry. 1994;**116**(5):980-985

[117] Li ZH, Matthews PD, Burr B, Wurtzel ET. Cloning and characterization of a maize cDNA encoding phytoene desaturase, an enzyme of the carotenoid biosynthetic pathway. Plant Molecular Biology. 1996;**30**(2):269-279

[118] Chamovitz D, Misawa N, Sandmann G, Hirschberg J. Molecular cloning and expression in *Escherichia coli* of a cyanobacterial gene coding for phytoene synthase, a carotenoid biosynthesis enzyme. FEBS Letters. 1992;**296**(3):305-310

[119] Cunningham FX, Chamovitz D, Misawa N, Gantt E, Hirschberg J. Cloning and functional expression in *Escherichia coli* of a cyanobacterial gene for lycopene cyclase, the enzyme that catalyzes the biosynthesis of β-carotene. FEBS Letters. 1993;**328**(1-2):130-138

[120] Sandmann G. [30] carotenoid analysis in mutants from *Escherichia coli* transformed with carotenogenic gene cluster and Scenedesmus obliquus mutant C-6D. Methods in Enzymology. 1993;**214**:341-347

[121] Hundle B, Alberti M, Nievelstein V, Beyer P, Kleinig H, Armstrong GA, et al. Functional assignment of Erwinia herbicola Eho10 carotenoid genes expressed in *Escherichia coli*. Molecular and General Genetics MGG. 1994;**245**(4):406-416

[122] Sandmann G, Misawa N. New functional assignment of the carotenogenic genes crtB and crtE with constructs of these genes from Erwinia species. FEMS Microbiology Letters. 1992;**90**(3):253-257

[123] Penfold RJ, Pemberton JM. Sequencing, chromosomal inactivation, and functional expression in *Escherichia coli* of ppsR, a gene which represses carotenoid and bacteriochlorophyll synthesis in Rhodobacter sphaeroides. Journal of Bacteriology. 1994;**176**(10): 2869-2876

[124] Kajiwara S, Kakizono T, Saito T, Kondo K, Ohtani T, Nishio N, et al. Isolation and functional identification of a novel cDNA for astaxanthin biosynthesis from Haematococcus pluvialis, and astaxanthin synthesis in *Escherichia coli*. Plant Molecular Biology. 1995;**29**(2):343-352

[125] Ohnuma SI, Suzuki M, Nishino T. Archaebacterial ether-linked lipid biosynthetic gene. Expression cloning, sequencing, and characterization of geranylgeranyl-diphosphate synthase. Journal of Biological Chemistry. 1994;**269**(20):14792-14797

[126] Keeler J. Understanding NMR Spectroscopy, 2nd ed. West Sussex, United Kingdom: Wiley; 2010. pp. 184-187. ISBN: 978-0-470-746080

[127] Maoka T, Otani M, Khan MZ, Takemura M, Hattan JI, Misawa N. Novel carotenoids produced on the interaction of the foreign carotenoid ketolase CrtW and endogenous epoxycarotenoids unique to sweetpotato tubers. Tetrahedron Letters. 2016;**57**(42):4746-4748

[128] Kim SH, Jeong JC, Park S, Bae JY, Ahn MJ, Lee HS, et al. Down-regulation of sweetpotato lycopene β-cyclase gene enhances tolerance to abiotic stress in transgenic calli. Molecular Biology Reports. 2014;**41**(12):8137-8148

[129] Song GQ, Honda H, Yamaguchi KI. Efficient Agrobacterium tumefaciens-mediated transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) from stem explants using a two-step kanamycin-hygromycin selection method. In Vitro Cellular & Developmental Biology-Plant. 2004;**40**(4):359-365

[130] Otani M, Mii M, Handa T, Kamada H, Shimada T. Transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) plants by *Agrobacterium rhizogenes*. Plant Science. 1993;**94**(1-2): 151-159

[131] Otani M, Shimada T. Efficient embryogenic callus formation in sweet potato (*Ipomoea batatas* (L.) Lam.). Japanese Journal of Breeding. 1996;**46**(3):257-260

[132] Otani M, Shimada T, Kimura T, Saito A. Transgenic plant production from embryogenic callus of sweet potato (*Ipomoea batatas* (L.) Lam.) using *Agrobacterium tumefaciens*. Plant Biotechnology. 1998;**15**:11-16

[133] Lou HR, Maria MS, Benavides J, Zhang DP, Zhang YZ, Ghislain M. Rapid genetic transformation of sweetpotato (*Ipomoea batatas* (L.) Lam) via organogenesis. African Journal of Biotechnology. 2006;**5**(20):1851-1857

[134] Kim SH, Ahn YO, Ahn MJ, Lee HS, Kwak SS. Down-regulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweetpotato. Phytochemistry. 2012;**74**:69-78

[135] Ling YU, Hong ZH, Wei CH, HE SZ, LIU QC. Cloning and functional analysis of lycopene ε-cyclase (IbLCYe) gene from sweetpotato, Ipomoea batatas (L.) Lam. Journal of Integrative Agriculture. 2013;**12**(5):773-780

[136] Lee P, Schmidt-Dannert C. Metabolic engineering towards biotechnological production of carotenoids in microorganisms. Applied Microbiology and Biotechnology. 2002;**60**(1):1-1

[137] Giuliano G, Aquilani R, Dharmapuri S. Metabolic engineering of plant carotenoids. Trends in Plant Science. 2000;**5**(10):406-409

[138] Guerin M, Huntley ME, Olaizola M. Haematococcus astaxanthin: Applications for human health and nutrition. Trends in Biotechnology. 2003;**21**(5):210-216

[139] Misawa N. Pathway engineering of plants toward astaxanthin production. Plant Biotechnology. 2009;**26**(1):93-99

[140] Zhu C, Naqvi S, Capell T, Christou P. Metabolic engineering of ketocarotenoid biosynthesis in higher plants. Archives of Biochemistry and Biophysics. 2009;**483**(2):182-190

[141] Hasunuma T, Miyazawa SI, Yoshimura S, Shinzaki Y, Tomizawa KI, Shindo K, et al. Biosynthesis of astaxanthin in tobacco leaves by transplastomic engineering. The Plant Journal. 2008;**55**(5):857-868

[142] Harada H, Maoka T, Osawa A, Hattan JI, Kanamoto H, Shindo K, et al. Construction of transplastomic lettuce (*Lactuca sativa*) dominantly producing astaxanthin fatty acid esters and detailed chemical analysis of generated carotenoids. Transgenic Research. 2014;**23**(2):303-315

[143] Jayaraj J, Devlin R, Punja Z. Metabolic engineering of novel ketocarotenoid production in carrot plants. Transgenic Research. 2008;**17**(4): 489-501

[144] Fujisawa M, Takita E, Harada H, Sakurai N, Suzuki H, Ohyama K, et al. Pathway engineering of Brassica napus seeds using multiple key enzyme genes involved in ketocarotenoid formation. Journal of Experimental Botany. 2009;**60**(4):1319-1332

[145] 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. Proceedings of the National Academy of Sciences. 2008;**105**(47): 18232-18237

[146] Yokoyama A, Shizuri Y, Misawa N. Production of new carotenoids, astaxanthin glucosides, by Escherichia coli transformants carrying carotenoid biosynthesis genes. Tetrahedron Letters. 1998;**39**(22):3709-3712

[147] Nishida Y, Adachi K, Kasai H, Shizuri Y, Shindo K, Sawabe A, et al. Elucidation of a carotenoid biosynthesis gene cluster encoding a novel enzyme, 2, 2′-β-hydroxylase, from Brevundimonas sp. strain SD212 and combinatorial biosynthesis of new or rare xanthophylls. Applied and Environmental Microbiology. 2005;**71**(8):4286-4296

[148] Shindo K, Hasunuma T, Asagi E, Sano A, Hotta E, Minemura N, et al. 4-Ketoantheraxanthin, a novel carotenoid produced by the combination of the bacterial enzyme β-carotene ketolase CrtW and endogenous carotenoid biosynthetic enzymes in higher plants. Tetrahedron Letters. 2008;**49**(20):3294-3296

[149] Breitenbach J, Bai C, Rivera SM, Canela R, Capell T, Christou P, et al. A novel carotenoid, 4-keto-α-carotene, as an unexpected by-product during genetic engineering of carotenogenesis in rice callus. Phytochemistry. 2014;**98**:85-91

[150] Maoka T, Takemura M, Tokuda H, Suzuki N, Misawa N. 4-Ketozeinoxanthin, a novel carotenoid produced in *Escherichia coli* through metabolic engineering using carotenogenic genes of bacterium and liverwort. Tetrahedron Letters. 2014;**55**(49):6708-6710

[151] Umeno D, Arnold FH. Evolution of a pathway to novel long-chain carotenoids. Journal of Bacteriology. 2004;**186**(5):1531-1536

[152] Kang L, Ji CY, Kim SH, Ke Q, Park SC, Kim HS, et al. Suppression of the β-carotene hydroxylase gene increases β-carotene content and tolerance to abiotic stress in transgenic sweetpotato plants. Plant Physiology and Biochemistry. 2017;**117**:24-33

[153] Li R, Kang C, Song X, Yu L, Liu D, He S, et al. A ζ-carotene desaturase gene, IbZDS, increases β-carotene and lutein contents and enhances salt tolerance in transgenic sweetpotato. Plant Science. 2017;**262**:39-51

[154] Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, et al. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. Journal of Experimental Botany. 2005;**56**(409): 81-89

[155] Ke Q, Kang L, Kim HS, Xie T, Liu C, Ji CY, et al. Down-regulation of

lycopene ε-cyclase expression in transgenic sweetpotato plants increases the carotenoid content and tolerance to abiotic stress. Plant Science. 2019;**281**: 52-60

[156] Gerjets T, Sandmann G. Ketocarotenoid formation in transgenic potato. Journal of Experimental Botany. 2006;**57**(14):3639-3645

[157] Kim SE, Lee CJ, Park SU, Lim YH, Park WS, Kim HJ, et al. Overexpression of the golden SNP-carrying Orange gene enhances carotenoid accumulation and heat stress tolerance in sweetpotato plants. Antioxidants. 2021;**10**(1):51

[158] Gama MI, Leite RP, Cordeiro AR, Cantliffe DJ. Transgenic sweet potato plants obtained by Agrobacterium tumefaciens-mediated transformation. Plant Cell, Tissue and Organ Culture. 1996;**46**(3):237-244

[159] Takahata Y, Tanaka M, Otani M, Katayama K, Kitahara K, Nakayachi O, et al. Inhibition of the expression of the starch synthase II gene leads to lower pasting temperature in sweetpotato starch. Plant Cell Reports. 2010;**29**(6): 535-543

[160] Kim SH, Ahn YO, Ahn MJ, Jeong JC, Lee HS, Kwak SS. Cloning and characterization of an Orange gene that increases carotenoid accumulation and salt stress tolerance in transgenic sweetpotato cultures. Plant Physiology and Biochemistry. 2013a;**70**:445-454

[161] Park SC, Kim SH, Park S, Lee HU, Lee JS, Park WS, et al. Enhanced accumulation of carotenoids in sweetpotato plants overexpressing IbOr-Ins gene in purple-fleshed sweetpotato cultivar. Plant Physiology and Biochemistry. 2015;**86**:82-90

[162] Kim SH, Kim YH, Ahn YO, Ahn MJ, Jeong JC, Lee HS, et al. Downregulation of the lycopene ϵ-cyclase gene increases carotenoid synthesis via the β-branch-specific pathway and enhances salt-stress tolerance in sweetpotato transgenic calli. Physiologia Plantarum. Apr 2013;**147**(4):432-442

[163] Ma DFLI. Selection of parents for breeding ed-ible varieties of sweetpotato with high carotene content. Agri-cultural Sciences in China. 2009;**8**(10):1166-1173

[164] Hwang SY, Tseng YT, Lo HF. Application of simple sequence repeats in determining the genetic relationships of cultivars used in sweet potato polycross breeding in Taiwan. Scientia Horticulturae. 2002;**93**(3-4):215-224

[165] Cervantes-Flores JC, Sosinski B, Pecota KV, Mwanga RO, Catignani GL, Truong VD, et al. Identification of quantitative trait loci for dry-matter, starch, and β-carotene content in sweetpotato. Molecular Breeding. 2011;**28**(2):201-216

[166] Laurie SM, Van Jaarsveld PJ, Faber M, Philpott MF, Labuschagne MT. Trans-β-carotene, selected mineral content and potential nutritional contribution of 12 sweetpotato varieties. Journal of Food Composition and Analysis. 2012;**27**(2):151-159

[167] Pillay K, Khanyile N, Siwela M. Acceptance of an orange-fleshed sweet potato complementary food by infant caregivers in KwaZulu-Natal Province—A preliminary study. South African Journal of Child Health. 2018;**12**(3):100-104